Particles under stress: Ultrasonication causes size and recovery rate artifacts with soil derived POM, but not with microplastics.

Frederick Büks[1], Gilles Kayser[2], Antonia Zieger[1], Friederike Lang[2], Martin Kaupenjohann[1]

[1]Chair of Soil Science, Dept. of Ecology, Technische Universität Berlin, 10587 Berlin, Germany
[2]Chair of Soil Ecology, University of Freiburg, 79085 Freiburg i.Br., Germany

Correspondence to: Frederick Büks (frederick.bueks@tu-berlin.de)

Author's response

Dear referee #1.

Many thanks for your mindful proofreading, the precise and very helpful comments. It has helped us to see some points which still need clarification. In the following, we want to explain how we propose to adjust our article based on the reviewer's comments and also explain why in some cases we do not agree with the reviewer's proposed changes. We also think that due to the added explanations the work exceeds the frame of a technical note or short communication. Our changes based on your suggestions are marked with green, referee #2 has pink, and our additional corrections are yellow.

Abstract

Line 11: delete "some"

[1] Done.

Introduction

Line 36: ultrasound is applied to a soil slurry by using a sonotrode
and Lines 36-37: "light" and "heavy" needs to be explained here

[2] We adjusted the Lines 36-37 „In studies on soil carbon pools, ultrasound is applied to a soil slurry to break down soil aggregates."

[3] and added the explanation of LF and HF (Line 38): „This disaggregation allows density fractionation of the free and occluded light fractions (fLF and oLF), which largely consist of material with densities below the fractionation medium, from the heavy fraction (HF), that has higher densities."

[4] Furthermore, „... and subsequent density fractionation of particulate organic matter ..." is added to Line 29 to introduce the fact that density fractionation is an integral part of the method.

Lines 38-42: split into two sentences

[5] Done.

Line 45: define "extractive performance" and give more reasoning why research in the field of soil contamination with microplastic is crucial.
and Line50: sentence is missing that connects this paragraph with the paragraph before

[6] We propose to split the paragraph at line 42 and rephrase and complement the following part: „Furthermore, the extracted POM fractions may not only contain the natural but also anthropogenic components such as microplastic. Recent studies reported soil microplastic concentrations between 1 mg kg^{-1} dry soil at less contaminated sites and 2 to 4 orders of magnitude above in samples from highly contaminated industrial areas (Fuller and Gautam, 2016; Rezaei et al., 2019). The agricultural application of sewage sludge, wastewater, compost as well as plastic mulching and the input of road and tire wear are discussed as important entry pathways to soils (Bläsing and Amelung, 2018). These origins of MP are characterized by a different composition of the size and shape of the extracted items (e.g. Zhang and Liu, 2018; Ding et al., 2020). In laboratory experiments, MP in the observed size range was shown to influence soil biogeochemical properties such as water holding capacity, soil structure, microbial activity and the health of soil biota, with strong dependence on the size and shape of the applied particles (de Souza Machado et al., 2018; Büks et al., 2020). Furthermore, the mobility within the soil pore space and preferencial flow channels, which is crucial for the accessibility of soil microplastic to ground and surface waters, is also highly dependent on particle size (O'Connor et al., 2019; Zubris and Richards, 2005). It is therefore a very topical task for both the impact assessment of given contaminations in landscapes and the design of robust experimental setups to have extraction methods with high yield and a low alteration of microplastic size and shape."

Line 49: "Büks et al., in review" is not a valid reference

[7] Now it is: Büks, F., van Schaik, N. L., and Kaupenjohann, M.: What do we know about how the terrestrial multicellular soil fauna reacts to microplastic?, SOIL, 6, 245–267, https://doi.org/10.5194/soil-6-245-2020, 2020.

Line63: punctuation mark is not at the correct spot

[8] Done.

Lines 91-93: Why do you assume this? You need to justify your assumption; otherwise, it is not a hypothesis. The phrase "we were curious" is a weak justification for doing research, provide here a solid hypotheses driven reasoning and provide prove for your claim that this has not studied before, based on what research (literature search?) you conclude this?

