Reply to Editor Comments on Manuscript egusphere-2022-1084

Title: Biochar promotes soil aggregate stability and associated organic carbon sequestration, and regulates microbial community structures in Mollisols from Northeast China

Author(s): Jing Sun et al.

Thank you very much for giving us the opportunity to revise our manuscript. We appreciate reviewer very much for the constructive comments and suggestions concerning to our manuscript. The comments are all valuable and very helpful for revising and improving our paper. We have provided a point-by-point response to the reviews including a list of all relevant changes made in the manuscript.

The main corrections in the paper and the response to the comments are as following:

Community 1:

The authors evaluated the effects of different biochar gradients combined with nitrogen fertilizer on the size, proportion, stability, and carbon content of Mollisols soil aggregates. In addition, they also examine how biochar affects the structure of microbial populations and identify the main factors influencing changes in microbial composition and recommend an optimal biochar application ratio to improve soil quality by modifying aggregates and the soil microbial community structure of Chinese black soils. They concluded that microbial abundance increased significantly when biochar and fertilizer were combined, and soil aggregation and SOC of black soil were significantly improved. The graphic description in the paper is relatively clear and intuitive, which well summarizes the conclusions expressed by the author.

1. Field experiments are a more objective reflection of actual production issues, and it is a pity that we did not see the author's research on crop yields, but it is still an impressive work, and the findings are essential if we want to improve our understanding of the improvement of soil quality through biochar. However, in my opinion, it is very necessary to make proper revisions before acceptance.
   ◆ Author response: Thank you very much for your careful review and constructive suggestions regarding our manuscript. The improvement of soil structure and quality is an important prerequisite for improving crop yield and quality. Therefore, this paper focuses on the analysis of the effect of biochar on soil improvement. However, we have not ignored the trends in crop yield and quality in the improved soils, which have been analyzed in detail in a subsequent manuscript.

2. Line 16-23, The summary of the Abstract section, the conclusions seem ambiguous. I suggest that the author provide a clear and reliable conclusion in concise language.

◆ Author response: Thanks for the advice. We have revised the "Abstract" part as you suggested. We combed through sentences that were relatively vague in meaning to make them clearer and more logical: "Results indicated that biochar combined with N fertilizer effectively increases soil carbon storage and aggregates stability ($P < 0.05$). And C2N treatment increased the aggregate contents of the > 2 mm and 0.25–2 mm fractions by 56.59% and 23.41%, respectively. The phospholipid fatty acids (PLFA) analysis revealed that microbial community structure was effectively improved with biochar combined with N fertilizer application ($P < 0.05$). The F/B ratio increased by 25.22% and the gram-positive (Gm^+)/gram-negative (Gm^-) ratio by 4.65% under the C2N1/2 treatment." **Line-15-21**

3. Line 44-47, There is large room for improvement in description and expression. Some sentences are long and contain a lot of repetitive information.
◆ Author response: Thanks for the advice. The sentence has been amended to: "The principal manifestations of soil degradation were a significant decline of soil organic carbon (SOC), destruction in soil aggregation (Zhang et al., 2018), and deterioration of soil structure (Luo et al., 2020; Zhang et al., 2019)." **Line-35-37**

4. Line 60-61, awkward sentence with controversial expression. Try to explain the limited time.
◆ Author response: Thank you for the valuable suggestion. We further explain the constraints of straw decomposition and add references to the literature.
The sentence has been amended to: "This possibly resulted from the low decomposition rate of returning straw due to long soil freeze durations, especially at higher latitudes in cold Chinese Mollisols with straw decomposition durations of a quarter to one year (Wang et al., 2011)." **Line-50-52**

5. Line 87, "Principal ecological activities including…", double check the term or phrase.
◆ Author response: Thanks for catching this. The phrase was modified as: "Principal ecological activities in soil". **Line-79-80**

6. Line 215, Please explain original profiled soil?
◆ Author response: The phrase was modified as: "The surface soil (0–10 cm) had the highest moisture content in the CK treatment". **Line-200-201**

7. Line 227, please define "bottom soil".
◆ Author response: Thank you for your suggestion. The "bottom soil" means "the 20–40 cm soil layer". To make the sentence clear and understandable.
The phrase was modified as: "First, the number of large aggregates fractions was lower in the 20–40 cm soil layer than in the 0–10 cm soil layer." **Line-213-214**

