Authors response to referee and editor comments on the manuscript: Effects of microplastic and microglass particles on soil microbial community structure in an arable soil (Chernozem)

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. 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, 92-93, 294-296 and 302-303 (Abstract, Discussion, Conclusion) that the amounts of microparticles used in our study indicating a worst-case scenario.

2. 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 269-279.

   "…Nevertheless, these 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 SiO_2, may also have inhibitory effects on protozoa. To our best knowledge no studies were performed in order to investigate this question…."

3. 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.

4. Some minor comments:

   - L. 164: Do not show any instead "show no"

   Response: done

   - 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.

   - 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 283-292).

   "…*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)…"*

   - 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.

- 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…."*

- 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.

Response to comments of anonymous referee #2

Received and published: 25 November 2019

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 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. 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. 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. 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 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. 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. 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, 92-93, 294-296 and 302-303 (Abstract, Discussion, Conclusion) that the amounts of microparticles used in our study indicating a worst-case scenario.

6. 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. 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 269-271.

"…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 SiO_2, may also have inhibitory effects on protozoa. To our best knowledge no studies were performed in order to investigate this question…."

8. Minor points:

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

Response: done

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.

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…."*

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 normal and an adequate way to use letters (or other symbols) to indicate homogeneous subset, which were defined 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 160-163 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. Dunn's 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 (Dunn, 1964) *Holm method was used to control the family-wise-error rate caused by the pairwise multiple comparisons* (Holm, 1979). *Different lowercase letters were used to illustrate significant differences between homogeneous subsets. …."*

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 117-118).

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

'WHC' should be defined.

Response: The analytical approach is described in line 116-118. 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.

Response to comments of the editor

Received and published: 04 March 2020

Editor comments:

1. Reviewer 2, point 7 needs addressing more thoroughly. The author response only deals with the 2nd part of the point on microglass. The first point the reviewer is making is that if you report non-significant effects of microplastics on PLFAs, then even if there are trends in the data, the discussion should focus on why there are no significant effects. This is especially true given the very high input rates of microplastics which have been used.
   Response: A paragraph containing a probable explanation for non-significant effects of microplastics on PLFAs was added (Line 209-214).

   "…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 e. g. prohibit the exposition of potential ecotoxic compounds. Nevertheless, the treatment of soil by different microparticles causes changes in microbial communities, albeit not significant…"

2. Reviewer 2, point 8 states: The paragraph in the discussion section on the effects of micro-particles on macrofauna seems irrelevant when the experiments were about testing microbial populations. Editor – I would agree with the reviewer that this paragraph should be removed or reduced to a single statement as it is not the focus of the paper.
   Response: The paragraph was removed.

3. Both reviewers indicate that figure 1 is unnecessary and should be removed. The figure contains information which can be gained from the references cited, and also the figure is not used as a conceptual framework for the experiments or referred to elsewhere in the paper, beyond one mention in the introduction. It should therefore be removed from the manuscript.
   Response: Figure 1 was removed and all paragraphs referring to the figure were modified or removed.

4. Regarding the attribution of specific PLFA indicators for general fungi and arbuscular mycorrhizal fungi these attributions are incorrect (see Frostegard et al 2011 for discussion and correct use). The authors use General fungi: 18:2ω6,9, 18:1ω9c, 20:1ω9c; and arbuscular mycorrhizal fungi (AMF): 16:1ω5c. For general fungi I have never seen 20:1ω9c ascribed to fungi so would question if this is accurate (Frostegard et al 2011). For arbuscular fungi it is widely published that PLFA 16:1ω5c can only be used in for AMF in non-soil systems as it also present in bacteria (Olsson et al 1999). It is the NLFA which should be used in soils (Frostegard et al 2011, Soil Biol Biochem). These attributions need correcting or strongly justifying.
   Response: Editor recommendations regarding to the attribution of specific PLFA indicators were implemented (Line 139-146). 20:1ω9c as fungal biomarker is no longer been used. 16:1ω5c as AMF

biomarker is now classed as marker for total PLFA due to discrepancy of the origin in soil systems (Frostegård et al., 2011; Olsson, 1999). 10Me16:0 was also classed as marker for total PLFA (reviewer 1 commented that 10Me16:0 is not only specific for ACT but also for S utilizing bacteria). Based on the modifications, affected figures (including the statistics) were updated.

References

Dunn, O. J.: Multiple Comparisons Using Rank Sums, Technometrics, 6(3), 241–252, doi:10.1080/00401706.1964.10490181, 1964.

Frostegård, Å., Tunlid, A. and Bååth, E.: Use and misuse of PLFA measurements in soils, Soil Biol. Biochem., 43(8), 1621–1625, doi:10.1016/j.soilbio.2010.11.021, 2011.

Holm, S.: A Simple Sequentially Rejective Multiple Test Procedure, Scand. J. Stat., 6(2), 65–70 [online] Available from: www.jstor.org/stable/4615733, 1979.

Olsson, P.: Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil, FEMS Microbiol. Ecol., 29(4), 303–310, doi:10.1016/S0168-6496(99)00021-5, 1999.

Phuong, N. N., Zalouk-Vergnoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C. and Lagarde, F.: Is there any consistency between the microplastics found in the field and those used in laboratory experiments?, Environ. Pollut., 211, 111–123, doi:10.1016/j.envpol.2015.12.035, 2016.

Rillig, M. C. and Bonkowski, M.: Microplastic and soil protists: A call for research, Environ. Pollut., 241, 1128–1131, doi:10.1016/j.envpol.2018.04.147, 2018.

Rillig, M. C., de Souza Machado, A. A., Lehmann, A. and Klümper, U.: Evolutionary implications of microplastics for soil biota, Environ. Chem., 16(1), 3, doi:10.1071/EN18118, 2019.

Rist, S. and Hartmann, N. B.: Aquatic Ecotoxicity of Microplastics and Nanoplastics: Lessons Learned from Engineered Nanomaterials, in Freshwater Microplastics : Emerging Environmental Contaminants?, edited by M. Wagner and S. Lambert, pp. 25–49, Springer International Publishing, Cham., 2018.

de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R. and Rillig, M. C.: Impacts of Microplastics on the Soil Biophysical Environment, Environ. Sci. Technol., 52(17), 9656–9665, doi:10.1021/acs.est.8b02212, 2018.

Zhang, S., Wang, J., Liu, X., Qu, F., Wang, X., Wang, X., Li, Y. and Sun, Y.: Microplastics in the environment: A review of analytical methods, distribution, and biological effects, TrAC Trends Anal. Chem., 111, 62–72, doi:10.1016/j.trac.2018.12.002, 2019.

