

Reply on RC2

Li Wang et al.

Author comment on "Soil bacterial communities triggered by organic matter inputs associates with a high-yielding pear production" by Li Wang et al., SOIL Discuss., <https://doi.org/10.5194/soil-2021-95-AC2>, 2022

Review2:

The authors of the manuscript soil-2021-95 "Soil microbial community triggered by organic matter inputs supports a high-yielding pear production" present a dataset based on 6 sites, each with a high-yield and low-yield treatment, named "yield-invigorating" and "yield-debilitating". Four composite samples (composite of nine individual samples) were taken per treatment. According to the introduction, the authors assume the higher yield at "yield-invigorating" pear orchards to be associated to a unique microbial community, which in turn is affected by abiotic factors. The objectives of this study were to i) identify differences in taxonomic diversity and composition of the bacterial community between the treatments, and ii) determine abiotic factors shaping the bacterial community composition. The broader implication stated would be the targeted manipulation of soil bacterial composition in order to support higher pear production. I want to highlight the impressive variety of statistical methods and results. I also appreciate the data provided in the supplemental material, which provides additional transparency. However, this variety of methods causes a lengthy results part, which sometimes loses the focus on the research question. While at the same time, important information is missing in the M&M section (i.e. a literature based metamodel explaining the rationales behind each pathway, how the model was fitted).

I have three major concerns:

[1] One major concern is the lack of a clear definition of the treatments "yield-invigorating" and "yield-debilitating", thus both terms remain vague from abstract to conclusions. In M&M it is stated, that yield-debilitating orchards received more chemical fertilizer under intensive management (unclear what intensive management refers to), while yield-invigorating orchards were "usually" amended with more organic fertilizer under integrated nutrient management. How did the NPK and OM inputs differ between the treatments. And how consistent were the treatments across the six sites? Is the fertilisation regime the only difference between the two treatments? The terms "yield-invigorating" and "yield-debilitating" indicate a trend in increased or reduced yield over time, however, Table S1 does only provide yield data for 2019, and yield is only given as average yield per plot and not given for each individual replicate, which results in a limited observation of 12 yields.

Response: Thanks for your nice comment. In the present study, yield-invigorating (YI)

orchard displays a higher pear fruit yield whereas yield-debilitating (YD) orchard shows a lower pear fruit yield compared that in orchard under local common managements according to the farm record. These terms indicate a trend of increase or decrease yield over time. In the present study, the orchard was classified according to the field survey and farm record especially annual production. In 2019, when soils were sampled, the yield of each orchard was calculated based on the farm record. Unfortunately, the farmers did not record the yield of each replicate, they only record the yield for the whole orchard. Therefore, we used the average yield of the whole orchard to represent the yield of each replicate. That is the limitation of our study. Anyway, combing the comment from another review, we introduced what the YI and YD means in the Introduction section in the revised manuscript.

We firstly performed a field survey to choose the pair-located orchards for further analysis. To minimize the effects of microclimate at each site, only pair-located pear orchards with invigorating and debilitating yield and at similar growth stage were selected for this research. In total, six pair-located yield-invigorating and -debilitating pear orchards distributed in four cities of Jiangsu province, China, were selected in the main pear production areas. Although these paired orchards differ in site, plant density and cropping years, however, the YI and YD orchards were the same except the soils sampled from Zhangjiagang. According to the farm records and our survey, for these paired orchards within each site, fertilization regime probably was the most different part during the agricultural management. The YI orchards usually received more organic fertilizer whereas YD orchards received more chemical fertilizer. We have added this information in the supplemental Table S2 and revised the concerned sentences in the revised manuscript.

[2] Furthermore, the conclusions drawn are very speculative, and correlation between distinct bacterial communities between treatments with yield (the main factor defining treatment) are interpreted as causal for higher yields. However, yield varies largely between the different sites, as do the bacterial communities. An increase in key taxa might be linked to treatment, thus fertilisation (which was indicated to vary between treatments), but does not necessarily cause higher yields. There is a lack on discussion on the direct effect of higher SOM on yield, plant growth and root exudation (which might also affect microbial community composition).