8. Line 230, "combined application"? unclear expression.
◆ Author response: Thank you for the valuable suggestion. Replaced "combined

application" with "Biochar combined with nitrogen fertilizer" **Line-215-216**

9. Line 288-290, Not necessary to simply repeat the numbers or values in the figures.
◆ Author response: The interpretation of the diagram has been simplified as requested. Replaced "The two RDA axes were significant ($P < 0.05$), accounted for 94.12% of the overall variation in the soil microbial characteristics. The first axis explained 85.83 % of the total variation in microbial community composition, while the second axis explained 8.29%." with "The two RDA axes were significant ($P < 0.05$), accounted for 94.12% of the overall variation in the soil microbial characteristics." **Line-272-273**

10. Line 342, macroaggregates, small macroaggregates? Please be uniform in the text.
◆ Author response: Thank you for your suggestion. Made the change as suggested. According to aggregates size divided into: large aggregates (> 2 mm), small aggregates (0.25–2 mm), microaggregates (0.053–0.25 mm), silt and clay (< 0.053 mm).

11. Line 410, Among the effects of biochar combined with N fertilizer on microbial communities, the authors suggest the effectiveness of reduced N fertilizer application to improve microbial richness, and the potential negative environmental effects after high doses of N fertilizer application should be further briefly discussed
◆ Author response: Thank you for the valuable suggestion. Based on your suggestions, the potential negative environmental effects have been supplemented: "In this study, C2N1/2 treatment had the best improvement effect on microbial community structure. The N source always an important source of microbial nutrients. However, when N fertilizer application exceeds crop absorption capacity and soil retention capacity, the excess N may be leached to deep soil and pollute groundwater. Meanwhile, the unbalanced C/N ratio also became the main factor for the significant decline of soil biodiversity (Yuan et al. 2017)." **Line-424-429**

12. Line 418, Please explain the difference between TOC and SOC. Both parameters are used.
◆ Author response: For TOC in soil (measured in the experiment), it consists of soil organic carbon (SOC) and soil inorganic carbon (SIC). SIC is generally present in the form of carbonate, which was negligible in the soil samples in this experiment, so TOC was used in this paper to represent the amount of organic matter in the samples. However, since microorganisms are unable to use SIC and mainly use SOC as carbon source. It is therefore generally more accurate to use the term SOC for the interpretation and justification of conclusions.

13. Line 426-428, Repeated statement.
◆ Author response: Thanks for your careful checks, we are very sorry for our carelessness. It has been corrected.

14. Line 430-432, the meaning of this sentence is unclear, rephrase the sentences to make the logic smooth and the meaning clear.

◆ Author response: Thank you for the valuable suggestion. The phrase was modified as: "The increased fungal abundance has been proposed as an important biological factor in soil aggregate formation (Yuan et al., 2015; Zheng et al., 2020). Previous research has shown that the improvement of aggregate stability and carbon storage are important prerequisites for enhancing microbial communities (Zhang et al., 2021)." **Line-431-434**

15. Line 454, delete "The PCA showed that".

◆ Author response: Made the change as suggested.

Referee 1:

Sun et al. conducted field experiments to explore the response of the stability mechanism of the soil aggregates, the dynamic properties of organic carbon, and changes in the microbial community structure to biochar. The results help to the sustainable utilization of Mollisols resources.

I have several suggestions that may improve the manuscript.

1. Line 37, (Zhang et al. 2018; Eswaran et al., 2011).

◆ Author response: Replaced "((Zhang et al. 2018; Eswaran et al., 2011)" with "(Zhang et al., 2018; Eswaran et al., 2011)". **Line-28-29**

2. Line 39, increased poor?

◆ Author response: Thanks for catching this. The sentence was rewritten as "However, the Mollisols has been severely degraded by intensive continuous cultivation and soil erosion, which has led to the destruction of agroecosystem and soil infertile, with far-reaching effects on global climate change" **Line29-31**

We have corrected the improper words, expressions, and the spelling and grammatical errors in the revised manuscript.

3. Line 94, E.-L. et al. 2014?

◆ Author response: We are very sorry for our carelessness. "(E.-L. et al. 2014)" has been changed to "(Ng et al. 2014)" **Line-86**

Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities?

