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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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Response to comments of anonymous referee #1 Received and published: 16 July 2019

Referee comments:

Increasing loads of microplastic waste potentially burden our soils. In this regard the paper is timely, as it investigates potential effects of microplastic and microglass pollution on soil microbial community in a laboratory incubation study. The manuscript is concise, very well written and organized, and it has improved in regard to a previous

version. However, still the paper includes the risk of presenting artificial results, which should be very openly discussed.

The shortcomings refer to:

1. Reviewer: Microplastic loads: The authors state that they refer to microplastic loads near industrial areas. However, 12 t ha-1 is a huge amount, far from being realistic. The authors should spell out clearly, also in abstract and conclusions, that their data refer to worst-case conditions that do not necessarily apply to common plastic and microglass loads in Discussion paper agricultural soils, because concentrations exceed natural loads at least by a factor of about 10.000!

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.

2. Reviewer: I like the finding for protozoa, and appreciate that an explanation is offered related to the hydrophilic surface. Nevertheless, why should this apply to glass but not to increased amounts of sand grains? Can enhanced amoutns of quartz grains also be toxic for protozoa and has this been published before? And if not, why should the glass be more toxic than pure sand? Here the authors should elaborate the physiological explanations a bit more in detail and also outline why microglass should be toxic whereas quartz particles in the fine sand fraction is apparently not (or is it?). It is also not clear why a specific toxicity should only apply for protozoa while one of their main food sources, bacteria, are not affected.

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. . .."

3. Reviewer: Experiment conditions: Usually soil has to be stored cool but should not be air-dried. Air-drying soil prior to incubation in known that it includes the risks of artifacts, even if pre-incubated. The authors should discuss this issue based on some literature which investigated related effects of sieving and air-drying for a range of microbial parameters

Response: The reviewer is right. However, the soil samples in our experiment were not air-dried prior to incubation. In our opinion, the reviewer misunderstood the description of the incubation setup (line 83-106). We modified the paragraph and add further information to prevent misunderstandings by readers.

"...Soil was immediately sieved (< 2 mm) after sampling and divided into subsamples for further basic soil analytics. Subsample material used for incubation was stored at approximately 8°C....Soil subsamples used for determination of soil basic properties were not used for incubation experiment..."

4. Some minor comments:

Reviewer: L. 164: Do not show any instead “show no”

Response: done

Reviewer: L. 204: What do you mean by ”trend” Please, show p-value

Response: We changed “trend” to “tendency”. In this case we cited results from another study (de Souza Machado et al. (2018)) to discuss our study results. In our opinion it does not create an added value to cite the results very detailed. Thus, we waived p-values from other studies, because the main focus lies on the confirmation of our results.

Reviewer: PLFA are only biomarkers, not as sensitive as DNA analyses for specific

taxa. The authors should be careful in taking each PLFA biomarker for granted, and they should add a discussion on potential misinterpretations and uncertainties, maybe in an extra paragraph towards the end of the methods section.

Response: We agree with the reviewer and added a paragraph, which shows limitation of the PLFA biomarker approach (line 294-303).

“. . .Nevertheless, it should be borne in mind that PLFA analyses and laboratory experiments always generate limited results. Fast change of PLFA pattern only allows a determination of actual state of the microbial community structure and it is unreliable to use single PLFA biomarker for taxa detection, which is feasible by deoxyribonucleic acid (DNA) analyses. But compared to gene sequencing or other DNA analyses, PFLA biomarker analysis is rapider and cheaper (Frostegård et al., 2011). Another problem may be the transferability of results generated on laboratory scale under ideal conditions (well-known homogenous plastic fabrics as treatments, simplified and controllable regimes, no rhizosphere, etc.). Also, the single addition of high amounts of microplastics does not reflect the ordinary way how microplastics enter an ecosystem. The accumulation of plastic particles in soils is rather a long and gradual process than a single event, which do not trigger sudden environmental impacts (Rillig et al., 2019). . .”

Reviewer: Note that 10Me16:0 is not only used for Actinomycetes, for instance, but has largely been suggested for S utilizing bacteria (see, e.g., work done by R. Evershed and others)

Response: We agree with the reviewer and exclude 10Me16:0 for calculating Actinomycetes. Based on the modification, affected figures (including the statistics) were updated. Please find attached the revised version.

Reviewer: Figure 1 is nice but it does not really relate to the contents of this paper. If the authors want to leave it, I suggest they should go a bit more into detail into the consequences of comparing the different sizes.

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: The stirring for microglass and microplastic incorporation into soil likely interfered with soil aggregation? Can it be that this stirring jointly with glass treatment also impaired protozoa? For me this would be a reasonable explanation for the results presented.

Response: All treatments (including the control treatment) were handled exactly the same way to compare effects between different treatments. Effects, caused by handling or laboratory routine, can never be completely eliminated, but due to the analogous sample preparation, potential effects should affect all sample replicates and treatments in a similar way. On the one hand, we are neither able to classify nor prove potential influences caused by handling, but on the other hand the results of the experiment show varying protozoa contents after treatment with different artificial

microparticles (e.g. LD-PE or PS show higher protozoa contents than PA12 or microglass). This indicates that it could be possible that stirring inhibit protozoa, but does not explain the question why protozoa are inhibited by microglass. This question still remains open and further research is needed.

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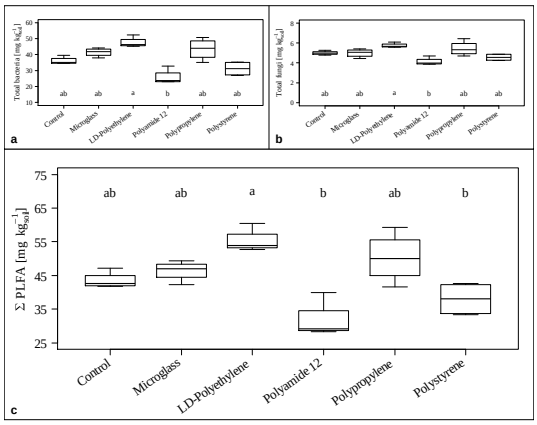

Figure 3. Phospholipid fatty acids as microbial marker in an incubated Chernozem after 80 days. a) Total bacterial-derived PLFA, b) Total fungal-derived PLFA and c) Sum of total fungal- and bacterial-derived PLFA. Different lowercase letters indicate significant differences between the treatment according to a multiple comparison by the Nemenyi test (n=4, $p < 0.05$). Please note varying ordinate scales.

Fig. 1.

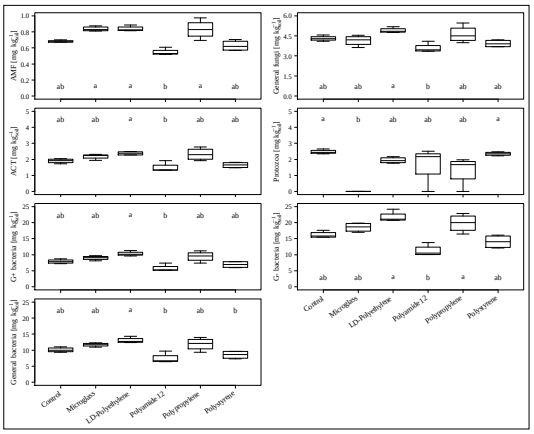

Figure 4. Microbial PLFA contents of the individual taxonomic groups of an incubated Chernozem after 80 days. Different lowercase letters indicate significant differences according to a multiple comparison by the Nemenyi test (n=4, $p < 0.05$). Please note varying ordinate scales.

Fig. 2.