[9] We really agree with the author's point, that we did not provide a sufficient hypothesis and therefore propose to add a new paragraph after Line 91: „In advance to the treatment, the nine materials showed different mechanical stabilities. Unlike all six types of plastic particles, the occluded POMs and the pyrochar were easily to grind between two fingers and therefore assumed to be prone to ultrasonication. An examination of the recent literature on microplastic extraction from soils showed that the stability of microplastic in face of ultrasound has not been studied yet, neither with weathered nor juvenile material. Experiments with polymer-based adsorber resins indicated fractures on microbead surfaces after treatment with 100 J s^{-1} at 40 kHz for 70 minutes (Breitbach et al., 2002). When exposed to the environment,

plastic undergoes weathering by UV radiation, mechanical comminution, microbial decay and chemical alteration (Kale et al., 2015; Andrady et al., 2017), which leads to embrittlement (Quelle). We therefore hypothesized, that unweathered microplastic particles will be prone to ultrasonic treatment in a degree less than weathered microplastic and much less than pyrochar or natural oPOMs.“

Materials and Methods

Lines 114-119: why did you use different particle sizes for soil POM, char POM, and plastic POM, please justify because different particle sizes might affect the outcome.

[10] The different sizes of the particles are caused by their origin. Data show, that a high percentage of MP in soils is <250 µm (e.g. Zhang and Liu, 2018). However, in laboratory PE, PET and PBAT are not comminutable to those sizes in larger extent with a passable expenditure of time by cryo-milling (several days of milling with permanent application of liquid N_2) or any other known method. Alternatively, an extraction of MP from soils would not lead to pure or unweathered material and requires the treatment of tens of kg of soil. Pyrochar, in contrast, is comminuted to a similar size spectrum as the MP, but with slightly higher proportion of small particles, only by gentle pestling. The oPOM samples were extracted to represent the size spectrum in natural soils and have a higher proportion of both small and large particles compared to MP. However, from our point of view this would not alter the quality of the results: Based on the theory of statistical brittle fracture (which is also applied to soil aggregates by Braunack et al., 1979), particles of the same material are statistically more fragile faced to mechanical stress if they have larger size and, thus, a higher probability of flaws within their structure. We therefore assume that by use of particle size spectra similar to that of the plastic particles, pyrochar and oPOMs would show a more distinct degree of comminution. On the other hand, smaller MP is not expected to be comminuted as larger particle remain intact. The qualitative statement, that natural POMs/pyrochar are more prone to mechanical stress than MP and size/recovery artifacts are highly probable, would not be altered.

Lines 119-121: the weathering approach is not clear to me, justify and explain in more detail, and according to Table 1 and 2 only microplastic samples were weathered, please clarify this here.

[11] We propose to add to Line 121: “..., which is the international industry standard for testing artificial weathering of polymere-based textiles and coatings (Pickett, 2018).” This approach is applied for pre-treatment of MP in current experiments knowing that also microbial processes might play a role in weathering of soil MP (Kale et al., 2015). However, there is no established method including this, yet.

Line 125: why this stress levels, please justify your selection and why do you use J/ml and not the more common J/cm3 unit?
and Line 227 (Discussion): what about above 500 J/ml?

[12] Both units J ml^{-1} and J cm^3 are common. If it is really wished, we will change it to J cm^{-3}.
[13] For justification of the chosen energy levels, we propose to insert the following text after Line 125: “The treatment with 0 J ml^{-1} was used as a control with no mechanical agitation and 10 J ml^{-1} represents a gentle stimulation, which is suggested not to disaggregate soil structure (Kaiser and Berhe, 2014). Macroaggregates are prone to 50 J ml^{-1} and 100 to 500 J ml^{-1} mark

the range of microaggregate disaggregation, as many studies stated full disaggregation of soils after application of ~500 J ml^{-1} (Kaiser and Berhe, 2014). Larger values were ruled out, although some studies applied energy levels above 500 J ml^{-1}, like Pronk et al. (2011) who could show that silt-sized microaggregates were not dispersed at energy levels ≤800 J ml^{-1}. However, small microaggregates often contain little or no POM (Tisdall, 1996), and energies >710 J ml^{-1} cause physical damage on mineral particles (Kaiser and Berhe, 2014). Therefore we focus on the range of 0 to 500 J ml^{-1} as a safe space for the extraction of POM with no other known artifacts."