E.-L. Ng [a,b,*], A.F. Patti [b], M.T. Rose [a,b], C.R. Schefe [c], K. Wilkinson [c], R.J. Smernik [d], T.R. Cavagnaro [a,d]

[a] School of Biological Sciences, Monash University, Victoria 3800, Australia
[b] School of Chemistry, Monash University, Victoria 3800, Australia
[c] Department of Environment and Primary Industries, Victoria, Australia
[d] School of Agriculture, Food and Wine, University of Adelaide Waite Campus, Urrbrae, South Australia 5064, Australia

Ng, E. L., Patti, A. F., Rose, M. T., Schefe, C. R., Wilkinson, K., Smernik, R. J., et al.: Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biology and Biochemistry. 70 (2014) 54-61. http://dx.doi.org/10.1016/j.soilbio.2013.12.004, 2014.

4. Line 166, $Wt = Mi/Mt \times 100\%$?

◆ Author response: Thank you for your suggestion. Made the change as suggested. **Line155**

5. Lines 186-188, remove these sentences as they are not methods.

◆ Author response: Made the change as suggested. These sentences have been deleted.

6. Line 222, in figure 1, the X axis represents different treatments, how to fit the data with the Y axis? So please remove the fit line in the figure. It is more suitable to use a box plot to show the results. Similar mistakes also occurred in figure 4 and figure 7, please revise carefully.

◆ Author response: Since the overall treatment at the cross-coordinate is not continuous, so my fitted curve is a segmented fit. In the segmented fit the treatment is continuous, with a continuous increase in biochar application as a regular change in environmental factors, where Figure 1 Figure 4 is reasonable. However, we are glad to accept the reviewer's suggestion. To avoid distressing the reader, we have used a box plot for Figure 7 to present our results. **Line280**
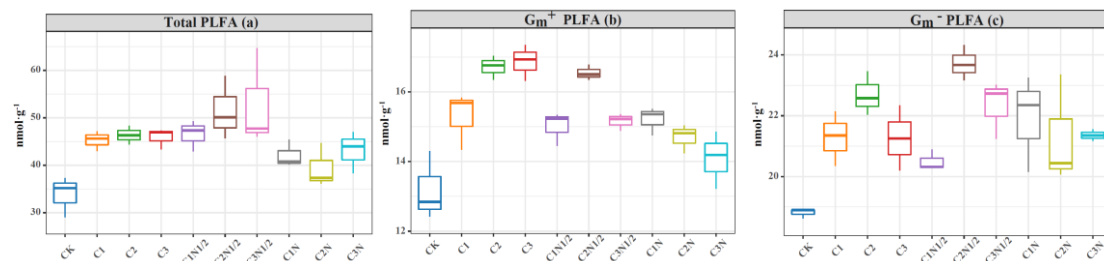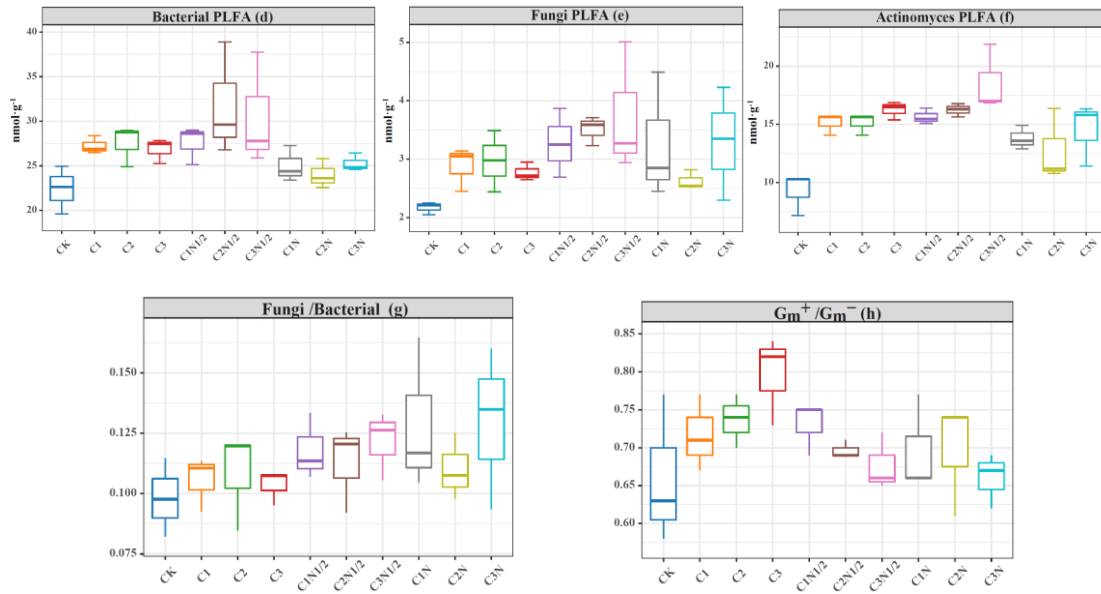
Figure 1 The concentration of the (a) total phospholipid fatty acids (PLFAs; nmol·g⁻¹), (b) gram-positive bacteria (Gm⁺) PLFAs, (c) gram-negative bacteria (Gm⁻) PLFAs, (d) bacteria PLFAs, (e) fungi PLFAs, (f) actinomycetes PLFAs, (g) ratio of the bacteria PLFAs/fungi PLFAs (F/B), and (h) ratio of the Gm⁺ to Gm⁻ bacteria of the microbial community in the soils under the treatments.

