Reply on RC2
Anne Daebeler et al.

Author comment on "Pairing litter decomposition with microbial community structures using the Tea Bag Index (TBI)" by Anne Daebeler et al., SOIL Discuss., https://doi.org/10.5194/soil-2021-110-AC2, 2021

I have read the manuscript “Pairing litter decomposition with microbial community structures using the Tea Bag Index (TBI) submitted by Daebeler and colleagues with great interest and appreciate the work that have been conducted. The manuscript is well written and nicely condenses the performed analyses to the most relevant points. I have some questions and recommendations related to the initial draft of the manuscript, which should be addressed before publication.

- Thank you for your assessment and the time you took to critically read our manuscript. We are very appreciative of your constructive comments and hope that the revised version will be acceptable for publication in SOIL.

1. One of the main points investigated by this study is the impact of the season on litter decomposition as well as on the related microbial community diversity and composition. I really appreciate this approach and agree with the authors that the investigation of decomposition over a complete year, i.e. including all seasons, has huge benefits, inter alia, for understanding decomposition dynamics and estimating annual rates. However, as for the three different soil types, it would be relevant to have an estimation of, how the four seasons differ from each other. Therefore, at least mean temperature and amount of precipitation for each of the seasons are crucial explanatory variables, which help to understand the obtained results. So far there is only a vague interpretation in this direction (e.g. in lines 203, 206ff, 216, 250) but it could be more refined by supporting weather data for each season from the “Agneshof” weather station.

- This is a good point and we have now added a new supplementary table with the seasonal data and refer to it in the Results. Naturally, with the plots being so close to each other, weather data is the same for all.

2. The second major point is related to k and S. The description of S in line 196 is not correct. The stabilization factor S reflect the proportion of the hydrolysable fraction that is not decomposed, i.e. stabilized. Accordingly, the statements made in lines 213 and 320f are wrong.

- Thank you for catching this mistake. We have changed the description of S and corrected the statements made in the results and discussion section.
2.1. I further ask the authors to check their calculation of \( S \), since the presented values in Figure 2 are rather high (besides the one for summer). If the calculations are correct, please discuss your results (\( S \) between 0.3 up to more than 0.5) with respect to the normal range for \( S \) (0.1-0.3) published by Keuskamp and colleagues (2013).

- The presented values are indeed higher than presented in Keuskamp et al., 2013, however, this is the first time that the values are also presented for different seasons and it seems logical that the \( S \) would be higher in the colder seasons than over the summer. The summer values are well in agreement with Keuskamp et al., 2013. Other researchers, e.g. Sarneel et al., 2020 (Science of the Total Environment) from tundra environments and Sandén et al., 2021 (Frontiers in Ecology and Evolution) from urban Austrian sites have also been reporting \( S \) values up to 0.4, which gives further support for our findings.

2.2. For \( k \) it is stated (L197) that the decomposition rates are given in g per day. According to non-linear degradation, which is the basis for the Tea Bag approach, this is not correct. The decomposition rate, \( k \), has a unit of relative weight loss per day, i.e. simply “d\(^{-1}\)”. Please also provide the unit at the y axis of figure 2.

- We apologize for this mistake and are glad you found it. The unit is now changed to d\(^{-1}\) throughout the manuscript.

2.3. In the description of figure 2 it is mentioned that some \( k \) values could not be calculated since the rooibos tea was no more in the first decomposition phase. I am wondering how the authors recognized that the first stage was already over and if so, why some rooibos samples obviously decomposed faster than all the others. One the other hand, I am curious about if and how it was checked whether the first decomposition phase of the green tea was completed at the low temperatures in the winter months. Otherwise \( S \) would be overestimated, which may partly explain the high values obtained for \( S \).

- The Tea Bag Index method assumes that rooibos tea is still in the first phase of decomposition while green tea is already in the second phase. With the weight loss of green tea, it can be calculated which fraction of rooibos tea potentially will break down and together with the weight loss of rooibos tea, it can be calculated how fast the initial decay rate of labile fraction is (\( k \)). Problems to calculate \( k \) arise when this assumption is violated. This may mean that the incubation length has been too long, or that decomposition has been very fast. This would mean that rooibos tea is no longer in the first, but in the second phase of decomposition, thus, the calculation will give a negative \( k \) or no \( k \) at all. Since soil is a complex matrix, it may be that decomposition varies somewhat even with the small distances between replicates that we had in our study. Since we wanted to stick to the method, and keep it the same for all seasons, we may have had too long incubation times for some tea bags. When working with school classes, it would have been difficult to have different incubation times for different seasons since the planning of the research activities was done before the school year started.

3. I am also slightly confused about the soil sampling and the abiotic results. First of all, I guess that the information given in lines 109f and 132f are redundant! Anyway, why does the number of collected composite samples vary between 1 and 3? How statistics can be performed with 1 sample? Regarding the results: Is there an explanation for the rather huge dynamics (up to 0.6% for Fluvisol) of the very slow changing soil property TOC?

- Thank you for this comment. Lines 109 and 132 were indeed redundant and they were now merged. For the purpose of soil chemical analysis we had 6 samples (2 reps per soil type) for seasons winter and spring and 9 samples (3 reps per soil type) for seasons summer and autumn. To overcome the low statistical power we’ve pooled all seasons and performed a single test per variable for the differences between plots. This is now clearly stated in L.181-184.
The dynamics of total organic carbon represent the natural differences in the samples that may occur for example be due to differences in plant cover between the seasons. At the Fluvisol and Luvisol sites we sampled in between wine rows (an area where tractors are driven and where other management, e.g. ploughing may take place), whereas at the Cambisol site we sampled an area at least 5 m from the neighboring vineyard. The Cambisol was a set aside grassland in between vineyards. All the sites were rather close to one another, as seen in Figure 1. All the soil samples were analysed in the same accredited soil laboratory at the Austrian Agency for Health and Food Safety (AGES) with the same method (ÖNORM L1080).