Line126: why 1% and 0.5%, please justify these amounts

[14] 1% is a low but common concentration of POM in soils as well as an amount of MP found in highly contaminated soils (Fuller and Gautam, 2016). We chose these amounts to use the POM economically on one hand and to use on the other hand enough material to find even small differences of the recovery rate. The use of only 0.5%, alas, is caused by an accident when the measurement had to be applied immediately. However, from our point of view, such slight differences in concentration would not affect the transmission of sound to the POM particles within the slurry. To account net weight differences, our data are in %.

Line 127: If you want to simulate the soil matrix, why did you used only fine sand and not a more heterogeneous mixture?

[15] We propose to add the following sentence into Line 127: "We chose acid-washed and calcinated fine sand to simulate the soil mineral matrix. This texture can be easily suspended by ultrasonication (coarse sand cannot), has a low tendency to coat POM or coagulate (like clay does) and shows a fast sedimentation when the sample is centrifuged. Fine sand, moreover, represents soils that originated from Weichselian sanders or aeolian sand deposition. In this methodical paper, our aim, however, was not to simulate a set of soil textures, but to have a proof of concept to find out if natural or artificial POM is damaged by ultrasonication. Then, quantities of 1 % w/w POM, and 0.5 % w/w in case of the oPOMs, were embedded into the fine sand matrix." An exact quantification of the degree of comminution goes beyond the scope of this, because it most probably depends not only on the texture, but also the degree of aggregation and the properties of occluded POM (as differences between forest and farm oLF showed. This will be part of a study in advance to this.

Results

Line 171: two times 100 J/ml

[16] The two "100 J/ml" refer to forest oPOM and pyrochar, respectively. We rearranged the sentence to make this more clear: "In sharp contrast, all other samples were decreasingly recovered along with increasing energy levels. Farmland POM, forest POM and pyrochar showed significant differences to the 0 J ml^{-1} treatment at ≥10 J ml^{-1}, ≥100 J ml^{-1} and ≥ 100 J ml^{-1}, respectively."

Figure 1 and Table 1 present the same data, so they are redundant, please remove Figure 1

[17] Removed.

For Table 1 and 2, from my prospective, a paired t-test requires independent samples but your samples are not independent (POM forest is from one soil, LD-PE from one plastic film, for example) based on that you can just state an increase or a decreaseor you go for mean values (per energy amount) from farm POM, arable POM, and py-rochar ("natural POM", n = 3) and mean values (per energy amount) from all plasticsamples ("microplastic POM", n = 6, this group could be further subdivided into weath-ered or not weathered), then energy amount or energy amount/ size ditribution can serve as factors in an ANOVA analysis,

[18] In this point we disagree with the referee. The 9 materials are independent samples. Both weathered and juvenile PE (e.g.) originated from the same raw material, but were differently treated in advance to the experiment (one was weathered, one not). In consequence, those are different collectives and all variants have 3 replicates and can be compared by use of a paired t-test. The comparison between the energy levels of all variants by an ANOVA is possible but not necessary, as our approach only focus on comparison between one energy level of a certain variant and its 0 J ml^{-1} control. This is adequately achieved by the t-test.

Captions for Figures 2 a and b: A, B, and C as well as mv need to be explained

[19] Done.

Discussion

Lines 181-195: this needs to be discussed in the light of the experimental settings, the artificial soil just contained POM and fine sand, how can these findings be applied to soils with much more clay or iron oxides?

[20] We deleted Lines 186-187 ("In consequence, particle size reduction will appear during most ultrasonic treatments aimed to extract oPOMs from soils."). Now the first paragraph is not that bold any more. Further points are mentioned in [15] (texture) and [25] (experimental settings).