7. Line 266, change C3+N1/2 to C3N1/2, please keep consistency in the MS

◆ Author response: Thank you for your suggestion. We also checked the accuracy of the words in the manuscript. "C3+N1/2" has been changed to "C3N1/2" **Line248**

8. Line 272, in figure 5, please remove the correlation coefficient with 1, no need to show.

◆ Author response: Thank you for your suggestion. Made the change as suggested. **Line255**
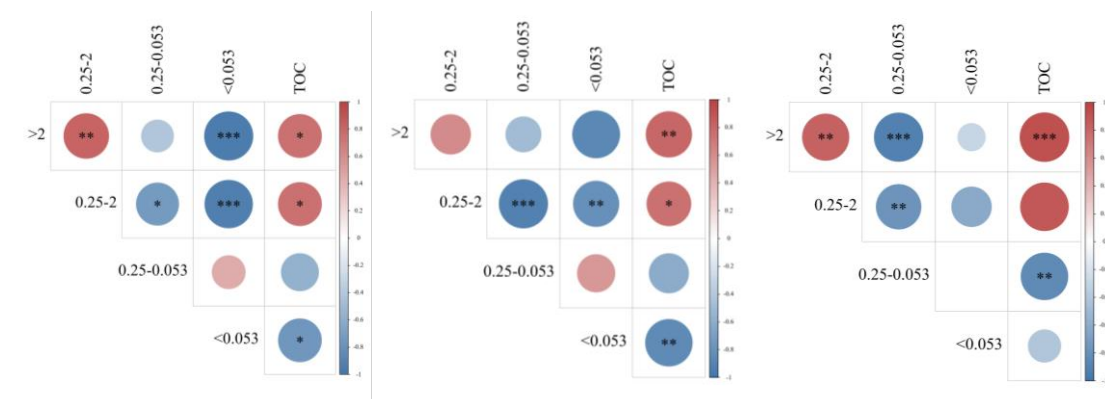
Figure 5 The correlation between the total organic carbon (TOC) and the aggregate contents of the different particle sizes in the soil profile (from left to right: 0–10 cm, 10–20 cm, and 20–40 cm).

9. Lines 305-306, the results showed that the first 3 principal components (F1-F3) explained 90.13% of the total variance.

◆ Author response: Thank you for your suggestion. Made the change as suggested. "The results showed that the cumulative variance contribution rate was 90.13%, which adequately explained the variation." has been changed to "The results showed that the first 3 principal components (F1-F3) explained 90.13% of the total variance." **Line290-291**

10. Line 376, where is Table 2?

◆ Author response: Thank you for reminding us. We have changed Table 2 to Table S2. **Line373**

11. Lines 445-446, the authors conclude that "Based on the sequestration of SOC and the sustainability and stability of the ecosystem, we selected C3N1/2 as the most reasonable biochar ratio.", however, according to table 1, C2N1/2 is the best.

◆ Author response: Thank you for your reminder. We have corrected it. "Based on the sequestration of SOC and the sustainability and stability of the ecosystem, we selected C2N1/2 as the most reasonable biochar ratio." **Line444-446**

12. More discussion is needed to explain why N addition affects soil aggregate stability.

◆ Author response: Thank you for your useful advice. We added an explanation of this section: "The N input enhancing aggregation possible is interpreted in three aspects. First, N fertilizer increased macro-aggregates and MWD stemmed from N-stimulation of root growth (Bai et al., 2021). Roots are a major driver of soil aggregation as they not only constitute the primary source of SOC, but also function as physical binding agents for aggregation (Sokol and Bradford, 2019); Second, N-increase of plant photosynthesis generally allows plant to allocate more photosynthates to the roots and their associated mycorrhizal fungi, which may physically facilitate the formation of soil macroaggregate through fine root and hyphal enmeshment of microaggregates (Miller and Jastrow, 1990; Bai et al., 2021); Third, the active functional groups in the N fertilizer adsorb soil Ca^{2+} and then tend to combine with clay minerals to form a clay-humus complex (Gao et al. 2019)." **Line356-364**