Effects of microplastic and microglass particles on soil microbial community structure in an arable soil (Chernozem)

Katja Wiedner[1] and Steven Polifka[2]

[1] SEnSol - Sustainable Environmental Solutions Consulting UG, Gleichen, Germany

[2] Physical Geography and Land Use Systems, Department of Geography, Ludwig-Maximilians-University, Munich, Germany

Correspondence to: Katja Wiedner (kawi.science@googlemail.com)

Abstract

Microplastic and microglass particles from different sources enter aquatic and terrestrial environments. The complexity of its environmental impact is difficult to capture and consequences on ecosystem components e.g. soil microorganisms are virtually unknown. Addressing this issue, we performed an incubation experiment by adding 1% of five different types of impurities (≤ 100 µm) to an agricultural used soil (Chernozem) simulating a worst-case scenario of contamination. The impurities are made of polypropylene (PP), low density polyethylene (LD-PE), polystyrene (PS) and polyamide12 (PA12) and microglass. After 80 days of incubation at 20°C, we examined soil microbial community structure by using phospholipid fatty acids (PLFA) as markers for bacteria, fungi and protozoa. The results showed that soil microorganisms were not significantly affected by the presence of microplastic and microglass. However, PLFAs tend to increase in LD-PE (28%), PP (19%) and microglass (11%) treated soil in comparison with untreated soil, whereas PLFAs in PA12 (32%) and PS (11%) treated soil decreased. Interestingly, PLFAs revealed significant differences PA12 (-89%) and PS (-43%) in comparison to LD-PE. Furthermore, variability of bacterial PLFAs was much higher after microplastic incubation whereby fungi seemed to be unaffected from different impurities after 80 days of incubation. Similar results were shown for protozoa, which were also more or less unaffected by microplastic treatment indicated by minor reduction of PLFA contents compared to control. In contrast, microglass seems to have an inhibiting effect on protozoa because PLFAs were under the limit of determination. Our study indicated, that high amounts of different microplastics may have contrary effects on soil microbiology. Microglass might have a toxic effect for protozoa.

1. Introduction

Microplastics are used e.g. for a range of consumer products or industrial application such as abrasives, filler, film and binding agents. The identification and quantification of sources and pathways of microplastics into the environment are highly diverse and difficult to detect. While different methods have been developed for synthetic polymer identification and quantification in sediments and water, analytical methods for soil matrices are still lacking or in an early experimental stage (e.g. Hurley et al., 2018). It is assumed that microplastics enter (agricultural) soils with soil amendments, irrigation and the use of agricultural plastic films for mulch applications, but also through flooding, atmospheric deposition and littering (Bläsing and Amelung, 2018; Hurley and Nizzetto, 2018; Kyrikou and Briassoulis, 2007; Ng et al., 2018; Weithmann et al., 2018). The extent of microplastics polluted soil ecosystems is probably much higher than previously thought. For instance, a recent study by Weithmann et al. (2018) found 895 plastic particles (> 1 mm) per kilogram dry weight in digestate from a biowaste digester used as soil fertilizer after aerobic composting. Li et al. (2018) detected an average microplastic concentration of 22.7 ± 12.1 ^{x} 10^{3} kg^{-1} dry weight in 79 sewage sludge samples from 28 wastewater treatment plants in China. The total amount of microplastics already entered soil habitats is uncertain, but Ng et al. (2018) estimated that 2.3 to 63.0 Mg ha^{-1} microplastic loadings from biosolids reached agroecosystems.

Properties of microplastics differ regarding its size, morphology, origin and chemical composition. A generally accepted definition for the term “microplastics” does not exist so far although essential for industry, research and political decision-makers. In several studies, microplastics are only defined as particles < 5 mm (5000 µm) and a contradistinction to nanoparticles is seldom given in environmental studies. Some environmental studies, however, specify microplastics in large (1 mm to 5 mm) and small (1 µm to 1 mm) particles (Wagner et al., 2014). The term “nanoplastic” and its definition is still controversial discussed. Gigault et al., (2018) specified nanoplastics and recommend 1 µm as upper size limit.

Microplastic particles are differentiated into primary microplastics (e.g. for abrasives, cosmetic additives or industrial resin pellets) and degraded secondary microplastics, which result from formerly larger plastic debris. Microplastic particles could be highly diverse regarding its morphology leading to a varying effects in environmental systems (Wagner et al., 2014).

More than 200 different types of plastic are known, which may have different properties e.g. regarding its reactivity or bioavailability in soil environment. Thus, differentiation of microplastic should not only base on size but also regarding its chemical (e.g. hydrophobicity scales) and physical properties (e.g. morphology) may affecting physicochemical soil properties and soil biology. For instance, De Souza Machado et al. (2018) showed, that 2% microplastic concentration in soil affects bulk density, water holding capacity, hydraulic conductivity, soil aggregation, water stable aggregates and microbial activity. This comprehensive study elucidates the complexity of processes triggered by the presence of microplastic particles in soil environment. Microglass is currently not part of the microplastics discussion although glass is very resistant to corrosion or weathering and can be thought as corrosion-proof (Papadopoulos and Drosou, 2012). Microglass is used as blasting abrasive, filling material and an additive of road markings. Thus, it enters the environment on similar ways than microplastics e.g. in sewage sludge or abrasive from roads. The effects on terrestrial ecosystems are equally unknown as those of microplastics. 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.

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.

The present study contributes to a deeper understanding of the impact of different types of microplastics and microglass (~100 µm) on soil microbial community structure in an agricultural soil. For this, different types of microplastics and microglass were arable soil and incubated for 80 days. In order to identify possible shifts in the microbial community structure we used phospholipid fatty analysis (PLFA). This study was guided by the following research questions:

1. Is it possible to observe distinct shifts in microbial community due to the presence of microparticles?
2. Do different plastic material properties stimulate microbial groups in diverse ways?
3. Does microglass affect the microbial community in a similar way to microplastics?

2. Material and Methods

2.1 Soil sampling and incubation experiment

Soil samples were taken on March 11, 2018 near Brachwitz (51°31'46" N, 11°52'41" E; 102 m above sea level), 10 km northwest of Halle (Saale) (Saxony-Anhalt, Germany). The samples were randomly taken at four different spots (A, B, C, D) from the first 10 cm of an arable topsoil in order to have four independent replicates, which served as basic substrate for the incubation experiment. 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. The soil subsamples were set at a water content of 60% water holding capacity (WHC) and pre-incubated for three weeks at 20°C.