Line 197: I do not really see a causal relationship here, please clarify

[21] We totally agree that, as we are not yet able to explain the underlying mechanism, causality cannot be stated, but only supposed. We therefore propose to alter Lines 196-198: "The concurrent decrease of particle size and recovery rate of soil derived POMs and pyrochar and its absence after ultrasonic treatment of microplastics might indicate a causal relationship of these measures. The underlying process, however, has not been studied before."

Line 199: this would mean that the fine sand particles form associations with small organic particles but I do not see any evidence for this or a paper cited that describes such phenomena, a reason might be that the density of natural POM is changing because of stronger solubilization processes of smaller particles in density solutions.
and Line 203: again, you only have mineral surface of fine sand particles, which are usually less involved in organic matter mineral associations, this needs to be clarified on a mechanistic level using appropriate literature if no own data can be used.
and Line 200: needs to be "specific surface area in cm2/g"

[22] Thank you very much for this interesting idea. After a new search for literature, we propose to replace the paragraph Line 199-207 with: "We assume a mechanism that prevents POM from density fractionation. This effect appeared in our experiment from energies around 50 J ml^{-1} with the beginning destruction of oPOM. Sparse data on molecular alteration of organic materials due to ultrasonication showed the transformation of lignin, a major constituent of plant cell walls. One hour of treatment caused the formation of a high molecular weight fraction of about 35% of the lignin content with molecular weights increased by the

450-fold (Wells et al., 2013). This may also increase the density of lignin and ligninoid fractions in soil POM towards the density of the fractionation medium and reduce their recovery rate."

[23] We also replaced the sentence in Lines 234-237 by: "Their smallest part, fibers and microfragments produced by physical, chemical and biological erosion within the soil, might also be affected by chemical alteration due to both weathering and ultrasonication causing enhanced retention in the sedimenting fraction."

Lines 206-207: why that? please provide more detailed explanations on potential effects on fPOM without any stress from sonication

[24] We deleted "and might also occur with small-sized fPOM during density fractionation without application of mechanical stress".

Lines 208-217: again, this is a very general statement but need to be seen in the perspective of your specific experimental settings, and what experiments would be necessary to get more general information

[25] We agree with you, that our statements have to be more specific and revised the paragraph in the following way: "As a consequence of the reduction of the recovery rate, farmland, forest and pyrochar POMs remain within a sandy matrix the stronger they are treated by ultrasound. If these findings are applied to ultrasonication/density fractionation of natural soils, not only an increasing number of particle size artifacts can be expected, but also the extraction of occluded POM is increasingly hindered at a certain energy level. After each extraction step, parts of the released oPOM remain within the heavy fraction, a carry-over artifact. This leads to an underestimation of the extracted oPOM fractions and an overestimation of the mineral-associated organic matter fraction (MOM), that natural part of the soil organic matter (SOM), which is adsorbed on mineral surfaces of the heavy fraction and mainly assumed to be molecular. According to our data, a reduction of recovery rates would appear at 10 J ml^{-1} in farmland soils and 100 J ml^{-1} in forest soils as well as at 100 J ml^{-1} when extracting pyrochar particles. Thus, the artifact would affect the extraction of oPOM from microaggregates of all samples and also the extraction of oPOM from macroaggregates in farmland soils. However, further research has to elucidate, if these results can be applied to natural soil samples."

Line 222: define "phenomenal influence"

[26] „phenomenal" deleted.

Lines 218-225: again, any recommendations how such effects could be minimized during fractionation.

[27] Unfortunately, we don't have. We propose to add after Line 225: "In respect to coming experiments, comminution and reduced recovery rate of the oPOM can possibly be avoided by not exceeding the energy levels mentioned here – or by determining a specific energy cut-off for each natural soil in preliminary studies. Regarding the application of higher energy levels, detailed investigation on the underlying mechanism are necessary to give such recommendations."