"Previous research proposed that biochar combined with N fertilizer promotes crop root growth, improves crop root fungi reproductive capacity. Fungal hyphae and root secretions promote soil aggregate by binding and entangling mineral particles to form large aggregates structures (Islam et al., 2021). Consistently, our data suggest that the increased stability of aggregate results from increased root activity and the significant role of exogenous carbon as a binder for soil particles (Wang et al., 2019)." **Line375-380**

13. In short, I think this study is interesting, however, more revisions are needed, as I listed above. Please also check English carefully and make the MS more concise, logical, and readable.

◆ Author response: As suggested, all the words, sentences and grammars have been carefully checked throughout the paper by us and native English speaker, in which the improper words, unclear terms, and grammatical errors have been edited. We believe that the written English and the readability substantially improved in the revised manuscript.

Referee 2:

This paper describes the effects of biochar and mineral fertilizer applications on soil aggregation, aggregates associated C, and microbial community structure. Few studies have examined the limpact of biochar on soil aggregation and microbial community structure that related to soil C and N cycles under field conditions. Furthermore, the study considers the interactions between biochar and mineral fertilizer on these processes linking to SOC sequestration. This paper provides important new information that will be of value to many researchers and students and the data are worthy of publication.

However, some issues should be revised before further consideration.

◆ Author response: We appreciate the reviewer very much for the positive and constructive comments and suggestions concerning to our manuscript. We have carefully studied the reviewers' comments and have made some revisions in the modified manuscript.

1. For the introduction section, some statements should be cited the references (E.g., L49-50, L65-66;)

◆ Author response: Thank you for your suggestion. We have added some references to the statements as has been mentioned.

Replaced "The SOC can promote the formation of large aggregates in soil, while increased soil aggregate stability promotes soil SOC sequestration" with "The SOC can promote the formation of large aggregates in soil, in turn, the increased

soil aggregate stability promotes soil SOC sequestration (Zhang et al., 2018)."**Line-41**

Zhang, Y., Li, X., Gregorich, E. G., Mclaughlin, N. B., Zhang, X., Guo, Y., et al.: No-tillage with continuous maize cropping enhances soil aggregation and organic carbon storage in northeast china. Geoderma 330, 204-211. https://doi.org/10.1016/j.geoderma.2018.05.037, 2018.

Replaced "Biochar can enhance SOC storage, soil granular structure, cation exchange capacity" with "Biochar can enhance SOC storage, soil granular structure, and cation exchange capacity (Hu et al., 2020; Mete et al., 2015)." **Line-57-58**

(Hu, L., Li, S., Li, K., Huang, H., Wan, W., Huang, Q.: Effects of Two Types of Straw Biochar on the Mineralization of Soil Organic Carbon in Farmland. https://doi.org/10.3390/su122410586, 2020.

Mete, F. Z., Mia, S., Dijkstra, F. A., Abuyusuf, M., Hossain, A., Agronomy, D. O.: Synergistic Effects of Biochar and NPK Fertilizer on Soybean Yield in an Alkaline Soil. Pedosphere 25(05),713-719. https://doi.org/10.1016/S1002-0160(15)30052-7, 2015.)

2. For L86, A summary is needed to grasp the research gaps here and other palaces in the introduction.

◆ Author response: Thank you for your suggestion. Following your comments, we have added a summary at the end of the paragraph: "Despite these benefits, a quantitatively understanding is scare on how combined effects between biochar and nitrogen fertilizer contribute to soil fertility by modifying microbe-soil interactions in agroecosystems." **Line-76-78**

3. For 322 to 328, these discussion should be mixed with your own data.

◆ Author response: Thank you for your helpful advice. We have freshened the discussions that were blend seamlessly with our data. The modified discussions are as follows:

