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Interactive comment on "Bacterial and fungal predator – prey interactions modulate soil aggregation" *by* Amandine Erktan et al.

Anonymous Referee #1

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The paper is overall interesting and covers a novel aspect in how soil aggregation can appear. Testing two different species that are located in two very distinct taxonomic groups of soil biodiversity and that represent diverse functional groups is nice and can provide a model system to observe some changes. The writing is good but could be a bit more concise in the introduction. Of course, these do not represent even a fraction of the full taxonomic and functional diversity of all soil organisms so it might be that the findings might not represent the function of most other taxa. This is fine but it would be good to acknowledge. Overall the idea behind the experiment are really interesting and relevant. But many aspects as shown below make me wonder if most of the conclusions can actually be drawn... Several things should be done to actually make this study publishable that I highlight below. It seems very important to investigate the feed-

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ing preference of the two species used. Many Acanthamoeba can indeed also feed on fungi (yeasts and spores) so it seems crucial to check if they feed only on bacteria or if they also change fungi. It should also be thoroughly checked if the prey is a good one to model all representative microbes (either bacteria or fungi)- Pseudomonas is often not the preferred prey of Acanthamoeba species. Please test this interaction in simple microcosm experiments to show that the protists grow on the bacteria. Furthermore, LB agar was used in the fungal treatment that is super beneficial for bacterial growth. How certain is it that fungal-feeding alone cause the observed effects or if it is a mix of bacteria/fungal effects? Some follow up tests are needed to confirm the feeding interactions of the collembola also with bacteria, in interaction with bacteria and fungi etc. the PLFA data provided are nice and partly cover some of the issues but still it is not ensured that competition between inoculated bacteria with other bacteria or between bacteria and fungi are not the actual cause of the experiments. Overall so far it can be said that something happened with predators but I doubt we can really link it to the suggested feeding interactions or energy channels. Please adjust and ideally follow up with some confirmative experiments. Please report more information on specific PLFAs to provide an overview on other bacteria that were potentially contaminating the system. The focus is only on some aspects in the collembola treatment. According to the analyses of gram negative bacteria, it seems that these increase with protists potentially suggesting that the inoculated Pseudomonas are not preyed upon. Please clarify and, as mentioned above, provide an experiment to show successful predation of amoeba on the exact Pseudomonas strain used here (please also report this one). Regarding the discussion, please adjust based on the changes in the results and comments above as I think many claims cannot be made with the current results (e.g.2nd line in discussion and the following- protists do not show a significant effect on aggregation). Similarly, a major discussion in the fungal system is based on a non-significant effect. As long as we use post-hoc statistics, this is determines our findings and what we should be reported. A repetition of the experiment with more replications could be done to increase the statistical power. Overall, throughout the discussion,

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conclusions and abstract, many major points are not supported by results and should be adjusted. In fig.1 it is shown that there is no significant difference in the aggregate stability in bacteria alone and bacteria with protist treatments. The same holds for the fungal treatment. As such, this should not be reported as an effect in several parts of the text! I wonder if the composition analysis done in Fig.3 makes sense... In the experiment you would expect a single PLFA marker in the fungal treatment (E.coli) and two in the bacterial treatment (E.coli and Pseudomonas- or even one as both are gram-negative). This figure shows that the setup seems very contaminated which make all results obtained little reliable. Also, I wonder what the relevance for these super simplistic approaches are as many things like interactions that cause biofilm production or integration of bacteria and fungi (and algae) into more stable structures might be needed to make ecological sense of aggregate formation? Please update the references and include some more recent references as only few are from 2018 and none from 2019 or 2020. A lot of work on soil biodiversity, especially on protists including interactions with bacteria, has been done in the last years- even including some papers by the authors that would bring the writing into a more novel context.

Minor comments L238: was higher IN these...

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