A respective amount of 1% (w/w) of polypropylene (PP), low density polyethylene (LD-PE), polystyrene (PS), polyamide12 (PA12) (Rompan, Remda-Teichel, Germany) and microglass (Kraemer Pigmente GmbH & Co.KG, Aichstetten, Germany) was added to each independent soil replicate and stirred manually for homogenization with a glass stirring rod. This quantity is equal to 12.6 Mg microparticles ha^{-1} (bulk density topsoil: 1.26 g cm^{-3}) indicating worst-case scenario. However, a study by Fuller and Gautam (2016) found similar contaminated soils closed to industrial areas. In addition, a control soil replicates were incubated without additives of microplastics or microglass. Due to the use of arable topsoil as incubation substrate, a microplastic contamination cannot be excluded. However, due to the high microplastic loads used in this the experiment a possible prior contamination is negligible. Microplastics were not pre-treated to cause degradation (e.g. with ultraviolet radiation) to simulate primary microplastic particles in soils. Incubation was performed in laboratory bottles for 80 days at 20°C without daylight. During this period all bottles were opened weekly for 30 s in order to secure aerobic conditions. Furthermore, the total weight of each bottle was monitored. In the case of any weight loss, an equivalent amount of water was replenished to provide a constant water holding capacity of 60%. According to manufacturer specifications sizes of microplastic and microglass particles ranged between 90-100 µm. The microplastics used in this study are commonly used for daily products and cosmetics (bottle caps, drinking straws (PP), plastic bags, milk bottles, food packaging film (LD-PE), disposable cups, packaging materials (PS), inks and clothing (PA)) and detected in high amounts in sewage sludge of Lower Saxony (Mintenig et al., 2017; Shah et al., 2008).

2.2 Soil basic properties

For soil basic characterization, soil subsamples (not samples for incubation) were air dried and sieved (< 2 mm). Total carbon (TC) and total nitrogen (TN) analysis were carried out with a vario Max cube CNS analyzer (Elementar Analysensysteme GmbH, Langenselbold, Germany). Electrical conductivity (EC) and pH values were analyzed by using suspensions of 0.01 M $CaCl_2$ and distilled H_2O at a soil solution ratio of 1 to 2.5. Soil particle size distribution was measured in a suspension using a Helos/KR laser diffractometer (Sympatec GmbH, Clausthal-Zellerfeld, Germany) equipped with a Quixel wet dispersion unit (Sympatec GmbH, Clausthal-Zellerfeld, Germany). Before analysis the sample material was treated with a dispersing agent (0.2 M tetra-Sodium diphosphate decahydrate). For the evaluation of water holding capacity, 10 g of soil was weighted into a plastic cylinder with fine-mesh on the bottom and placed in water. After 24 hours, saturated samples were drained until water release stopped and weighted again for calculation of water holding capacity. Soil subsamples used for determination of soil basic properties were not used for incubation experiment.

Soil chemical properties of the Chernozem topsoil (IUSS Working Group WRB, 2015) were as follows: Total organic carbon (TOC) 28.6 ± 1.8 g kg^{-1}, Total nitrogen (TN) 2.48 ± 0.13 g kg^{-1}, C:N 11.56 ± 0.15, EC 170 ± 9 µS cm^{-1} and pH_{CaCl2} 5.13 ± 0.02. Proportions of clay, silt and sand were 7.0 ± 0.2 %, 58.5 ± 3.6 % and 34.5 ± 3.7 %, respectively and the soil texture was classified as silt loam (FAO, 2006). Water holding capacity was 0.218 ± 0.005 g_{H2O} $g_{dry\ weight}^{-1}$.

2.3 Phospholipid fatty acid analysis

For phospholipid fatty acid (PLFA) analysis, 6 g of fresh soil were extracted with a single-phase trichloromethane/methanol/citrate buffer system (1:2:0.8; v/v/v). 19:0 was added as first internal standard (IS1) to each sample for later quantification of the phospholipids. Extracts were centrifuged for 15 minutes at 4000 rpm. The supernatants were separated using a liquid-liquid extraction. Lipid fractionation was performed using a silica based solid phase extraction. Remaining phospholipid fractions of the samples and the external standards were treated by an alkaline saponification using 0.5 M sodium hydroxide in methanol followed by a methylation with boron trifluoride in methanol (12%). For separation of the PLFA methyl esters a liquid-liquid separation with saturated sodium chloride solution and hexane was used. For quality control 5-α-cholestane was added as second internal standard (IS2) after the phase separation. Analytes were transferred with isooctane into GC autosampler vials and analyzed by a GC 2010 capillary gas chromatograph (Shimadzu Ltd., Tokyo, Japan) equipped with Supelco SPB-5 fused silica capillary column (30m x 0.25 mm x 0.25 µm film thickness) and flame ionization detector. All PLFA contents were corrected for dry mass due to the use of fresh soil for extraction. For this purpose, WHC was determined subsequent to sample weighing.

Single PLFA were assigned to taxonomic groups according to following pattern: Total fungi: 18:2ω6,9, 18:1ω9c; protozoa: 20:4ω6c; general bacteria: 14:0, 15:0, 16:0, 17:0, 18:0; gram-positive bacteria: i14:0, a14:0, i15:0, a15:0, i16:0, a16:0, i17:0, a17:0; gram-negative bacteria: 16:1ω7c, cy17:0, 18:1ω7c, cy19:0; Actinomycetes (ACT): 10Me18:0 (Frostegård et al., 1993; Olsson et al., 1999; Zelles, 1999; Zelles et al., 1992). These biomarkers are not entirely specific for their taxonomic groups and therefore must be interpreted cautiously (Zelles, 1997). For total bacteria the sum of general, gram-positive, gram-negative and ACT was calculated. Sum of PLFA describes the sum of measured contents of fungal-derived, bacterial-derived, protozoa and the unspecific PLFA markers 16:1ω5c and 10Me16:0.

2.4 Scanning Electron Microscopy (SEM)

Microplastic samples were fixed on an object slide and coated with gold using a Q150R ES rotary pumped sputter coater (Quorum Technologies Ltd., Laughton, United Kingdom) in a low vacuum atmosphere. The SEM images were taken with a Tabletop Microscope TM4000Plus (Hitachi Ldt., Tokyo, Japan).

2.5 Statistical analysis

Statistical analysis and graphical design were carried out using R 3.5.0 (R Core Team, 2018). Prior test assumption of normally distributed data was examined using Shapiro-Wilk test. Because of mostly non-normal distributed data Brown-Forsythe test was used for checking for homoscedasticity in the groups. 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. Dunn's 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 (Dunn, 1964). Holm method was used to control the family-wise-error rate caused by the pairwise multiple comparisons (Holm, 1979). Different lowercase letters were used to illustrate significant differences between homogeneous subsets. Interquartile range of boxplot whiskers is 1.5.

3. Results

3.1 Morphology and size of microparticles

The SEM images of the microplastics (PP, LD-PE, PS, PA12) and microglass are shown in Fig. 1, illustrating the heterogenic morphology between but also within the same type of microplastic. Furthermore, according the manufacturer specifications size of microplastics and microglass should range between 90 to 100 µm. Many particles are, however, much bigger (up to 200 µm) or smaller (down to 10 µm). Especially LD-PE, PA12 and PP have a slag-like structure leading to pore formation, whereas PS has a plate shaped structure with fringed or even sharp edges. Pointy and sharp edges are also shown for LD-PE, PA12 and PP. In contrast, microglass particles appear with a few exceptions more regular than the microplastic ones and could described as microspheres.

3.2 Impact of microplastics and microglass on soil microbial community structure

The total PLFA contents do not show significant differences between single specific microparticles compared to the control (Fig. 2). Nevertheless, the PLFA contents of microglass, PP and LD-PE treated soil tend to increase compared to the control by 11, 19, and 28%, respectively, whereas PA12 and PS show lower PLFA contents compared to the control by 32 and 11%. The comparisons of single plastic types show that PLFA contents of PA12 and PS are with 89% and 43%, respectively, significant lower compared to LD-PE (Fig. 2). A similar pattern is also observable in treatment distribution of each group PLFA content of bacteria and fungi. Although, the fungi show a more inexplicit pattern compared to bacteria. This might imply that a positive and negative stimulations of the single microplastics affect bacteria as well as fungi in a comparable way. Compared to the control bacterial-derived PLFA contents show an increase in soil treated with microglass (19%), PP (25%) and LD-PE (32%). On the other hand, decline of total bacteria has been determined in soil treated with PA12 (-33%) and PS (-11%, Fig. 3). Fungal PLFA contents, however, show a smaller increase compared to the control by 9% (microglass), 15% (PP), 24% (LD-PE) and a lower decrease by -22% (PA12) and -9% (PS; Fig. 3). The treatment effect variability

of bacterial-derived PLFAs are multiple times higher compared to fungal-derived PLFAs. For instance, the highest positive median deviation of total bacterial-derived PLFAs to the control is 32% (LD-PE), whereas the highest negative deviation is 33% (PA12). In contrast, positive deviation of fungal-derived PLFAs compared to the control is only 24% (LD-PE) and negative deviation is only 22% (PA12, Fig. 3).

Regarding a whole comparison of all treatments, with the exception of protozoa, the increase of PLFA contents could be observed for all fungal and bacterial (Gram-negative, Gram-positive, ACT, general) groups when incubated with microglass, LD-PE and PS (Fig. 3). The significant lower PLFA contents of PA12 compared to LD-PE are also shown continuously in all microbial groups (Fig. 3). In contrast to the fairly consistent pattern of the fungi and bacteria, protozoa show a different pattern. Protozoa PLFA contents decreased for all microplastics by up to 21% (LD-PE) compared to the control (Fig. 3). PA12 and PP show a comparatively high data variability compared to the other treatments. Most interestingly, PLFA content of protozoa was under the limit of determination for all replications incubated with microglass.

4. Discussion

High amounts of artificial soil impurities (12.6 Mg microplastics or -glass ha^{-1}) do not have a significant effect on soil microbial community structure within the incubation time of 80 days. However, there is a conspicuous tendency that different types of microplastics may have promoting (LD-PE, PP) or reducing effects (PA12, PS) on soil microorganisms (Fig. 2 and 3). Furthermore, different plastics have obviously various effects on individual taxonomic groups as indicated by the significant lower values of treatment PA12 and PS compared to LD-PE (Fig. 2 and 3). As mentioned in Section 3.2, the variability of bacterial-derived PLFA are much higher than fungal-derived PLFAs, which possibly indicates that bacteria are more susceptible to interference. However, this is not surprisingly because bacteria respond relatively fast on environmental changes (e.g. changing water conditions, temperature, etc.) e.g. due to its rapid reproduction rate (e.g. Fierer et al., 2003).

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 e. g. prohibit the exposition of potential ecotoxic compounds. 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. However, de Souza Machado et al. (2018) investigated the microbial activity after the addition of different amounts of polyester and polyacrylic fibers as well as polyethylene fragments by measuring the enzyme activity with fluorescein diacetate (FDA). The study showed that polyester and polyacrylic fibers reducing microbial activity whereas the soil incubated with polyethylene fragments showed no clear tendency. The effects might be caused e.g. through changes in soil bulk density, water holding capacity or aggregate changes (de Souza Machado et al., 2018). The reasons for the observed promoting and also inhibiting effects on microorganisms from different plastic types, remain a matter of speculation and further research is necessary addressing these issues. The causes mentioned by de Souza Machado et al. (2018) are essential reasons effecting soil microbiology.

Nevertheless, the morphology and surface properties of microplastics should not be underestimated. The slag-like structure of LD-PE, PA12 and PP form wrinkles and pores (Fig. 1) may act as habitat for soil microorganisms. This in turn may have a promoting effect on the soil microbial community composition of soil as known from pore rich soil additives e.g. such as charcoal (biochar). For instance, fungal hyphae or bacteria penetrate in pores and wrinkles and are protected from predators (Lehmann et al., 2011; Thies and Rillig, 2009). Furthermore, McCormick et al. (2014) showed that microplastic particles could be act as habitat for bacteria in rivers. Umamaheswari et al. (2014) found fungi hyphae from *Penicillium sp., Fusarium sp.* and *Aspergillus sp.*, which colonized and grew on the surface of soil buried PS after 70 days. The potential colonization of microorganism on the surface of LD-PE was clearly reviewed by (Kumar Sen and Raut, 2015), who also mentioned the penetration of the microplastic surface by fungi hyphae. Similar colonization of bacteria were reported by Harrison et al. (2014), who found rapid attachment of microorganisms onto LD-PE microplastics within coastal marine sediments after 14 days. In sum, LD-PE seems to benefit the bacterial and fungal colonization. Both bacteria and fungi tend to increasing populations in our experiment. LD-PE may also act as habitat as well as carbon source. The extent of these functions is mostly controlled by abiotic for example ultraviolet irradiation and temperature (Kumar Sen and Raut, 2015). Thus, the provided habitat seems to be the most important factor for enhanced PLFA in our experiment, because abiotic factors were either excluded (no ultraviolet irradiation) or kept usual (stabile temperature at 20°C). However, colonization on microplastic surfaces after incubation was not determined in this experiment and currently it is still uncertain, if colonized microplastic surface areas could also act as a hotbed for extensive soil colonization. Furthermore, it remains uncertain why PA12 seems to inhibit microorganisms in this experiment through having similar surface properties compared to e.g. LD-PE, which tends to promote the microorganisms. According to Galloway et al. (2017), organic compounds, nutrients and pollutants can accumulate on microplastic surface in aquatic ecosystems. It can be assumed that this also occurs in terrestrial ecosystems such as soil environments. Furthermore, it is conceivable that also humic substances accumulate on microplastic surfaces leading to an increased colonization of specific microorganisms and in consequence to the formation of a bacterial biofilm. The accumulation of nutrients and water on a surface is the precondition for the formation of biofilms consisting of extracellular polymeric substances derived from bacteria (Flemming and Wingender, 2010). The formation of biofilms may occur within three weeks, as shown by Lobelle and Cunliffe (2011) investigated the surface of PE particles in marine environment. Due to the constant (water)conditions in this study, the formation of biofilms on microplastic surfaces cannot be excluded at least on LD-PE and PP particles as well as microglass indicating promoting effects on soil microorganisms reflected by increased PLFA contents. Future research on the role of artificial microparticles in soil microcosm is urgently needed to clarify potential risks, intensities of soil microbiological disturbance by microplastics due to promoting colonization of specialized (and harmful) microorganism, toxicity due to released harmful chemicals or a direct damage after entering microorganism as secondary nanoparticles (Lu et al., 2019).