"Our results demonstrated that soil bulk density of Mollisols had a negative correlation with biochar application rate. The C3 treatment reduced soil bulk density by up to 12.69% (Fig. 1). The bottom soil bulk density was on average 18.88% higher than surface soil by the biochar amendment, though the improvement in the bottom soil bulk density was not significant ($P > 0.05$), which are consistent with Xiu et al. (2019). This trend might be due to the unique properties of biochar, such as complex microporous structure, large specific surface area, and light texture, et al. (Zhang et al. 2015)." **Line-308-313**

"The soil water content gradually increased with increasing biochar application rate. This improvement was the largest during the single application of biochar, with an average increase of 18.07%. The two-factor ANOVA showed that the increase in soil

water content was mainly attributed to biochar, though there was also a synergistic effect of biochar and nitrogen fertilizer on the increase in soil moisture content (Table. S1)." **Line-320-324**

4. L334-345, this statement should be supported by citation.
◆ Author response: Thank you for your suggestion. We have added references to the statement as suggested.

Replaced "The porosity, hydrophilic domains, and huge specific surface area of biochar may aid in water retention." with "The porosity, hydrophilic domains, and huge specific surface area of biochar may be favorable the improvement of water retention (Leonard et al. 2014)." **Line-328-329**

Leonard, Githinji.: Effect of biochar application rate on soil physical and hydraulic properties of a sandy loam. Archives of Agronomy & Soil Science. https://10.1080/03650340.2013.821698, 2014.

5. L338, In the end of subsection, a summary is heavily needed to conclude what they get from the complete discussion.
◆ Author response: Thank you for your suggestion. As suggested, we have added an additional summary at the end of the discussion section: "The input of biochar combined with N fertilizer actively participates in the formation of soil large aggregates, which enhances the soil water-holding capacity and alleviates soil erosion by improving the soil aggregate structure (Gaia et al. 2020; Islam et al. 2021)." **Line-332-334**

6. L447, The novelty and significance of these findings should be mentioned.

◆ Author response: Thank you for your useful advice. We have added the novelty and significance in the "conclusion" section: "This study highlighted that biochar combined with N fertilizer application could be a potential option for mitigating soil degradation, reasonable applicating N, and enhancing soil carbon storage, which would support sustainable use of Mollisols. In the future, we will further investigate the long-term effects of biochar application on soil C and N cycles in agroecosystem." **Line-453-457**

attached file:

● label 1. please specify the meaning the C2N?
  ◆ Author response: Thank you for your suggestion. The C2N represents the biochar application rate (19.6 Mg C ha^{-1}) combine with the nitrogen fertilizer application rate (600 kg N ha^{-1}), which is clearly mentioned in the Abstract: "The biochar input levels were C1, C2, and C3 (9.8, 19.6, and 29.4 Mg C ha^{-1}, respectively), while the nitrogen fertilizer rates were N1/2 (300 kg N ha^{-1}) and N (600 kg N ha^{-1})."

- label 2. please keep the integer.
  - Author response: Made the change as suggested. "56.59" has been changed to "56.59%" **Line-17**

- label 3. showed
  - Author response: "shows" has been changed to "indicated" **Line-15**

- label 4. please check this citation
  - Author response: We are very sorry for our carelessness. "(E.-L. et al. 2014)" has been changed to "(Ng et al. 2014)" **Line-85**

Soil Biology & Biochemistry xxx (2013) 1–8

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities?

E.-L. Ng [a,b,*], A.F. Patti [b], M.T. Rose [a,b], C.R. Schefe [c], K. Wilkinson [c], R.J. Smernik [d], T.R. Cavagnaro [a,d]

[a] School of Biological Sciences, Monash University, Victoria 3800, Australia
[b] School of Chemistry, Monash University, Victoria 3800, Australia
[c] Department of Environment and Primary Industries, Victoria, Australia
[d] School of Agriculture, Food and Wine, University of Adelaide Waite Campus, Urrbrae, South Australia 5064, Australia

Ng, E. L., Patti, A. F., Rose, M. T., Schefe, C. R., Wilkinson, K., Smernik, R. J., et al.: Does the chemical nature of soil carbon drive the structure and functioning of soil microbial communities? Soil Biology and Biochemistry. 70 (2014) 54-61. http://dx.doi.org/10.1016/j.soilbio.2013.12.004, 2014.

- label 5. In the end of subsection, a summary is heavily needed to conclude what they get from the complete discussion. Line-409

  - Author response: As suggested, we have added a summary at the end of this discussion section: "Therefore, biochar in combination with nitrogen fertilizer as amendments effectively improve the soil aggregates and carbon sequestration." **Line-408-409**