Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-48-AC1, 2020

Biogeosciences Discussions
Open Access
EGU

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

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Received and published: 28 April 2020

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 #1 Received and published: 16 March 2020 (1) The paper is over-

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all 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. (2) We thank you for the positive comments. We agree that the introduction is sometimes repetitive. (3) We re-wrote most of the introduction. In particular, we broaden the scope of our study to the effects of predator-prey interactions on microbial community composition, C dynamics and soil aggregation. By doing so, the part on soil aggregation was reduced and repetitions were avoided.

(1) 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. (2) We agree that our simplified system does not capture the complex interactions occurring in soils. However, we believe that microcosm experiments with simplified interactions are valuable tools to decipher representative mechanisms which occur in more complex systems, but can't be directly studied in real soil system because of their complexity. (3) We made clearer that the simplification of our experimental system is a limitation and made sure not to abusively generalize results.

(1) 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. (2) We thank you for pinpointing the interest of our study as well as for your valuable comments. Please, see our point by point response for an in-depth response to your comments.

(1) It seems very important to investigate the feeding 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. (2) Acanthamoeba has indeed been reported to be a generalist consumer under lab

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conditions. In our experimental setup, however, we did not observe a significative change in fungal PLFA markers in response to the addition of A. castellanii (Figure 1 B). The amount of fungal PLFA markers did not vary in the bacterial system, and was constantly low. We thus conclude that A. castellanii did not significantly feed on fungi in our system.

(1) 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. (2) In fact, we know that Acanthamoeba castellanii feeds on Pseudomonas fluorescens, but they prefer non-toxic strains (Jousset et al. 2009). In our study, we used exactly the same wild-type strain as in the study of Jousset et al. (2009), who showed that A. castellanii preferably feed on non-toxic strains (signal blind, non-toxic gacS-deficient mutants of P. fluorescens), but also fed to a lesser degree on the wild-type P. fluorescens. In our study, we selected P. fluorescens (wild-type) as it often occurs in high frequency in soils (Dubuis et al., 2017), produces mucilage (we checked visually with the ink method) and is known for its soil aggregating properties (Caesar-TonThat et al. 2014). Moreover, P. fluorescens is known to react to consumption by A. castellanii by producing antibiotic phenolic compounds (Jousset and Bonkowski, 2010), which can modify the soil microbial community (Jousset et al., 2010). Besides, phenolic compounds recently also have been proven to be involved in soil aggregation (Yoshikawa et al. 2018), but no links to protist-bacteria interactions were made. We also expected that P. fluorescens modulates mucilage production in response to protozoan consumption, as it is a common strategy of bacteria in response to protist predation (Matz and Kjellberg, 2005; Queck et al. 2006), with expected consequences on soil aggregation because mucilage is playing a central role in soil particle cohesion. Caesar-TonThat, T. C., Stevens, W. B., Sainju, U. M., Caesar, A. J., West, M., Gaskin, J. F. 2014. Soil-Aggregating Bacterial Community as Affected by Irrigation, Tillage, and Cropping System in the Northern Great Plains. Soil Sci., 179 (1), 11-20. Dubuis, C., Keel, C., Haas, D. 2007. Dialogues of root-colonizing bio-

control pseudomonads. European Journal of Plant Pathology 119, 311-328 Jousset, A., Rochat, L., Péchy-Tarr, M., Keel, C., Scheu, S., Bonkowski, M. 2009. Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. The ISME journal, 3(6), 666-674. Jousset, A., and Bonkowski, M. 2010. The model predator Acanthamoeba castellanii induces the production of 2, 4, DAPG by the biocontrol strain Pseudomonas fluorescens Q2-87. Soil Biol. Biochem., 42(9), 1647-1649. Matz, C., Kjelleberg, S. 2005. Off the hook–how bacteria survive protozoan grazing. Trends microbiol., 13(7), 302-307. Queck, S. Y., Weitere, M., Moreno, A. M., Rice, S. A., Kjelleberg, S. 2006. The role of quorum sensing mediated developmental traits in the resistance of Serratia marcescens biofilms against protozoan grazing. Env. Microbiol., 8(6), 1017-1025. Yoshikawa, S., Kuroda, Y., Ueno, H., Kajiura, M., Ae, N. 2018. Effect of phenolic acids on the formation and stabilization of soil aggregates. Soil Science and Plant Nutrition, 64(3), 323-334. (3) We specified in the material and methods why we chose P. fluorescens and A. castellanii. We refer to the study of Jousset et al. (2009) and note that P. fluorescens is usually a toxic non-preferred prey, but also is consumed by A. castellanii. In the discussion, we now discuss how the choice of P. fluorescens may have driven the effects of A. castellanii on soil aggregation in our system.