Response: Thanks for your warm suggestion. In this study, we found that YI and YD orchards differed in their soil chemical properties, especially the contents of soil organic matter, which leading to the variation of soil microbiome. And the manipulation of soil microbiome correlated to the improvement of pear yield. Based on the result of structural equation modelling, we observed that soil organic matter, beta diversity of bacterial community, and network connector were key indicators in supporting high-yield pear production based on the structural equation modelling results. Worth to mention, soil organic matter was not directly linked to the yield in the constructed model, indicating that soil organic matter maintain the high-yielding pear production probably via the indirect ways. Therefore, we proposed that yield-invigorating soils harbour unique bacterial communities that may improve soil biological fertility, which could be driven by soil organic matter and manipulated by keystone species (Chloroflexota) through altering the bacterial interactions. In this study, we hypothesized that high input of organic fertilizer could improve soil structure and modify chemical properties, which leading to YI soils harbor unique bacterial communities that maintains the high-yielding pear production. We have re-organized our Result and Discussion sections to improve the writing of our manuscript.

[3] Third, statistical analyses miss to investigate important effects of SOM on yield, and of pH on microbial community composition. The structural equation model approach could promote valuable insights and help to disentangle direct and indirect effects of SOM on yield. However, this would need an inclusion of a direct path from SOM to yield. Furthermore, the authors assume that soil pH (major factor influencing bacterial community) is not of importance in the study cited. However, including pH into the model would allow to prove this assumption. In general, the SEM is lacking the information how much of variation in yield is explained. From Fig. 5A it looks like yield explained ~8% of variation in bacterial community. Vice versa, this questions the conclusion that the bacterial community structure affects growth.

Response: Thanks for your comment. The R^2 is 0.739 for this SEM and we have added it in the revised manuscript. The raw data used for SEM construction was selected because these index may play key roles in supporting a high-yielding pear production based on the previous result in the present study. In this study, we firstly used the VPA analysis to show soil chemical properties play key roles in determining soil microbial communities. Next we used RDA analysis and stepwise to select key soil chemical properties driving soil microbial communities. Meanwhile, we used random forest analysis to explore the key soil chemical properties in predicting pear yield. Combining all these results together, soil organic matter was recognized as most important factor in driving soil microbial community and determining pear yield. For soil chemical properties, soil organic matter was only the factor selected to build the SEM mainly due to the following reasons: 1) only OM was significant differed between YI and YD soils, 2) OM was top parameters for predicting the orchard yield, 3) OM within the module was identified as the top important soil property that determines the composition of bacterial community as evidenced by the RDA vectors. Therefore, soil pH or another factors were not shown in our manuscript when building the SEM. And the direct correlation between soil OM and pear yield was added in the revised manuscript. the detailed information about the parameters used for SEM construction was added in the revised manuscript. Following your suggestion, we also added a conceptual model in the supplemental material.

Worth to mention, the built model including soil chemical and properties may explain 73.9% variation of yield based on the SEM result. As for microbial community, the result of VPA showed soil chemical properties together may explain 36.8% variations of microbial community while location and yield only explain a small proportion. In our opinion, this probably location and yield were not direct factor in shaping the changes of soil microbial communities. They are mainly affected the composition of soil microbial communities first through changing soil properties. And our results are in line with to many previous VPA results that soil chemical properties explaining the most of the variations of microbial communities (Wu et al., 2021, 161: 108374; Yang et al., 2017, 215: 756-765; Zhao et al., 2013, 67: 443-453). Anyway, we have added this point in our Discussion section.

Unfortunately, I cannot recommend publication in its current form and therefore propose a rejection. I hope the authors still take the time to consider my recommendations regarding the revision of this manuscript.

[4] Title The shown data does not allow to assume causality between distinct bacterial community upon organic fertiliser inputs and high yield. Therefore, the title should be revised.

Response: Thanks for your warm suggestion. We have revised the title as “Soil bacterial community triggered by organic matter inputs was associated with a high-yielding pear production”.

Abstract

[5] e.g. L33 Try to write the abstract in a way that it is understandable to a broader audience, without using too many very terms specific to a certain method (meta-modules).

Response: Thanks for your nice reminder. Meta-module usually refers to a group of modules functionally interrelated as the original references (Langfelder and Horvath, 2007, Oldham et al., 2008). We have revised this sentence in the abstract as your suggestion.

[6] L37 I don't agree on conclusion that the factors presented are causal for higher yields.

