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# *Interactive comment on* "Effects of microplastic and microglass particles on soil microbial community structure in an arable soil (Chernozem)" *by* Katja Wiedner and Steven Polifka

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Referee comments:

This manuscript investigates the effects of microplastic and microglass particles on the structure of microbial communities in soil using soil microcosms that have been spiked with these contaminants. The issue of micro-particles in the environment is very topical, and while there is a lot of information about the impact of macro-plastics



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on wildlife e.g. marine animals, there is relatively little information on the impact of microparticles on microbial populations in terrestrial environments. In this respect, this manuscript is timely. However, the explanation of the experimental design was lacking, and therefore the results should be interpreted with care.

1. Reviewer: Are the authors confident that an incubation period of 80 days was sufficient to observe full effects of the addition of micro-particles?

Response: Bacteria are one of the fast crowing organisms on the world – for instance within a few days, agar plates are fully colonized with bacteria (and fungi). Fungi are, of course a bit slower in its reproduction, but fast enough in order to see an effect after 80 days of incubation. We created an optimal environment for the microorganism (water and temperature conditions). Under natural conditions microorganisms (fast changing wet and dry soil conditions) need a fast reproduction rate in order to survive. Thus, in our opinion 80 days are adequate time to establish a steady microcosm. Although, the microcosm is very artificial (no rhizosphere, macrofauna or variations in temperature or water content).

2. Reviewer: How was 80 days selected as the end point of the experiment? Was it based on published literature or observations?

Response: As in every study, the end point is often set by time and money. In addition, we checked different studies and found, that many of them dealing with soil microorganisms used even much less time.

3. Reviewer: The apparent lack of significant alterations in the bacterial and fungal communities may be due to a relatively short incubation time.

Response: As already explained, microorganisms are fast crowing. Therefore, microbial ecotoxicology test last mostly 7 to 28 days depending on the experimental design. In our opinion, the time period of 80 days is sufficient to establish a stable microcosm and provoke potential treatment effects. But it is conceivable that other microplastic





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types (e.g. secondary microplastics) cause stronger impacts on soil microbiology after 80 days as mentioned in line 209-216. In this case, further research is needed.

"...Reasons for missing significant effects between microparticle treatments and the untreated control after 80 days may be found in the conscious choice of primary microplastics, which were not pre-treated to cause a physical degradation (e.g. ultraviolet radiation). Subsequently, microplastics are mostly chemically inert during the experiment due to unaltered chemical and physical properties, which prohibit the exposition of potential ecotoxic components. Nevertheless, the treatment of soil by different microparticles causes changes in microbial communities, albeit not significant. The observed effects are based on complex soil-impurity interactions and studies dealing with the impact of microplastics on soil microbiology are still lacking (Rillig and Bonkowski, 2018; Zhang et al., 2019) and, to our best knowledge, published PLFA or even DNA based studies are still missing..."

4. Reviewer: In addition, the authors did not consider the effects of transfer of the field soil into the lab environment and compartmentalisation of the soils as a cause of the observed changes in PLFAs. This could be remedied if the authors provide PLFA profiles before the soils were used in the microcosms for comparison, or consider such changes in the discussion section.

Response: Potential transfer effects from field to laboratory are not interesting, because all samples are handled the same way und the use of a control version is exactly the reason of your mention and in order to compare effects. Transfer effects can never be completely eliminated, but due to the analogous sample preparation, potential effects should affect all sample replicates in a similar way. Thus, systematic errors are only minor problem in this experimental design.

5. Reviewer: The amounts of microparticles used in the microcosms (1%) is very high compared to what is observed in the field. The authors state that this is comparable to an industrial site, but this is a rare case, and so these results will not be relevant for

most environmental scenarios.

Response: We include line 14-15, 306 and 314 (Abstract, Discussion, Conclusion) that the amounts of microparticles used in our study indicating a worst-case scenario.

6. Reviewer: If the authors think that colonisation on the microplastics could explain the increase in PLFAs, they could use SEM to confirm this, especially when they had already used SEM to characterise the micro-particles at the beginning of the experiment.

Response: Unfortunately, Cryo-SEM is necessary for fungal and bacterial SEM microscopy. This kind of instrumentation is not available in-house and the study was financially limited due to their pioneering character. Thus, we mentioned that our study is a basis for further studies (e. g. line 327-328). Our discussion attempts to explain our observations, but does not prove the assumptions, which is not unusual for experiments dealing with microorganisms.

7. Reviewer: In the discussion section, the authors discuss the changes of PLFAs after the addition of microparticles, but also state that overall, soil organisms were not significantly affected. If the latter is true, then the relative changes are of no consequence. Instead, there should be a discussion on the apparent lack of impact of microplastics on the microbial communities, especially when the literature that they cite points to the contrary. On the other hand, the PLFA may not be able to detect finer microbial community changes that e.g. a DNA-based method will be able to detect – there needs to be a discussion on this. There should also be more of a discussion on why microglass should only affect protozoa and not bacteria. The authors only cite one paper, but it confuses matters as they found that microglass inhibits bacterial growth, which was not the case in the experiment.

Response: We conceive the idea of the reviewer and tried to find studies dealing with effects of natural or artificial particles, which are made of quartz, on protozoa. To our best knowledge no studies were performed in order to investigate this question. We

added a critical evaluation of this result in line 259-261.

"...This harmful effects of microglass particles on protozoa observed in our study are surprisingly, because this indicates that e.g. sand grains in soil, which consist of SiO2, may also have inhibitory effects on protozoa. To our best knowledge no studies were performed in order to investigate this question...."

8. Minor points:

Reviewer: The manuscript could do with a native English speaker to correct the grammar.

Response: done

Reviewer: The paragraph in the discussion section on the effects of micro-particles on macrofauna seems irrelevant when the experiments were about testing microbial populations.

Response: We understand the reviewer's point of view, but our intentions of discussing effects on macrofauna are based on the SEM analyses and show further potential problems caused by microparticles in soil. In the first section of our study we showed the morphology of different microparticles in SEM pictures. We thus included a short discussion on soil fauna due the possible harmful effects on soil fauna originating from those particles.

Reviewer: Figure 1 does not add anything to the manuscript.

Response: In our opinion, figure 1 is an essential feature of the introduction. We mention that no clear definition exists – regarding the size and structure (even in scientific paper) of microplastics (line 65 et seqq.). Therefore, we tried to get a definition for microparticles especially for future studies dealing with the effects of microparticles on soil fauna and flora. Figure 1 serves as a graphical overview about a potential size classification described in different review paper dealing with micro- and nanoplastics. Furthermore, figure 1 displays the potential interaction potentials between soil mineral

phase, biosphere and artificial microparticles, which are relevant for our interpretation of the results.

"...The difficulty of highly diverse study structures and test environments due to heterogenic material properties is already reported in related research disciplines like marine and freshwater ecology (Phuong et al., 2016; Rist and Hartmann, 2018). To create a standardize study structure in soil science, we highly recommend for future scientific studies dealing with the effect of artificial microparticles on soil flora and fauna to use the definition and size comparison shown in Fig. 1. Furthermore, a detailed description of microparticle characteristics should be mandatory to show potential interactions between biotic or abiotic soil components and microparticles on different size scales..."

Reviewer: I don't understand the use of lowercase a and b to denote p-values. Better to state the p-values.

Response: In figures showing graphs it is an adequate way to use letters (or other symbols) to indicate homogeneous subset, which were defined by using a multiple comparison between the different treatment level (Post-Hoc Test). Using p-values instead of homogenous subsets would require tables instead of box-plot graphs, which we do not prefer due to (in our opinion) a better visibility of several statistical parameters. We add detailed information in line 162-163 to enhance the comprehension.

"...Residuals of each linear model were checked graphically for homoscedasticity and normal distribution to validate the model performance. Because of widespread heteroscedasticity and bad model performances, differences in PLFA marker contents between treatments of each taxonomic microbial group were statistically evaluated using the Kruskal-Wallis rank sum test. Nemenyi test was performed for multiple comparison between the treatment levels in case of a significant ($p \leq 0.05$) treatment effect in the Kruskal-Wallis test. Different lowercase letters were used to illustrate significant differences between homogeneous subsets..."

Reviewer: The use of the plastic cylinders to adjust water holding capacity will also

contaminate the soils with plastic.

Response: Subsamples were used for detection of water holding capacity, which were not used for the incubation. Thus, a risk of contamination with microplastic can be excluded. We add further information to prevent misunderstandings by readers (line 118-119).

"...Soil subsamples used for determination of soil basic properties were not used for incubation experiment..."

Reviewer: 'WHC' should be defined.

Response: The analytical approach is described in line 107-110. In our opinion, the target group of this journal have professional expertise in soil science. Thus, function of water holding capacity in soil is generally known.

References:

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