(1) 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? (2) We agree that the effect of bacteria often complement the one of fungi (Aspiras 1971; Bonfante and Anca 2009). In our study, we did not observe an increase in bacterial PLFA markers when LB agar was added, alone in the “remaining microbial background + Collembolan wash” treatment or with C. globosum in the “remaining microbial background + Collembolan wash + C. globosum” treatment as shown in Figure 1 C. As a consequence, we conclude that the addition of LB agar did not induce a significant increase in bacterial growth in our study, and did not function as major driver of soil aggregation. Aspiras, R. B., Allen, O. N., Harris, R. F., and Chesters, G. (1971). Aggregate stabilization by filamentous microorganisms.

Soil Sci. 112, 282–284. Bonfante, P., Anca, I.-A. (2009) Plants, mycorrhizal fungi, and bacteria: A network of interactions. Annual Review of Microbiology 63, 363-383 (3) In the discussion, we point out more clearly how the interactions between bacteria and fungi could be the main reason for changes in soil aggregation.

(1) Some follow up tests are needed to confirm the feeding interactions of the collembola also with bacteria, in interaction with bacteria and fungi etc. (2) It has been shown in previous experiments that H. nitidus feeds on C. globosum and also reproduces when fed with this fungal species (Pollierer et al. 2019). We now refer to this paper in the manuscript. In the same study it has been shown that H. nitidus feeds as well on bacteria, notably Pseudomonas fluorescens. However, when fed with bacteria, H. nitidus did not reproduce indicting that bacteria are of lower food quality than C. globosum. These results also nicely illustrate 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 the preferred resources are absent. We now explicitly refer to these studies when discussing our findings. 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 also have been due to consumption of bacteria.

(1) 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. (2) We agree we overlooked the effects of microbial competition, or more generally interactions between the remaining microorganisms in the microcosms and the added bacterial and

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fungal strains. We fully agree that this is an important aspect to understand variations in the composition of the microbial community, and how this can relate to soil aggregation. (3) We consider these interacting microbial processes in the revised version of the manuscript. We renamed the all the treatments to show that a remaining microbial background was present in our systems because of our non-sterile set-up. The treatment with E. coli thus was not only colonized by E. coli, but contained the remaining microbial background and E. coli. We also detailed more in the results and discussion how such interactions between microbes, with or without the presence of consumers can be the underlying cause of changes in soil microbial community composition, and soil aggregation.

(1) 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. (2) We agree that our interpretation of the results was too simplistic in some parts of the manuscript. (3) We added information about feeding preference of A. castellanii and H. nitidus, notably related to their feeding on P. fluorescens and C. globosum, respectively. For the bacterial system, we stress that effects of A. castellanii on soil aggregation were likely to be more closely related to the production of bacterial defense in response to the attack from A. castellanii than its consumption itself. For the fungal system, we stress that both changes in fungal biomass and bacterial community composition likely were important drivers of soil aggregation.

(1) 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. (2) We agree that a more in-depth description of the microbial community was needed. (3) In the results, we added the variations of Gram+ bacteria, as well as the F : B ratio and the Gram+ / Gram– ratio. In addition, we used as well the PLFA 15:0 as general bacterial marker.

(1) 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). (2) We agree that the lack of decrease in Gram – bacteria when the amoebae were added suggests that P. fluorescens was not or little consumed by A. castellanii. (3) The fact that P. fluorescens is a non-preferred prey for A. castellanii is thoroughly discussed in the revised version of the manuscript. We refer to the study of Jousset et al. (2009) investigating predation of A. castellanii on P. fluorescens. Jousset, A., Rochat, L., Péchy-Tarr, M., Keel, C., Scheu, S., & Bonkowski, M. (2009). Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. The ISME journal, 3(6), 666-674.

(1) 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, 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! (2) We agree that A. castellanii and H. nitidus induced significant changes on soil aggregate formation, but not on soil aggregate stability as indicated by direct comparison of the treatments with P. fluorescens or C. globosum with and without their associated predators. However, we observed that the significant increase in soil aggregate stability in response to the addition of P. fluorescens and C. globosum vanished when their associated predators were added. Overall, this indicates that A. castellanii and H. nitidus weakly reduced the positive effect of P. fluorescens and C. globosum

on soil aggregate stability. (3) We adjusted our description of results, as well as the discussion to underline that the reduction of soil aggregate stability is only a trend. We highlighted the stronger effect of soil aggregate formation in the conclusion as well.

(1) 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). (2) Bacteria do have a number of PLFAs including specific and non-specific. This is shown e.g. for Bacillus and Pseudomonas (Ruess et al. 2005, Ecology 86, 2075-2082). PLFA markers are thus not specific enough to trace specific microbial strains. Therefore, it is not possible to use PLFA markers to specifically trace E. coli and P. fluorescens. Ruess, L., Schütz, K., Haubert, D., Häggblom, M. M., Kandeler, E., & Scheu, S. (2005). Application of lipid analysis to understand trophic interactions in soil. Ecology, 86(8), 2075-2082.

(1) 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? (2) We agree that presenting the trophic interactions as one predator (protist or collembolan) feeding on one microbial strain (P. fluorescens or C. globosum) was confusing. We did not set-up sterile microcosms, meaning that there was a residual (because of autoclaving) microbial background present in the microcosms. Although our systems are simplified, we argue that the presence of such microbial background in fact helps to link our results to more realistic and complex conditions. (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 the residual microbial background present 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.

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(1) 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. (2) We agree that recent studies investigated effects of higher trophic levels on microbial communities and also on the effects of microbes on soil aggregation and that the manuscript would greatly benefit from these recent research inputs. (3) We linked our work to the recent studies linking higher trophic levels to soil microbial communities. We added most of the references suggested by reviewer 2, as well as others, such as Thakur and Geisen (2019), Lehmann et al. (2020) and Coulibaly et al. (2019). 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 Lehmann, A., Zheng, W., Ryo, M., Soutschek, K., Roy, J., Rongstock, R., Maaß, S., Rillig, M. C. 2020. Fungal Traits Important for Soil Aggregation. Front. Microbiol. 10:2904 Thakur, M. P., Geisen, S. 2019. Trophic regulations of the soil microbiome. Trends in Microbiology, 27(9), 771-780.

(1) Minor comments L238: was higher IN these... (2) We apologize for this typo (3) The paragraph was fully rephrased

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Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-48, 2020.

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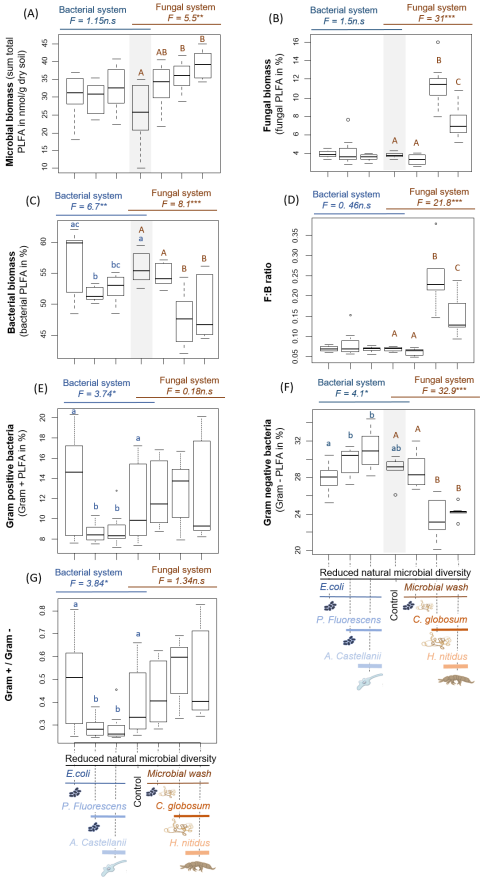

Fig. 1. Figure 1 Effect of bacterial and fungal predator-prey inoculations on microbial biomass and composition.

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