Response: Thanks for your suggestion. Agreed with your opinion, there are lots of biotic and abiotic factors that determine the pear yield. However, in our present study, we analyzed the soil physiochemical properties and microbial composition, and used different statistical methods to discover the potential key factors responding for yield improvement. Here is the result from structural equation modelling. We lack of a further-step experiment to explore if these factors were really the causal for higher yields. Anyway, our result could give a basic information about which index probably links to yield improvement. Therefore, we revised our manuscript as following: “Structural equation modelling showed that soil organic matter, beta diversity of bacterial community, and network connector (Chloroflexi) were key factors in explaining the high-yield pear production.”

introduction

[7] L52 Consider adding a sentence on the role of fungi in perennial agroecosystems (e.g. orchards).

Response: Thanks for your suggestion. We have added the information as following: “Fungi participate in decomposition of organic matter and deliver nutrients for plant growth (Frac et al., 2018), however, considering that bacteria are the most diverse and abundant group of microorganisms in soil, bacterial communities and their functions can be pivotal indicators for crop production in agroecosystems (van der Heijden et al., 2008).”

[8] L59 Which monoculture system does Lu et al. 2013 refer to? Be more precise.

Response: Thanks for your suggestion. We have revised it as your suggestion.

[9] L64 I miss the logical link to Ls 62-63. Why is it desirable to identify indicators of bacterial community composition in response to high-yielding crop production?

Response: Thanks for your suggestion. There are many microbial indices that probably respond to yield improvement such as a higher microbial diversity, interaction patterns, and enrichment of beneficial microbes as we introduced in the L55-L62. However, the relative contributions of microbial diversity, interactions among community members, or enrichment of key taxa to crop production remain largely unknown. Therefore, we mentioned here that it is highly desirable to identify pivotal indicators of bacterial community composition in response to high-yielding crop production.

[10] L70 What does "forms" mean?

Response: Thanks for your suggestion. Here means the form of soil nutrient. We have revised the sentence as "forms and contents of soil nutrient".

[11] L75 Delete therefore. Is not logically linked to the sentence in Ls 73-75.

Response: Thanks for your suggestion. We have deleted it.

[12] L75-L77 Be more precise. Do you mean, that it depends on the scale which chemical properties are related to bacterial community composition? Does this refer to your assumed absence of pH as relevant factor in your soils? (L378-L379)

Response: Thanks for your suggestion. Here we mean the driver of soil microbial community was depending on the scales of soil samples investigated. At a large distance even at continental level, the assemblage of soil microbiome was determined by soil pH. At small scale, especially in agricultural soils, the assemblage of soil microbiome was determined by many soil factors. We revised the sentence to be more precise as following: "Key soil chemical properties identified in controlling the distribution and abundance of bacterial community is largely depending on the geographical distributions of soils".

[13] L80-L87 Reduce paragraph on pear to one sentence.

Response: Thanks for your suggestion. We have revised it as following: "'Sucui No. 1' pear, is an early-maturing variety bred by the Jiangsu Academy of Agricultural Sciences, China, and has been popularly cultivated in Eastern and Central China, due to the advantages including easy to produce, adaptable to the environment, and has good quality and high economic benefits (Lin et al., 2013).".

[14] L88-L89 What do you mean by that? It is unclear. Revise. Plus Zorz et al. 2019 refer to lake microbiomes. Consider choosing a reference related to agroecosystems.

Response: Thanks for your suggestion. We have revised it as your suggestion.

[15] L93-L94 It is unclear from introduction, why yield-invigorating bacterial communities should differ or be unique. Revise introduction so that your hypothesis has a better fundament.

Response: Thanks for your suggestion. We have revised it as following:

“In this study, compared to local average yield, orchard showing higher pear yield production was recognized as yield-invigorating (YI) orchard while orchard displaying lower pear yield production was regarded as yield-debilitating orchard (YD). After field surveys accomplished in 2019, six yield-invigorating and adjacent yield-debilitating pear orchards in total were selected for further analysis of soil chemical properties and microbiome. We hypothesized that high input of organic fertilizer could improve soil structure and modify chemical properties, which leading to YI soils harbor unique bacterial communities that maintains the high-yielding pear production. To address this, soil bacterial communities and edaphic properties of the study sites were compared were compared to (1) decipher the differences of taxonomic diversity, and composition of the bacterial community, and (2) determine the contributions of environmental variables to the changes in the structure of bacterial communities.”

methods

[16] L116-L121 I miss a clear definition of the treatments. What does it mean, that yield-invigorating orchards were “usually” amended with more organic fertiliser. Did yield-debilitating orchards also receive organic fertiliser sometimes? Was the fertilisation regime/ the treatments consistent over all six sites? Table S1 does not provide “detailed information”. It just provides information stated in L103. Could you provide detailed information on the amount of NPK and organic fertiliser inputs for each site?

Response: Thanks for your suggestion. For both yield-invigorating and yield-debilitating orchards, chemical fertilizer and organic fertilizer were applied. Moreover, the amounts of these fertilizers were also differed in different locations due to the farmer accustomed fertilization. However, for the paired yield-invigorating and yield-debilitating orchards, the total amount of applied chemical fertilizer was similar but more organic fertilizer (usually two or three folds compared the amount of organic fertilizer applied to the yield-debilitating orchards) was applied into the yield-invigorating orchards according to the farm record. We have added the formation about the fertilization regimes as Table S2 in the supplemental material.

[17] L168 Provide references for all statistical software and packages used.

Response: Thanks for your suggestion. We have added it in the revised manuscript.

[18] L204-L208 When applying structural equation modelling it is good practice to provide a conceptual meta-model summarising underlying theoretical pathways, plus a table providing references to the hypothesised pathways. Please consider adding this information. Additionally, you should include a fit index, which is robust to sample size, such as the comparative fit index. Consider citing Grace (2006).

Response: Thanks for your suggestion. We have added the fit index in the figure. The reference was also cited in the revised manuscript. Moreover, a conceptual meta-model

summarising underlying theoretical pathways was also added in the supplemental material.

Results

[19] L222-L223 The PCoA analysis shows clustering between sites. However, the PCoA does not clearly separate the treatments (Fig 2A). Revise.

Response: Thanks for your suggestion. We have revised the “treatment-based” as “location-based”.

[20] L237-L241 Long sentence. Revise.

Response: Thanks for your suggestion. We have revised it as following: “A total of 2546 unique ASVs with 53,222 sequences were found in all yield-invigorating orchards while 2308 unique ASVs with 44,389 sequences were observed in all yield-invigorating orchards. Among these unique ASVs, almost 70% of ASVs were shared between yield-invigorating orchards and -debilitating orchards.”.

[21] L244 Please check <https://ncbiinsights.ncbi.nlm.nih.gov/2021/12/10/ncbi-taxonomy-prokaryote-phyla-added/> for new standards in taxonomic names.

Response: Thanks for your suggestion. We have revised it through our manuscript and figures.

[22] L258-L262 Move to M&M.

Response: Thanks for your suggestion. We have moved this sentence to Material and Method section.

[23] L272 What are the threshold values Z_i and P_i ?

Response: Thanks for your suggestion. The threshold values of Z_i and P_i was 0.62 and 2.5, respectively. And we have added this information in the Material and Method section.

[24] L289-L291 Why is pH not included. Not explained so far. And probably not meaningful to exclude soil pH, due to it’s important influence on microbial community composition.

Response: Thanks for your comment. As we all know, soil pH was s main driver for the assemblage of soil bacterial community in many cases. However, we used forward stepwise to build a module explaining the variation of soil microbial community. After

selection, the modules include soil OM, TN, alkaline N, AP and AK, available calcium (Ca), copper (Cu) and manganese (Mn) explained the majority of the variation in bacterial community composition. Other soil properties were removed probably due to the collinearity or not important roles in this study. As we discussed in the manuscript, the variation of soil pH was not very large in the agricultural soils. This maybe the reason why pH was not in the module.

[25] L291 RDA1 does only explain 17.4% of variation in bacterial community composition this does not support a strong influence of OM on community composition. Revise wording.

Response: Thanks for your comment. We have revised it as "As evidenced by the RDA vectors, OM within the module was identified as the top soil property that determines the composition of bacterial community."

discussion

[26] L311 I don't agree that Fig.1C suggests a direct significant correlation between bacterial community and pear yield. Why do you assume it to be direct? Both could be correlated to SOM. The distinct bacterial community composition was not associated to yield but to treatment (as far as I understood). Yield differed across sites.

Response: Thanks for your comment. We have deleted the word of "directly".

[27] L316 Shared across six sites?

Response: Thanks for your comment. We have deleted the word of "Shared".

[28] L317 Well-organized not neutral à "more interactive"?

Response: Thanks for your suggestion. We have revised the it as your advice.

[29] L332 Delete "always".

Response: Thanks for your suggestion. We have revised it.

[30] L336-L338 Incomplete sentence.

Response: Thanks for your comment. We have revised it.

[31] L339 Define what you mean by soil "biological" fertility.

Response: Thanks for your comment. We have revised it. Here we want say that Planctomycetota may promote plant production through improving soil fertility, *i.e.*, involving nitrogen cycling. Therefore, we delete the word of "biological" to make it clearer.

[32] L345 See my major concern on the conclusion drawn on causality.

Response: Thanks for your comment. As we responded to the comments [2], we decide to delete this sentence.

[33] L354 What do you mean by "small world"?

Response: Thanks for your comment. We have added this information in this paragraph.

[34] L355 Revise conclusions.

Response: Thanks for your comment. We have revised it as "Our comparative network analysis indicated that microbial co-occurrence patterns in soils links to different pear production.".

[35] L361 Basal shifts in network architecture linked to fertilisation regime and or OM quality?

Response: Thanks for your comment. We have no evidence about this question. However, we agree with you that the basal shifts in network architecture linked to fertilisation regime and/or OM quality. Anyway, this needs farther works.

[36] L365 Which study does "this" refer to?

Response: Thanks for your comment. We have revised it as "In our study".

[37] L370 How does the fertilisation regime in the cited study relate to the fertilisation regime in the presented study?

Response: Thanks for your comment. In the cited reference, the soil was fertilized with different treatments, including miner fertilizers, miner fertilizers plus organic fertilizer. The result was conclude based on the integrated result of these soils from different treatments. In our study, soils were fertilized with miner fertilizer plus organic fertilizer too. These two studies were roughly similar.

[38] L380-L374 Too speculative. Revise.

Response: Thanks for your comment. Considering soil pH was introduced in the

Introduction section and the comments from another review, we have deleted this sentence.

[39] L378 Two points on a log-scale already are a huge difference in terms of soil pH. However, more important than the range across sites would be the difference between treatments – which looks small, indeed. However, best would be to include pH in the analysis and to show, that it really plays a minor role in determining bacterial community upon treatments.

Response: Thanks for your comment. As we all know, soil pH was s main driver for the assemblage of soil bacterial community in many cases. However, we used forward stepwise to build a module explaining the variation of soil microbial community. After selection, the modules include soil OM, TN, alkaline N, AP and AK, available calcium (Ca), copper (Cu) and manganese (Mn) explained the majority of the variation in bacterial community composition. Other soil properties were removed probably due to the collinearity or not important roles in this study. As we discussed in the manuscript, the variation of soil pH was not very large in the agricultural soils. This maybe the reason why pH was not in the module. Considering the comments from another review, we have deleted this sentence.

[40] L380 Not only a question of higher SOM content, but probably also associated to quality. Discuss.

Response: Thanks for your comment.

Response: Thanks for your comment. We totally agreed with your comment. However, we did not measure the quality of soil organic matter in this study. Anyway, we have revised this sentence to discuss this point as following: "Despite the quality of soil organic matter was not evaluated in this study, the quality of soil organic matter was associated with the diversity of microbial community (Ding et al., 2015), which implies more attentions should be paid to illustrate the relationship between the quality of soil organic matter and microbial community in our future work."

[41] L384 Fierer 2004 does not state that diversity increases with higher C content. He states that diversity depends on SOM quantity and quality.

Response: Thanks for your comment. We have revised it as your suggestion.

[42] L385 see comments on SEM.

Response: Thanks for your comment. We have responded to this comment in the response to comment [3].

[43] Fig 1 Separate A from B and C and make it an own figure. Does not belong to results.

Figure 1 C Why are there so many dots? If microbial composition distance was calculated

for each replicate, it should be 48 dots. Additionally, yield data only represents orchard average per tree, thus it should be only 6 points (or lines, where the 48 dots of microbial composition distance align). Why are there more than 6 levels of yield distance? The relationship does not look linear. Consider choosing another colour for the dots.