Line 226: again, very general statement, define "plastic"

[28] We added: „Microplastic particles, whether they are weathered following DIN ENISO4892-2/3 or pristine, are ..."

Line 227: I recommend to avoid statements like “no carry-over”, for such a bold statement the data are not sufficient

[29] We replaced the „no“ by „significantly less“.

Conclusion

[30] “... fractions only extractable with higher energy levels or were bound to ...” (Line 246) and “... at the mineral phase...” (Lines 250-251) deleted.

Best regards,

Dr. Frederick Büks
M.Sc. Gilles Kayser
M.Sc. Antonia Zieger
Prof. Dr. Friederike Lang
Prof. Dr. Martin Kaupenjohann

Additional references

Andrady, A.L.: The plastic in microplastics: a review, Mar. Pollut. Bull., 119, 12–22, https://doi.org/10.1016/j.marpolbul.2017.01.082, 2017

Bläsing, M. and Amelung, W.: Plastics in soil: Analytical methods and possible sources, Science of The Total Environment, 612, 422–435, https://doi.org/10.1016/j.scitotenv.2017.08.086, 2018.

Braunack, M., Hewitt, J. and Dexter, A.: Brittle fracture of soil aggregates and the compaction of aggregate beds, Journal of Soil Science, 30, 653--667, https://doi.org/10.1111/j.1365-2389.1979.tb01015.x, 1979.

Breitbach, M., Bathen, D., Schmidt-Traub, H. and Ebener, H.: Stability of adsorber resins under mechanical compression and ultrasonication, Polymers for Advanced Technologies, 13(5), 391-400, https://doi.org/10.1002/pat.203, 2002.

Büks, F., Loes van Schaik, N., and Kaupenjohann, M.: What do we know about how the terrestrial multicellular soil fauna reacts to microplastic?, SOIL, 6, 245–267, https://doi.org/10.5194/soil-6-245-2020, 2020.

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, Environmental Science & Technology, 52, 17, 9656–9665, https://doi.org/10.1021/acs.est.8b02212, 2018.

Fuller, S. and Gautam, A.: A procedure for measuring microplastics using pressurized fluid extraction, Environ. Sci. Technol., 50, 5774–5780, https://doi.org/10.1021/acs.est.6b00816, 2016.

Kale, S. K., Deshmukh, A. G., Dudhare, M. S., and Patil, V. B.: Microbial degradation of plastic: a review, J. Biochem. Technol., 6, 952–961, 2015.

Pickett, J. E. (2018). Weathering of plastics. In *Handbook of Environmental Degradation of Materials* (pp. 163-184). William Andrew Publishing.

Pronk, G. J., Heister, K., and Kögel-Knabner, I.: Iron Oxides as Major Available Interface Component in Loamy Arable Topsoils, Soil Science Society of America Journal, 75(6), 2158, https://doi.org/10.2136/sssaj2010.0455, 2011.

Rezaei, M., Riksen, M. J., Sirjani, E., Sameni, A., and Geissen, V.: Wind erosion as a driver for transport of light density microplastics, Sci. Total Environ., 669, 273–281, https://doi.org/10.1016/j.scitotenv.2019.02.382, 2019.

Tisdall, J.: Formation of soil aggregates and accumulation of soil organic matter, Structure and organic matter storage in agricultural soils, 57-96, 1996.

Wells, T., Kosa, M., & Ragauskas, A. J.: Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications, Ultrasonics Sonochemistry, 20(6), 1463–1469, https://doi.org/10.1016/j.ultsonch.2013.05.001, 2013.

Zubris, K. A. V., and Richards, B. K.: Synthetic fibers as an indicator of land application of sludge, Environmental Pollution, 138(2), 201–211, https://doi.org/10.1016/j.envpol.2005.04.013, 2005.

Dear referee #2.

Many thanks for your proofreading and your helpful comments. We added the requested data to our manuscript and supplements. In the following, we want to explain how we propose to improve our explanations as in your favor. Our changes based on your suggestions are marked with pink, referee #1 has green, and our additional corrections are yellow.