4. Furthermore, the authors stated that red tea was a stronger selector of microbial colonizers than green tea due to a stronger relative enrichment of fungal orders (stated e.g. in lines 40ff, 315f). According to the underlying figure 3, indeed the mean relative differences in the abundance of the fungal orders is higher, but there is only one order that is enriched in each tea compared to the surrounding soil. The relative increase for prokaryotic orders is reasonably lower since there are simply more orders, both, in total as well as the ones enriched in the tea. If the differences of the significantly increased orders of bacteria are summed up, they will end up with even higher relative increase than fungal orders in both teas.

- Thank you for this comment. We have reworded the respective sections and no longer conclude that fungi were selected for stronger than bacteria.

5. Finally, this directly leads me to the last point, which is related to the fact that an interesting finding is partly hidden. In the second hypothesis it is stated that the two different teas are colonized by different subsets of microbes, which was confirmed as stated e.g. in line 323. However, it should be more clearly stated that, while the majority of studied order were enriched or decreased in a more or less equal way in both teas, especially Rhizobiales and the fungal orders Hypocreales and Helotiales show contrasting colonization of green and rooibos tea. I consider this information as a very relevant one, since these orders could function as indicator species for future decomposition studies.

- Thank you for this point. We agree that the exclusive enrichment of bacterial and fungal orders in either green tea or rooibos tea is an interesting finding. In the revised version of the manuscript we report and discuss this more clearly (L347-353)

Further points:

6. L44: What exactly is meant by “the active component of the microbial community”?

- The active component of the microbial community involved in litter decomposition. This has been clarified in the text.

7. L93: Please provide a reference at the end of this sentence (instead of a double blank character)

- Corrected

8. L198: What about the statistic effects between seasons if you sum up the three soil types?

- The conclusion remains the same. The sentence has been revised to: “no significant differences could be found between sites or seasons, neither when pooled (P = 0.15), nor at each site (P = 0.45; Fig. 2).”

9. L198: Closing bracket is missing after Fig.2
10. L200: double parenthesis

11. L219: I recommend to reconsider the wording here. I doubt that the performed approach can really say which species are indeed “involved” in the decomposition for two reasons. (i) we only see a snapshots of the community after three months, which is likely not equal to the community in the weeks and month before. (ii) as stated later only (line265ff) a certain part of the detected community is really involved in the degradation, while others may only benefit from the generated products. Therefore I recommend to soften the wording here and in the whole manuscript.

Thanks for bringing up this important issue. Indeed our method cannot differentiate between ‘direct’ litter degraders (i.e. cellulolytic) and ‘indirect’ one who consume degradation products or the cellulolytic organisms themselves. However, we can be quite positive that our method captures actively growing organisms that benefit from litter degradation, because the organisms must grow to a certain population size before we can detect them. Second, it is false to dismiss the secondary consumers as not important for the process because in many cases they are crucial for the continuation of the degradation process through removal of products through syntrophy or symbiosis. We rephrased this line and others to reflect this.

12. L224: typo in shows

13. L268: “minority” might be not the best word for more than 30% of prokaryotes and about 15% of fungi. As stated before, the authors should be more careful in their interpretation and also consider taxa which may just be enriched in the community due to the usage of secondary products generated by decomposing taxa.

True. We revised the sentence as follows: “...we conclude that the litter material in the teabags selected for colonisation by a sizeable portion of the bacterial, archaeal and fungal population acting as the active litter decomposers and associated populations.”

14. L272 (and elsewhere): consider to use “substrate or litter type” instead of “sample type”

Thanks for the suggestion, we replaced “sample type” with “soil or litter type” throughout the manuscript.

15. L275: I might be wrong, but I asked the authors to recheck the explained-variance values since according to Fig S3 the fungal communities seems to show more differences between green and rooibos tea than the one of prokaryotes. If the values are, however, correct, the statement made in the sentence starting in line 315 does not fit.

We have double-checked the analysis. The reported variances are correct. Two things contribute to the fact that it might seem like that the fungal communities show more differences between green tea and rooibos: 1. The total variance explained by the fungal ordination (first 2 axes) is smaller than that of bacteria and 2. That the ordination only shows the first two principal coordinates and not all of them (as is captured by ADONIS).

16. L280: Here it should be proposed, which locally, rather stable factors do hide the commonly observed impact of different soil types (climate!?)
Thanks for the suggestion. The sentence has been revised to: “This contrasts with the recent findings of (Pioli et al., 2020), in which a large effect of soil type on the community composition was reported, though not surprising considering that this study was performed on a very local scale, with identical climate and similar soil types and management practices.”

17. L289: typo in Beta[...]les
   ● Corrected. This order to bacteria is now known as Burkholderiales

18. Table 1: please include information of vegetation for each site/soil
   ● Table 1 was moved to the supplementary, and the vegetation has been included there.

19. Table 1: is not really helpful and other things might be more important. Consider to put Table 1 in the Supplementary and instead Figure S2 or S3 into the main text
   ● Table 1 was moved to the supplementary. Fig. S2 was moved to the main text.

20. Fig S3 description: consider “obtained” instead of “contained”
   ● Corrected.