Response: Thanks for your comment. We have revised the figure as new figure 1 and figure 2. The reason for many dots are mainly because the figure was plotted based on the baycurtis distance (a kind of beta diversity, the result was a matrix) not simply linear correlation.

[44] Fig 2 Shapes in A too small. Do not abbreviate yield-invigorating and yield-debilitating wherever the space allows to write the name (plus consider changing the treatment names).

Figure 2 A The PCoA analysis shows clustering between sites. However, the treatments does not clearly separate the treatments. Revise L222-L223.

Response: Thanks for your comment. We have revised it according to your suggestions.

[45] Figure 3 B Consider moving this to supplement. Does not add much to the main story.

Response: Thanks for your comment. We have removed this part into the supplemental material.

[46] Fig 4 You show many results and provide a lot of valuable information. However, I am not sure how this Figure adds to the research question. **Additionally, size too small. Hard to read.**

Response: Thanks for your comment. Network analysis could provide very useful information about the potential interactions among the soil microbiome, which have been shown that play key roles in determining soil ecosystem functions. Therefore, we report this result in our manuscript. Following your suggestion, we have revised our manuscript to make it easier to read.

[46] Fig 5 Important figure.

Figure 5 A Implications are a bit unclear for Fig 5 A. Does this implicate if yield only explains 2% of bacterial community composition variation, only 2% of yield can be explained by the community composition?

Figure 5 B Soil pH should be included. See previous comment on that.

Figure 5 D Again, why are there so many points?

Response: Thanks for your comment. In this study, we firstly used the VPA analysis to

show soil chemical properties play key roles in determining soil microbial communities. Next we used RDA analysis and stepwise to select key soil chemical properties driving soil microbial communities. Meanwhile, we used random forest analysis to explore the key soil chemical properties in predicting pear yield. Combining all these results together, soil organic matter was recognized as most important factor in driving soil microbial community and determining pear yield. For soil chemical properties, soil organic matter was only the factor selected to build the SEM mainly due to the following reasons: 1) only OM was significant differed between YI and YD soils, 2) OM was top parameters for predicting the orchard yield, 3) OM within the module was identified as the top important soil property that determines the composition of bacterial community as evidenced by the RDA vectors. Therefore, soil pH or another factors were not shown in the RDA result after VIF selection using vegan package. As for microbial community, the result of VPA showed soil chemical properties together may explain 36.8% variations of microbial community while location and yield only explain a small proportion. In our opinion, this probably location and yield were not direct factor in shaping the changes of soil microbial communities. They are mainly affected the composition of soil microbial communities first through changing soil properties. And our results are in line with to many previous VPA results that soil chemical properties explaining the most of the variations of microbial communities (Wu et al., 2021, 161: 108374; Yang et al., 2017, 215: 756-765; Zhao et al., 2013, 67: 443-453). Anyway, we have added this point in our Discussion section. The reason for many dots are mainly because the figure was plotted based on the baycurtis distance not simply linear correlation. And the regression was also revised in the manuscript.

[47] Fig 6 Structural equation model seems a good approach to address the question whether bacterial community composition improves pear yield. However, to address this question a direct path from SOM to yield must be included in the model. Additionally, soil pH as a major driver of community composition, should be included as well, even if hypothesised to play a minor role in this study. The major lack of this SEM is that no information on R^2 is provided. How much of the yield is explained by the SEM? All these points are needed to address the cited research question and to draw the conclusion drawn. Additionally, what does beta diversity PCoA mean? Unclear. The comparative fit index, which is not sensitive to sample size should be included. It remains unclear, how the model was developed and fitted.

Response: Thanks for your comment. The R^2 is 0.739 for this SEM and we have added it in the revised manuscript. The raw data used for SEM construction was selected because these index may play key roles in supporting a high-yielding pear production based on the previous result in the present study. For soil chemical properties, soil organic matter may play the most important roles as different analysis pointed. As for microbial indices, the microbial abundance, key taxa and interactions were selected due to they were recognized as potential key components in determining pear yield. The detailed information about the parameters used for SEM construction was added in the revised manuscript. Following your suggestion, we also added a conceptual model in the supplemental material.

Please also note the supplement to this comment:

<https://soil.copernicus.org/preprints/soil-2021-95/soil-2021-95-AC2-supplement.pdf>