Beside the morphology of microplastic, its surface chemistry has effects on soil physicochemical processes. In comparison to LD-PE, PP and PS, which show hydrophobic characteristics, PA12 combines hydrophobic and hydrophilic surface groups (Schmidt et al., 2015) whereby microglass has a hydrophilic surface. A study by Marangoni et al. (2018) showed, that glass microspheres (4 µm, 7-10 µm and 30-50 µm; micoglass addition of 1-5% v/v) reduced the mobility of water reflected in a large decrease of the spin-spin relaxation time of water protons, decreases in the self-diffusion coefficient of water molecules, a lower water activity, and strengthening of O-H bonds. The study further showed that glass microspheres have an inhibiting effect on *Escherichia coli* growth and

the germination of *Medicago sativa* seeds. In our experiment, an inhibiting effect of microglass could not be shown for the most microorganisms with the exception of protozoa (Fig. 3). Based on the results by Marangoni et al. (2018) is conceivable that protozoa respond in a similar way to the presence of microglass like *Escherichia coli*. Nevertheless, these 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 SiO_2, may also have inhibitory effects on protozoa. To our best knowledge no studies were performed in order to investigate this question.

Another important fact is the heterogeneity of microplastics. The wide variance between the several types of plastic and just as the heterogeneity of different sources prevent a generalization of scientific results. For example Cao et al. (2017) visualized polystyrene using SEM. The showed image of PS differs strongly from the plastic used in this study. The way of producing, the pathway to environment and the degradation status of microplastics play an important role for evaluating the behavior of microplastics in soil or other environments. Furthermore, it remains ambiguous if primary microplastics added to soils cause similar effects compared to secondary microplastics, which result from the decomposition of larger plastic debris. Depending on the parent plastic material and environmental variables, highly diverse plastic surfaces result from uncontrolled surface modification due to decomposition processes. This fact is already known from the comparison of primary and secondary nanoplastics properties (Gigault et al., 2018). Especially in view of the fact that already emitted macro- and microplastics will degrade in terrestrial ecosystems right up to nanoscales.

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). Thus, this first study should only serve as a basic work, which stimulates future microbial studies dealing with microparticles in soils or sediments. So, further research is needed to link laboratory and environmental conditions to enhance the environmental relevance of microplastic research. High amounts were chosen to show worst-case effects on highly contaminated place (industrial areas or floodplains in vicinity of urban areas). On the other hand, agricultural land is treated regularly with compost, sewage sludge and other microplastics containing soil amendments or plastic mulches are used in vegetable production. Due to their recalcitrance plastic tend to accumulate in soil. So, a worst-case scenario is able to illustrate future soil statuses on an undefined time scale.

5. Conclusion

This study aimed the question, whether high amounts of microplastics and -glass have effects on soil microbial community structure by using PLFAs as microbial markers. High amounts were added to soil in order to show a worst-case scenario in highly contaminated soils (e.g. industrial areas or floodplains in vicinity of urban areas). On the other hand, agricultural land is treated regularly with compost, sewage sludge and other microplastics

containing soil amendments. Furthermore, plastic mulches used for fruit and vegetable production are further sources of microplastic in soils. Due to its high recalcitrance, plastic tend to accumulate in soil. Thus, our worst-case scenario may illustrate future soil statuses at an undefined time scale. The use of microbial markers in laboratory incubation experiments, describing microbial soil communities always act as a simplification of complex natural environmental systems. This study provides first insights into soil microcosm disturbed by different microparticles. The results provide hints that after 80 days of incubation microorganisms are either promoted or inhibited depending on the type of the impurities. Different microplastic types seem to have contrary effects on soil microorganisms depending on the origin and the properties of the plastics, which influence the morphological and chemical appearance of the microplastics. On the other hand, microglass seems to be even highly toxic for protozoa. Within this study we cannot clarify why bacteria and protozoa show different reaction on quartz glass microparticles. Changes in soil microbiology induced by plastic pollution have unexpected consequences for soil ecosystems. This study should therefore be considered as basis for further research which is urgently needed in order to understand the long-term consequences of microplastics in soils and other terrestrial ecosystems.

Data availability.

All data compiled in this study is published in figures. Detailed primary data and underlying research are available by request from the corresponding author.

Author contributions.

KW conceptualized and carried out the experiment. Laboratory work was performed by KW and SP. Statistical analysis and data visualization was carried out by SP. KW prepared the manuscript with contributions from SP.

Competing interests.

The authors declare that they have no conflict of interest.

Acknowledgements.

We acknowledge Bruno Glaser (Soil Biogeochemistry, Martin Luther University Halle-Wittenberg) for providing the Soil Biogeochemistry laboratories for PLFA analysis. We are grateful to Aline Brosch for supporting the incubation experiment. Furthermore, we thank Tobias Bromm (Soil Biogeochemistry, Martin Luther University Halle-Wittenberg) for supporting the GC measurements. We also thank Gregor Borg (Economic Geology and Petrology, Martin Luther University Halle-Wittenberg) and his scientific staff Andreas Kamradt and Tim Rödel for providing the SEM. Finally, we are grateful to the anonymous reviewers and editor for their critical and detailed comments.

References.

Bläsing, M. and Amelung, W.: Plastics in soil: Analytical methods and possible sources, Sci. Total Environ., 612, 422–435, doi:10.1016/j.scitotenv.2017.08.086, 2018.

Cao, D., Wang, X., Luo, X., Liu, G. and Zheng, H.: Effects of polystyrene microplastics on the fitness of earthworms in an agricultural soil, IOP Conf. Ser. Earth Environ. Sci., 61(1), 012148, doi:10.1088/1755-1315/61/1/012148, 2017.

Dunn, O. J.: Multiple Comparisons Using Rank Sums, Technometrics, 6(3), 241–252, doi:10.1080/00401706.1964.10490181, 1964.

FAO: Guidelines for Soil Description. Fourth Edition, Rome, Italy. [online] Available from: http://www.fao.org/3/a0541e/A0541E.pdf, 2006.

Fierer, N., Schimel, J. P. and Holden, P. A.: Influence of Drying-Rewetting Frequency on Soil Bacterial Community Structure, Microb. Ecol., 45(1), 63–71, doi:10.1007/s00248-002-1007-2, 2003.

Flemming, H.-C. and Wingender, J.: The biofilm matrix, Nat. Rev. Microbiol., 8(9), 623–633, doi:10.1038/nrmicro2415, 2010.

Frostegård, Å., Bååth, E. and Tunlio, A.: Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis, Soil Biol. Biochem., 25(6), 723–730, doi:10.1016/0038-0717(93)90113-P, 1993.

Frostegård, Å., Tunlid, A. and Bååth, E.: Use and misuse of PLFA measurements in soils, Soil Biol. Biochem., 43(8), 1621–1625, doi:10.1016/j.soilbio.2010.11.021, 2011.

Fuller, S. and Gautam, A.: A Procedure for Measuring Microplastics using Pressurized Fluid Extraction, Environ. Sci. Technol., 50(11), 5774–5780, doi:10.1021/acs.est.6b00816, 2016.

Galloway, T. S., Cole, M. and Lewis, C.: Interactions of microplastic debris throughout the marine ecosystem, Nat. Ecol. Evol., 1(5), 0116, doi:10.1038/s41559-017-0116, 2017.

Gigault, J., ter Halle, A., Baudrimont, M., Pascal, P.-Y., Gauffre, F., Phi, T.-L., El Hadri, H., Grassl, B. and Reynaud, S.: Current opinion: What is a nanoplastic?, Environ. Pollut., 235, 1030–1034, doi:10.1016/j.envpol.2018.01.024, 2018.

Harrison, J. P., Schratzberger, M., Sapp, M. and Osborn, A. M.: Rapid bacterial colonization of low-density polyethylene microplastics in coastal sediment microcosms, BMC Microbiol., 14(1), 232, doi:10.1186/s12866-014-0232-4, 2014.

Holm, S.: A Simple Sequentially Rejective Multiple Test Procedure, Scand. J. Stat., 6(2), 65–70 [online] Available from: www.jstor.org/stable/4615733, 1979.

Hurley, R. R. and Nizzetto, L.: Fate and occurrence of micro(nano)plastics in soils: Knowledge gaps and possible risks, Curr. Opin. Environ. Sci. Heal., 1, 6–11, doi:10.1016/j.coesh.2017.10.006, 2018.

Hurley, R. R., Lusher, A. L., Olsen, M. and Nizzetto, L.: Validation of a Method for Extracting Microplastics from Complex, Organic-Rich, Environmental Matrices, Environ. Sci. Technol., 52(13), 7409–7417, doi:10.1021/acs.est.8b01517, 2018.

IUSS Working Group WRB: World Reference Base for Soil Resources 2014, update 2015. International soil classification system for naming soils and creating legends for soil maps, Rome, Italy. [online] Available from: http://www.fao.org/3/i3794en/I3794en.pdf, 2015.

Kumar Sen, S. and Raut, S.: Microbial degradation of low density polyethylene (LDPE): A review, J. Environ. Chem. Eng., 3(1), 462–473, doi:10.1016/j.jece.2015.01.003, 2015.

Kyrikou, I. and Briassoulis, D.: Biodegradation of Agricultural Plastic Films: A Critical Review, J. Polym. Environ., 15(2), 125–150, doi:10.1007/s10924-007-0053-8, 2007.

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C. and Crowley, D.: Biochar effects on soil biota - A review, Soil Biol. Biochem., 43(9), 1812–1836, doi:10.1016/j.soilbio.2011.04.022, 2011.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G. and Zeng, E. Y.: Microplastics in sewage sludge from the wastewater treatment plants in China, Water Res., 142, 75–85, doi:10.1016/j.watres.2018.05.034, 2018.

Lobelle, D. and Cunliffe, M.: Early microbial biofilm formation on marine plastic debris, Mar. Pollut. Bull., 62(1), 197–200, doi:10.1016/j.marpolbul.2010.10.013, 2011.

Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z. and Jin, Y.: Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health, Sci. Total Environ., 667, 94–100, doi:10.1016/j.scitotenv.2019.02.380, 2019.

Marangoni, A. G., Al-Abdul-Wahid, M. S., Nicholson, R., Roma, A., Gravelle, A. J., De Souza, J., Barbut, S. and Spagnuolo, P. A.: Water immobilization by glass microspheres affects biological activity, Sci. Rep., 8(1), 9744, doi:10.1038/s41598-018-28123-4, 2018.

McCormick, A., Hoellein, T. J., Mason, S. A., Schluep, J. and Kelly, J. J.: Microplastic is an Abundant and Distinct Microbial Habitat in an Urban River, Environ. Sci. Technol., 48(20), 11863–11871, doi:10.1021/es503610r, 2014.

Mintenig, S. M., Int-Veen, I., Löder, M. G. J., Primpke, S. and Gerdts, G.: Identification of microplastic in effluents of waste water treatment plants using focal plane array-based micro-Fourier-transform infrared imaging, Water Res., 108, 365–372, doi:10.1016/j.watres.2016.11.015, 2017.

Ng, E.-L., Huerta Lwanga, E., Eldridge, S. M., Johnston, P., Hu, H.-W., Geissen, V. and Chen, D.: An overview of microplastic and nanoplastic pollution in agroecosystems, Sci. Total Environ., 627, 1377–1388, doi:10.1016/j.scitotenv.2018.01.341, 2018.

Olsson, P. A., Thingstrup, I., Jakobsen, I. and Bååth, E.: Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field, Soil Biol. Biochem., 31(13), 1879–1887, doi:10.1016/S0038-0717(99)00119-4, 1999.

Papadopoulos, N. and Drosou, C.-A.: Influence of weather conditions on glass properties, J. Univ. Chem. Technol. Metall., 47, 429–438, 2012.

Phuong, N. N., Zalouk-Vergnoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C. and Lagarde, F.: Is there any consistency between the microplastics found in the field and those used in laboratory experiments?, Environ. Pollut., 211, 111–123, doi:10.1016/j.envpol.2015.12.035, 2016.

R Core Team: R: A Language and Environment for Statistical Computing, [online] Available from: http://www.r-project.org, 2018.

Rillig, M. C. and Bonkowski, M.: Microplastic and soil protists: A call for research, Environ. Pollut., 241, 1128–1131, doi:10.1016/j.envpol.2018.04.147, 2018.

Rillig, M. C., de Souza Machado, A. A., Lehmann, A. and Klümper, U.: Evolutionary implications of microplastics for soil biota, Environ. Chem., 16(1), 3, doi:10.1071/EN18118, 2019.

Rist, S. and Hartmann, N. B.: Aquatic Ecotoxicity of Microplastics and Nanoplastics: Lessons Learned from Engineered Nanomaterials, in Freshwater Microplastics : Emerging Environmental Contaminants?, edited by M. Wagner and S. Lambert, pp. 25–49, Springer International Publishing, Cham., 2018.

Schmidt, J., Sachs, M., Fanselow, S., Wirth, K.-E., Peukert, W. and Kolberg, S.: Funktionalisierung von Polymermaterialien für Laserstrahlschmelzverfahren, in Neue Entwicklungen in der Additiven Fertigung, pp.

25–40, Springer Berlin Heidelberg, Berlin, Heidelberg, Germany., 2015.

Shah, A. A., Hasan, F., Hameed, A. and Ahmed, S.: Biological degradation of plastics: A comprehensive review, Biotechnol. Adv., 26(3), 246–265, doi:10.1016/j.biotechadv.2007.12.005, 2008.

de Souza Machado, A. A., Lau, C. W., Till, J., Kloas, W., Lehmann, A., Becker, R. and Rillig, M. C.: Impacts of Microplastics on the Soil Biophysical Environment, Environ. Sci. Technol., 52(17), 9656–9665, doi:10.1021/acs.est.8b02212, 2018.

Thies, J. E. and Rillig, M. C.: Characteristics of Biochar: Biological Properties, in Biochar for Environmental Management: Science and Technology, edited by J. Lehmann and S. Joseph, pp. 85–105, Earthscan Ltd., London, United Kingdom., 2009.

Umamaheswari, S., Murali, M. and Thiyagarajan, R.: Role of Fungi Inhabiting Soil, Cow Dung and Sewage in Degrading Polyethylene Terephthalate and Polystyrene Foam, J. Pure Apllied Microbiol., 8(3), 2465–2471 [online] Available from: https://microbiologyjournal.org/archive_mg/jmabsread.php?snoid=1952&month=&year=, 2014.

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A. D., Winther-Nielsen, M. and Reifferscheid, G.: Microplastics in freshwater ecosystems: what we know and what we need to know, Environ. Sci. Eur., 26(1), 12, doi:10.1186/s12302-014-0012-7, 2014.

Weithmann, N., Möller, J. N., Löder, M. G. J., Piehl, S., Laforsch, C. and Freitag, R.: Organic fertilizer as a vehicle for the entry of microplastic into the environment, Sci. Adv., 4(4), doi:10.1126/sciadv.aap8060, 2018.

Zelles, L.: Phospholipid fatty acid profiles in selected members of soil microbial communities, Chemosphere, 35(1–2), 275–294, doi:10.1016/S0045-6535(97)00155-0, 1997.

Zelles, L.: Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review, Biol. Fertil. Soils, 29(2), 111–129, doi:10.1007/s003740050533, 1999.

Zelles, L., Bai, Q. Y., Beck, T. and Beese, F.: Signature fatty acids in phospholipids and lipopolysaccharides as indicators of microbial biomass and community structure in agricultural soils, Soil Biol. Biochem., 24(4), 317–323, doi:10.1016/0038-0717(92)90191-Y, 1992.

Zhang, S., Wang, J., Liu, X., Qu, F., Wang, X., Wang, X., Li, Y. and Sun, Y.: Microplastics in the environment: A review of analytical methods, distribution, and biological effects, TrAC Trends Anal. Chem., 111, 62–72, doi:10.1016/j.trac.2018.12.002, 2019.

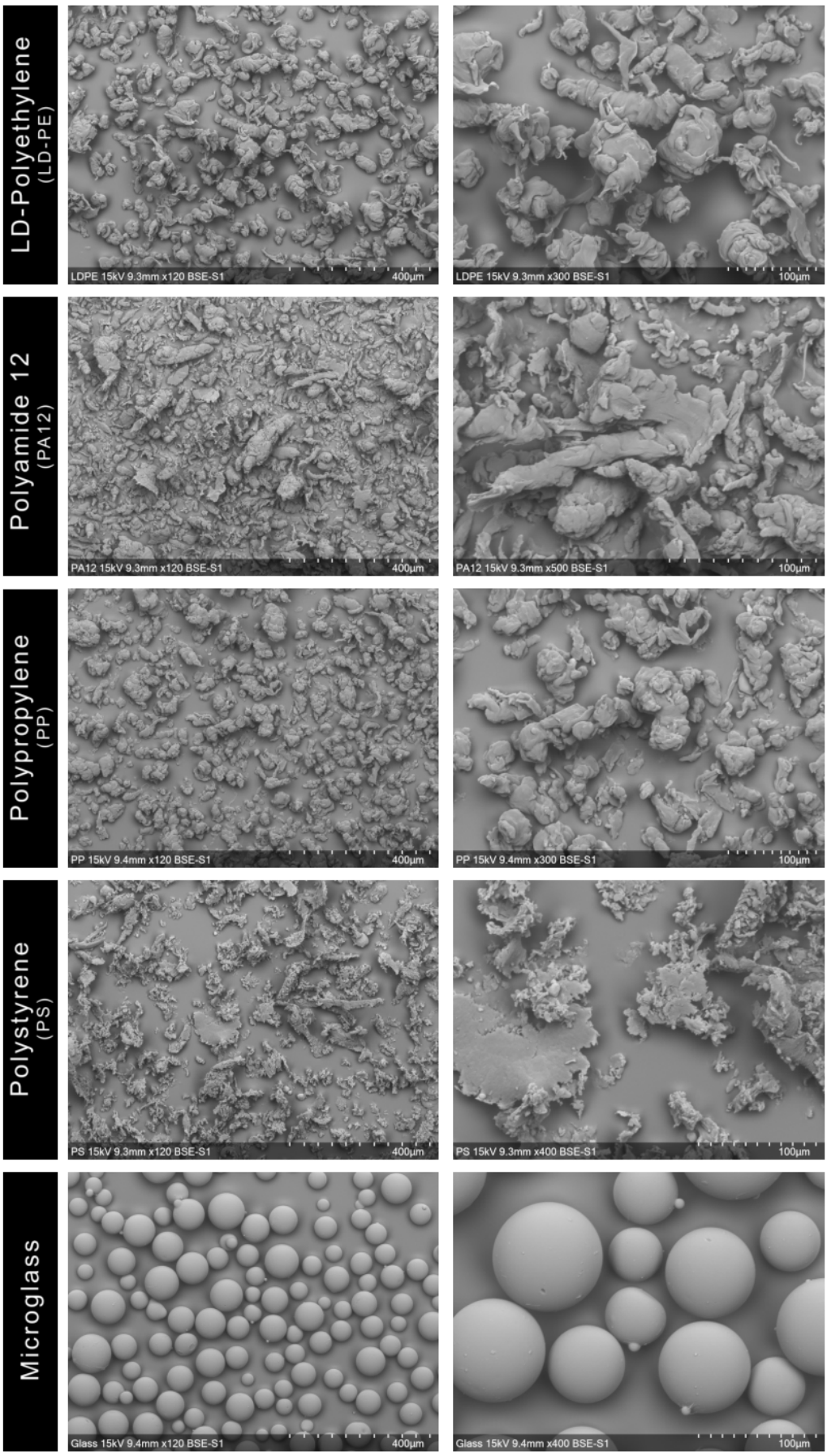

Figure 1. Heterogenic particle size distribution and morphology depending on the microparticle type visualized by SEM.

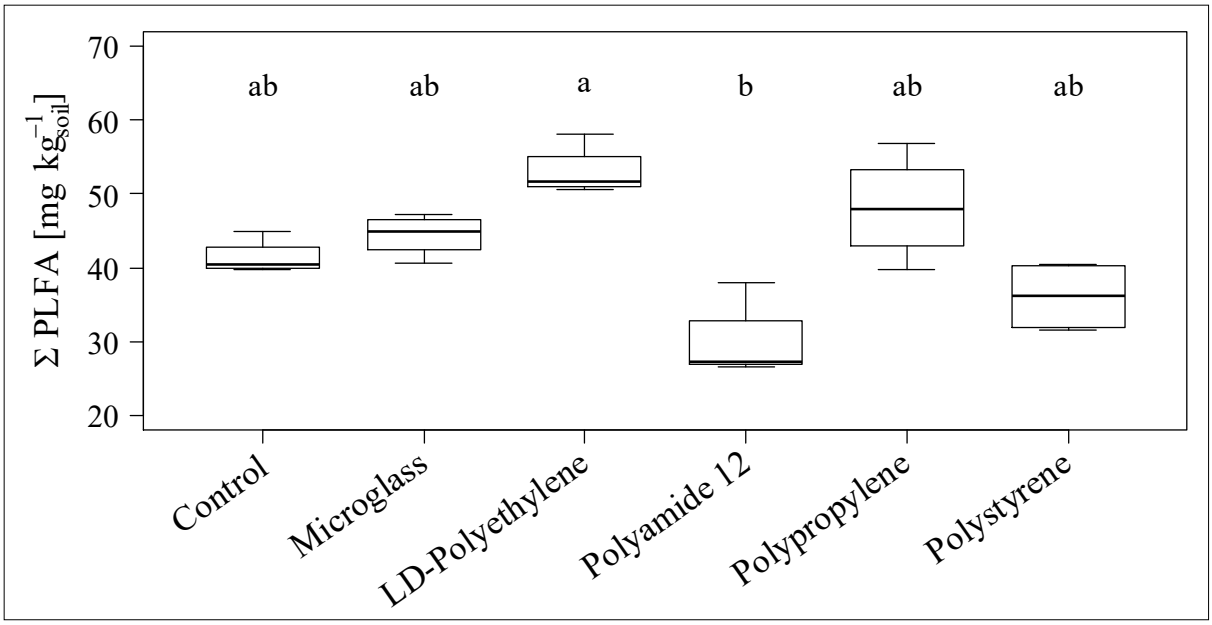

Figure 2. Sum of total phospholipid fatty acids as microbial marker in an incubated Chernozem after 80 days. Different lowercase letters indicate significant differences between the treatment according to a multiple comparison by Dunn's test (n=4, $p < 0.05$). Please note varying ordinate scales.

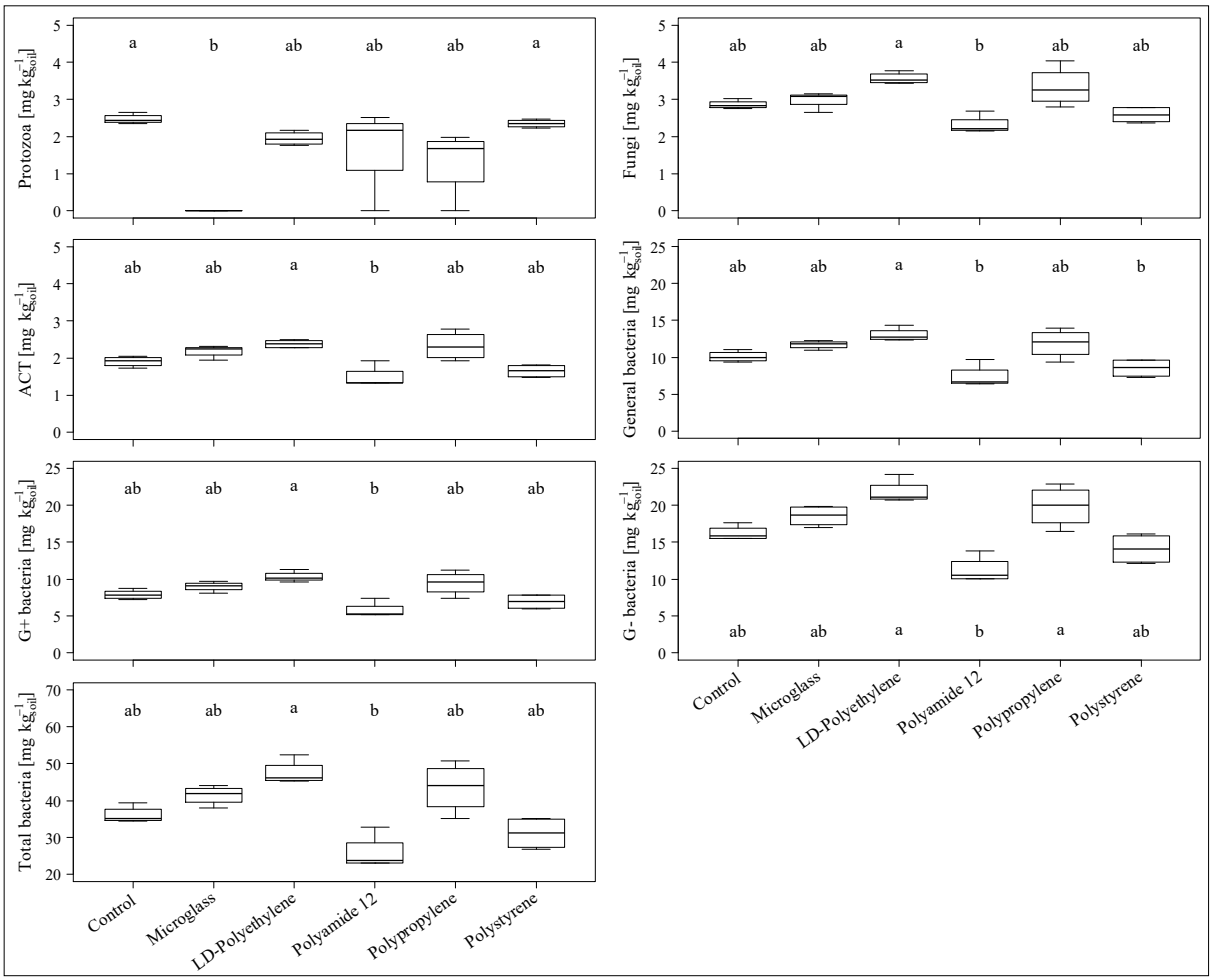

Figure 3. Microbial-derived phospholipid fatty acid contents of the individual taxonomic groups of an incubated Chernozem after 80 days. Different lowercase letters indicate significant differences according to a multiple comparison by Dunn's test (n=4, $p < 0.05$). Please note varying ordinate scales.