Comment 1: For oPOM and pyrochar, the recovery rate decreased with the increment of ultrasonication power. The cause was supposed to be an increase of new active surface to absorb the comminuted oPOM after disintegration of soil aggregates. However, this explanation is unlikely applicable to pyrochar.

[1] We hope that we understood your comment correctly. Pyrochar has an enormous internal surface, but we assume that also pyrochar receive a larger outer surface if particles are comminuted. However, based on the comment of referee #1 on the lack of evidence in literature regarding the association of small organic particles with sand grains we refraine from this explanation and instead added reply [22] to referee #1.

Comment 2: As mentioned in Ince (2001) and confirmed in Kaiser & Berhe (2014), ultrasonication induced high temperature may reduce total C content due to oxidative reactions. If this happens,the conclusion of "counting up to around 36.2 to 64.2 % of POM to the MOM" is really overestimated. I would like to know how much oPOM was lost and how much was transferred to MOM in this study.

That is a very interesting question, which is really improving our work. We did measurments in this regard and [2] added after line 164 into the material & methods section: "2.5 organic matter balance: A second set of triplicates of pyrochar and farm soil oPOM were treated similarly at 0 and 500 J/ml to balance the complement of the recovered POM. For this purpose, the C concentration within the lyophilized sediment was measured by use of a CNS analyzer and converted to POM mass by use of the C content (%) of the respective organic matter. In addition, the mass gain of the cellulose acetate filters was measured after rinsing the sample and drying the filter at 70°C for 24 hours. The DOC concentration of the filtrate was measured and converted to DOM by use of an assumed 50% C content. The difference of these and the recovered fractions compared to the initial weight of organic particles is termed the balance loss during the extraction procedure."

Corresponding to that, we [3] added the following to the resuls section after Line 179: "3.3 Mass loss: The treatment of pyrochar triplicates with 500 J/ml resulted in a recovery rate of 54.3±5.2 % after density fractionation. In turn, 34.9±3.7 % of the POM remained in the sediment, 0.6±0.1 % into the DOM fraction and <0.5 % onto the filter, leading to a balance loss of 10.2±2.1 % (Fig. 2). The respective data of farm oPOM are 54.6±1.9 %, 20.3±3.1 %, 5.1±0.2 %, <0.5 % and 20.0±1.5 %. Samples treated with 0 J/ml instead showed a significantly higher recovery rate and lower retention compared to the 500 J/ml samples. In contrast, the balance loss remained constant between 0 and 500 J/ml." The data are shown in an additional figure.

We furthermore [4] supplemented our comment [22] to referee #1 in the discussion section as follows: "We assume a mechanism that prevents POM from detection. This effect appeared in our experiment from energies around 50 J ml-1 with the beginning destruction of oPOM. As mentioned in Ince et al. (2001) and confirmed in Kaiser and Berhe (2014), ultrasonication induced high temperature may reduce total C content due to oxidative reactions, but the balance loss, constant between 0 and 500 J/ml in both pyrochar and farm oPOM, implies that there is no burning of organic matter due to ultrasound treatment. The recovery rate decreases in the same degree as the retention in the sediment increases when ultrasound is applied, while filter residues and lost DOM, which doubled on a low level, play a minor role. Extreme thermal conditions occuring during ultrasoincation, however, may explain the increased retention of POM within the sediment. Sparse data on molecular alteration ..."

Comment 3: "About 100 mg POM were suspended" for particle size analysis. However, the initial quantity of oPOM in each vessel is 20 g 0.5% = 100 mg. Therefore, with a recovery rate may be as low as 50%, it is unlikelyto get 100 mg of oPOM for particle size analysis.*

[5] We are sorry for this phrase has escaped our notice. It actually means "up to 100 mg" and refers to the plastic samples, which had an initial weight of 0.2 g and were recovered by nearly 100%. For pyrochar and the oPOMs the QicPic used a smaller amount according to the extracted matter. The actual range of sample weight is therefore "30 to 100 mg", which is to correct in line 149.

Comment 4: The farm and forest soils used for this experiment were from an organic horticulture and a spruce/beech mixed forest. However, soil organic C content was only 4.9 and 7.3 g kg-1. Please check these data.

[6] Thank you for your mindful reading. It is indeed 4.93% and 7.32% (or 49.3 mg/kg and 73.2 mg/kg) and will be corrected in Lines 100 and 102.

Comment 5: Is the weight of POM measured or the C content measured?

The recovery rates base on POM weights. That is because (1) this work focus on mass losses and (2) C analytic is destructive and would have doubled the operational effort with respect to the following particle sizing.

Comment 6: There are some grammar errors, including explanation of the calculation of CF.

We thoroughly reread our manuscript and corrected some grammatical errors that had escaped our notice.

Best regards,

Dr. Frederick Büks
M.Sc. Gilles Kayser
M.Sc. Antonia Zieger
Prof. Dr. Friederike Lang
Prof. Dr. Martin Kaupenjohann

Particles under stress: Ultrasonication causes size and recovery rate artifacts with soil derived POM, but not with microplastics.

Frederick Büks[1], Gilles Kayser[2], Antonia Zieger[1], Friederike Lang[2], Martin Kaupenjohann[1]

[1]Chair of Soil Science, Dept. of Ecology, Technische Universität Berlin, 10587 Berlin, Germany
[2]Chair of Soil Ecology, University of Freiburg, 79085 Freiburg i.Br., Germany

Correspondence to: Frederick Büks (frederick.bueks@tu-berlin.de)

Abstract. The breakdown of soil aggregates and the extraction of particulate organic matter (POM) by ultrasonication and density fractionation is a method widely used in soil organic matter (SOM) analyses. It has recently also been used for the extraction of microplastic from soil samples. However, the investigation of [1]~~some~~ POM physiochemical properties and ecological functions might be biased, if particles are comminuted during the treatment. In this work, different types of POM, which are representative for different terrestrial ecosystems and anthropogenic influences, were tested for their structural stability in face of ultrasonication in a range of 0 to 500 J ml^{-1}. The occluded particulate organic matter (oPOM) of an agricultural and forest soil as well as pyrochar showed a significant reduction of particle size at ≥50 J ml^{-1} by an average factor of 1.37±0.16 and a concurrent reduction of recovery rates by an average of 21.7±10.7 % when being extracted. Our results imply that increasing ultrasonication causes increasing retention of POM within the sedimenting phase leading to a misinterpretation of certain POM fractions as more strongly bound oPOM or part of the mineral-associated organic matter (MOM). This could e.g. lead to a false estimation of physical stabilization. In contrast, neither fresh nor weathered polyethylene (PE), polyethylene terephthalate (PET) and polybutylene adipate terephthalate (PBAT) microplastics showed a reduction of particle size or the recovery rate after application of ultrasound. We conclude that ultrasonication applied to soils has no impact on microplastic size distribution and thus provides a valuable tool for the assessment of microplastics in soils and soil aggregates.

1 Introduction

The mechanical disintegration of soil aggregates by use of ultrasonication following the method of Edwards and Bremner (1967a) [4]and subsequent density fractionation of particulate organic matter is widely used in the assessment of soil organic matter (SOM) stability. This includes characteristics such as aggregate composition and stability (Edwards and Bremner, 1967b), the constitution of SOM pools (Golchin et al., 1994), the stabilization of SOM in forest ecosystems (Graf-Rosenfellner et al., 2016) and the occlusive strength of particulate organic matter (POM) (Büks and Kaupenjohann, 2016). Ultrasonication is also applied to assess quantities and qualities of anthropogenic contaminants such as microplastics (Zhang and Liu, 2018; Zhang et al., 2018).

In studies on soil carbon pools, [2]ultrasound is applied to a soil slurry to break down soil aggregates. ~~sonotrodes are applied to break down soil aggregates and separate the free and occluded light fractions (fLF and oLF) from the heavy fraction (HF)~~. [3]The disaggregation allows density fractionation of the free and occluded light fractions (fLF and oLF), which largely consist of material with densities below the fractionation medium, from the heavy fraction (HF), that has higher densities. These operational fractions largely correspond to the free particulate organic matter (fPOM), the occluded particulate organic matter (oPOM) and the mineral-associated organic matter (MOM). [5]This organic matters are assigned to the labile, intermediate and stable carbon pool, respectively, and have turnover times of <1 year (labile) to several thousands of years (stable) (Lützow et al., 2007).

Furthermore, the extracted POM fractions may not only contain the natural but also anthropogenic components such as microplastic. [6]Recent studies reported soil microplastic concentrations between 1 mg kg^{-1} dry soil at less contaminated sites and 2 to 4 orders of magnitude above in samples from highly contaminated industrial areas (Fuller and Gautam, 2016; Rezaei et al., 2019). The agricultural application of sewage sludge, wastewater, compost as well as plastic mulching and the input of road and tire wear are discussed as important entry pathways to soils (Bläsing and Amelung, 2018). These origins of MP are characterized by a different composition of the size and shape of the extracted items (e.g. Zhang and Liu, 2018; Ding et al., 2020). In laboratory experiments, MP in the observed size range was shown to influence soil biogeochemical properties such as water holding capacity, soil structure, microbial activity and the health of soil biota, with strong dependence on the size and shape of the applied particles (de Souza Machado et al., 2018; Büks et al., 2020). Furthermore, the mobility within the soil pore space and preferencial flow channels, which is crucial for the accessibility of soil microplastic to ground and surface waters, is also highly dependent on particle size (O'Connor et al., 2019; Zubris and Richards, 2005). It is therefore a very topical task for both the impact assessment of given contaminations in landscapes and the design of robust experimental setups to have extraction methods with high yield and a low alteration of microplastic size and shape. ~~Its quantification and characterization is a very~~

~~topical task in the growing research on microplastic contamination of soils and requires a high extractive performance. In addition, when optical methods are used to determine the size and shape of the microplastic, the extraction should also cause the least possible damage to the extracted material, because both attributes provide information about the source (Zhang and Liu, 2018; Ding et al., 2020), the mobility within the soil pore space (O'Connor et al., 2019) and the ingestibility of microplastic by soil organisms (Büks et al., in review).~~

The common method of ultrasonication is carried out with a pieco-electric converter, that uses electric energy to generate axial vibration of a sonotrode, which is dipped into a flask containing a fluid and a submerged soil sample. The oscillating sonotrode emits acoustic pulses within the fluid. In front of the shock-waves the medium is compressed, and the increased pressure causes an increased gas solubility. Behind the wave the medium relaxes and the pressure drops below the normal level leading to an explosive outgassing (Ince et al., 2001). This so called cavitation effect produces lots of exploding micro-bubbles between particles and within cavities of the soil matrix generating very local pressure peaks of 200 to 500 atm accompanied by temperatures of 4200 to 5000 K (Ince et al., 2001). It provokes the detachment of physiochemical bondings between soil primary particles and soil aggregates and, thus, causes disaggregation. Depending on the type and settings of the device, the vibration frequency can vary up to 10000 kHz, but low frequencies around 20 to 100 kHz are recommended for soil aggregate dispersion to avoid chemical alteration of OM, and the use of 40 kHz is very common (Kaiser and Berhe, 2014; Graf-Rosenfellner et al., 2018)[8].

As an artifact of the method, ultrasonication is known to provide mechanical and thermal stress strong enough to comminute mineral particles at energy levels >700 J ml^{-1} (Kaiser and Berhe, 2014). Also, the destructive influence on POM was tested in different studies and appears even at energy levels much lower than 700 J ml^{-1}. Without application of a solid mineral matrix, Balesdent et al. (1991) found >60 % of the POM in suspension comminuted after application of 300 J ml^{-1}. Amelung and Zech (1999) treated natural soils with 0 to 1500 J ml^{-1} and performed a separation into size fractions of <20 µm, 20 to 250 µm and >250 µm. At ