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Biogeosciences
Discussions

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

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We thank the reviewers for the very detailed comments which helped us to improve our manuscript. The response to comments from the two referees is organized according to the Biogeosciences's guidelines as follows: (1) comments from Referees, (2) author's response, (3) author's changes in manuscript. We did not directly quote updated text nor the exact location of the changes made in the updated manuscript as co-authors are still working on it and minor changes can still occur. According to the guidelines of Biogesociences, we submit the answer to the comments from the two referees first and then the updated manuscript.

Anonymous Referee #2 Received and published: 17 March 2020

(1) General: In general I find this study to be well executed and of interest. Including trophic structure in our assessments of soils is an important and understudied topic. While most of the work is well executed, the authors spend a large portion of their discussion talking about bacterial mucosal production, but this is never actually tested. If a huge portion of the work depends on understanding how trophic structure influences bacterial mucosal production, then it would be important that this is assessed. I would be hesitant to focus so intently on this interpretation, and spend more time addressing the various components you did test. Additionally, I believe that the 13C portion of this analysis to determine differences in soil and litter derived C needs to be expanded on. This could be an important conclusion, but it is unclear how this work was done, and whether or not labeled litter was added. (2) We agree that the discussion about the mucosal effect was too much developed as we did not measure mucilage it in this experiment. We agree that the use of the two C sources by the microbes can be expanded and more detailed. (3) We reduced the discussion about mucilage production in the discussion. We expanded the part about microbial C use throughout the manuscript by integrating it in the main objectives and not only as a side aspect of the article. Please see more detailed response to the 13C part in the response to the specific comment on this measurement.

(1) Abstract: No specific comments. Intro: In general, this is a well written introduction. The authors lay out multiple factors on how microbes and mesofauna influence soil aggregation. It is at times a bit repetitive though, consistently focusing on the lack of trophic structure assessment to soil aggregation. Additionally, I believe that the authors focus on soil aggregation limits the scope of this study. The authors are assessing multiple components of the soil environment, and therefore, it would be ideal if they could expand their introduction of topics beyond soil aggregation. The authors explore the influence of trophic interactions on soil microbial community formation and on the incorporation of C and on CO2 emissions. If the authors were more concise, they would have room to include additional dimensions to their work. (2) Thank you very much for this insightful comment. We agree that the scope of our study was too narrow and

restricted to soil aggregation. There is a current trend towards microbe-centered approaches linking microbial communities to higher trophic levels (Thakur and Geisen, 2019; Coulibaly et al., 2019; Lucas et al., 2020) and our work would benefit from being presented as a contribution to this effort. We apologize for the introduction being a bit repetitive sometimes. Coulibaly, S.F.M., Winck, B.R., Akpa-Vinceslas, M., Mignot, L., Legras, M., Forey, E., Chauvat, M. 2019. Functional Assemblages of Collembola Determine Soil Microbial Communities and Associated Functions. Front. Environ. Sci. 7:52. doi: 10.3389/fenvs.2019.00052 Lucas, J. M., McBride, S. G., Strickland, M. S. 2020. Trophic level mediates soil microbial community composition and function. Soil Biol. Biochem., 143, 107756. Thakur, M. P., Geisen, S. 2019. Trophic regulations of the soil microbiome. Trends in Microbiology, 27(9), 771-780. (3) We broadened the scope of our study and included effects of trophic interactions on microbial communities, C dynamics (microbial C use, SOC concentrations and CO2 emissions) and soil aggregation. We re-wrote most of the introduction, results and discussion to better balance the coverage of each of these aspects.

(1) Methods: Line 101: Can you clarify this detail a bit more? I think the point is that when you add mesofauna they introduce new microbial organisms, and to account for this you also added microbes to the control treatments, but I am not entirely clear on this detail. How did you detail the Predator associated microbiota? (2) Collembolans are not sterile, they are associated with microbiota on their body surface (Anslam et al. 2016). When we added collembolans to the microcosms, we also added the microbes that were attached to their body surface. It is possible that such microbial addition modify the soil microbial community composition. To tease apart the effects of collembolans on the soil microbial community composition due to (i) the addition of their associated microbiota and to (ii) consumptive and other non-consumptive effects, we added a microbial wash of the collembolans to the fungal treatment. By doing so, the differences between the fungal and fungal + collembolan treatment provide the effect of consumptive and non-consumptive effects not related to the transport of microbes on the body of the collembolan. The effect of the transport of microbes on the body

of collembolan is tested separately in the “collembolan wash” treatment. Anslam, S., Bahram, M., Tedersoo, L. (2016) Temporal changes in fungal communities associated with guts and appendages of Collembola as based on culturing and high-throughput sequencing. Soil Biology and Biochemistry 96, 152-159. (3) We detailed the potential consumptive and non-consumptive effects of collembolans on the microbial community in the introduction. In the discussion, we now stress how the compensatory addition of the collembolan wash allowed to trace the effect of the transport of microbes on the body surface of the collembolans.

(1) I find the 13C-12C comparison protocol confusing. Could you expand your discussion of how you are able to differentiate between soil and litter sources? In particular, how are you assessing the final amount of 13C in your soils. Are you obtaining this information from GCMS work, or are you specifically measuring them using an isotopic analysis device? Additionally, I am unclear as to how you are able to ultimate differentiate whether the 13C in you sample came from litter or soil, unless you inoculated with 13C labeled litter. (2) We apologize for the lack of clarity. We used the natural difference in 13C/12C of C4 and C3 plants to trace C in microbial PLFAs originating from soil (mainly wheat origin, C3 plant), and added chopped litter (mainly maize, C4 plant). The isotopic 13C/12C ratios of the PLFAs was measured using a trace gas chromatograph (GC; Thermo Finnigan, Bremen, Germany), equipped with a DB5-DB1 column combination (30 m and 15 m, both 0.25 μm ID, Agilent), and coupled via a GP interface to a Delta Plus mass spectrometer (Thermo Finnigan, Bremen, Germany). (3) We modified the material and methods to make clear that the different 13C/12C of C3/C4 plants (wheat/maize) was used to trace C origin in microbial communities.

(1) Results: Are the control treatments truly just E. coli? I presume that because they were made from field soil, there is also a natural microbial community. This is not necessarily a problem, but if you are labeling these as E. coli only, that may be misleading. (2) We agree that labelling E. coli was confusing and that a microbial background remained in the microcosms after autoclaving. (3) We made clearer that

the community consumed by A. castellanii and H. nitidus were not composed only of the added strains, but also included remaining microorganisms in the microcosms. We described and discussed how inoculation steps modified the microbial community, and how this in turn can be linked to soil aggregation.

(1) Line 211: Awkward phrasing, maybe adjust to “Neither soil aggregate formation nor stability differed” and break this sentence up into two different sentences. (2) We apologize for the awkward phrasing. (3) We re-wrote the entire paragraph and the sentence was deleted.

(1) Lines 210-214: This paragraph starts with fungal results, but then also addresses other treatments. Maybe split this into two paragraphs, as it is difficult to follow the portion of the results in the second half of this paragraph. (2) We agree that the description of the controls should have been separated from the effect of the fungal treatment. (3) This paragraph was fully re-written.

(1) Discussion: While PLFA is an acceptable method, its ability to measure more fine scale changes in community composition is limited. It is possible that changes did occur, but they were not obvious with PLFA analysis. (2) We agree that finer changes in microbial community composition may have occurred and could have been identified using metagenomic analyses. However, while 16S rRNA metagenomics shows higher precision in depicting changes in bacterial community composition compared to PLFAs, both methods are of similar power in linking microbial community composition to soil functioning (C and N cycles, response to land-use, etc.) (Orwin et al., (2018). Orwin, K. H., Dickie, I. A., Holdaway, R., Wood, J. R. (2018). A comparison of the ability of PLFA and 16S rRNA gene metabarcoding to resolve soil community change and predict ecosystem functions. Soil Biology and Biochemistry, 117, 27-35. (3) We added a sentence in the discussion to acknowledge for possible changes in microbial community composition not detected in PLFAs.

(1) Line 223: missing a) (2) We apologize for the typo (3) The paragraph was fully

re-written in the revised version of the manuscript

(1) Is the collembolan species used known to also feed on bacteria? If so, how would this influence the results? (2) We know that H. nitidus can feed on bacteria, notably P. fluorescens (Pollierer et al., 2019). But when fed on bacteria, H. nitidus is not able to reproduce. This indicates that bacteria are of inferior food quality than fungi for H. nitidus. This also nicely illustrates the concept of food flexibility (Briones et al., 2018) indicating that soil animals often prefer certain food resources but also feed on other resources if their preferred food source is absent. In our case, as C. globosum (which is their preferred food source, see response to reviewer 1, Pollierer et al., 2019) is provided as food source, we expected collembolans to preferentially feed on fungi. This is consistent with the decrease in fungal PLFA markers observed when collembolans were added (Figure 1 B). Of course, this does not exclude that collembolans may also have ingested some bacteria and this at least partly may explain the observed changes in bacterial community composition when H. nitidus was added. Briones, M.J.I. (2018) The Serendipitous Value of Soil Fauna in Ecosystem Functioning: The Unexplained Explained. Front. Environ. Sci. 6:149. doi: 10.3389/fenvs.2018.00149 Pollierer, M. M., Larsen, T., Potapov, A., Brückner, A., Heethoff, M., Dyckmans, J., & Scheu, S. (2019). CompoundâĂŘspecific isotope analysis of amino acids as a new tool to uncover trophic chains in soil food webs. Ecological Monographs, 89(4), e01384 (3) We specified in the material and methods the feeding preference of H. nitidus for C. globosum. In the discussion, we stress that the changes in the bacterial community composition after the addition of H. nitidus may as well have been due to consumption of certain bacteria. In addition, we link changes in bacterial community composition to changes in soil aggregation in the fungal-based system.

(1) Why are the CO2 respiration amounts not mentioned throughout the study? It seems like this would be of interest considering that these metrics are often used to estimate microbial biomass. (2) We initially thought of measuring CO2 emission only as a control to check that living organisms were respiring during the incubation. This

is indeed a good idea to exploit these results more in our study, especially because C use, microbial biomass and soil aggregation may relate to CO2 emissions. In our case, we can't use CO2 emissions to estimate the microbial biomass as our systems do not have only microbes. The CO2 emitted results from the respiration of microbes, but also protists and collembolans. (3) The effect of predator-prey interactions on CO2 emissions was presented in figure 3, together with the effects on SOC concentrations. We re-organized the manuscript to present and discuss the effects of microbial C use, SOC concentrations and CO2 emissions together as C dynamics.

(1) Additional literature to consider including: Bradford, M.A., 2016. Re-visioning soil food webs. Soil Biology and Biochemistry 102, 1–3. Bailey, V.L., Fansler, S.J., Stegen, J.C., McCue, L.A., 2013. Linking microbial community structure to -glucosidic function in soil aggregates. The ISME journal 7, 2044. Crowther, T.W., Thomas, S.M., Maynard, D.S., Baldrian, P., Covey, K., Frey, S.D., van Diepen, L.T.A., Bradford, M.A., 2015. Biotic interactions mediate soil microbial feedbacks to climate change. Proceedings of the National Academy of Sciences 112, 7033-7038. Grandy, A.S., Wieder, W.R., Wickings, K., Kyker-Snowman, E., 2016. Beyond microbes: Are fauna the next frontier in soil biogeochemical models? Soil Biology and Biochemistry 102, 40-44. Jiang, Y., Liu, M., Zhang, J., Chen, Y., Chen, X., Chen, L., Li, H., Zhang, X.-X., Sun, B., 2017. Nematode grazing promotes bacterial community dynamics in soil at the aggregate level. The ISME Journal 11, 2705-2717. Lucas, J.M., McBride, S., Strickland, M.S.S., 2020. Trophic level mediates soil microbial composition and function. Soil Biology and Biochemistry. (2) Thank you very much for these suggestions. We carefully read these articles, which helped us to better introduce our study and discuss the results. (3) We added all these references in the revised manuscript, expect the one of Bailey et al. (2013) which considered soil aggregate as a habitat for microbes, while our focus is more on the effect of soil microbes on soil aggregation. We also added several recent references to better integrate our work into the current research linking higher trophic levels to microbial communities.

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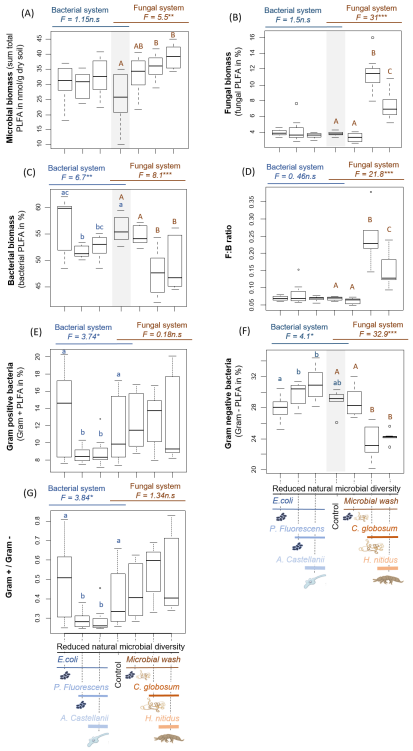

Fig. 1. Figure 1 Effect of bacterial and fungal predator-prey inoculations on microbial biomass and composition.

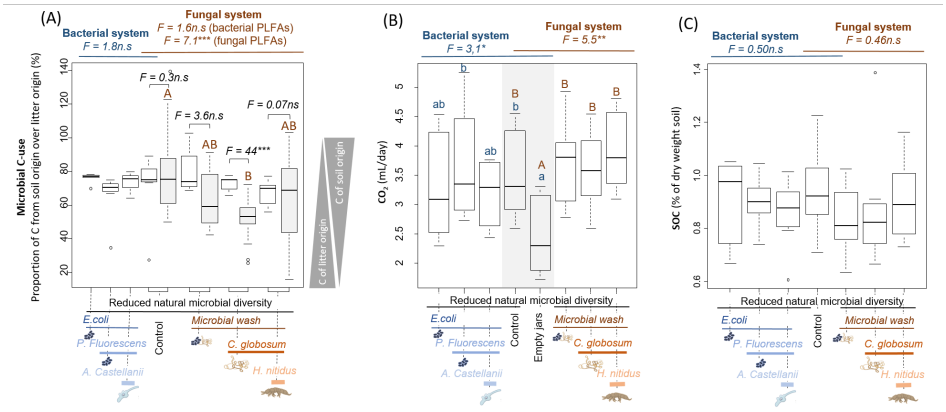

Fig. 2. Figure 3 Effect of bacterial and fungal predator-prey inoculations on C dynamics.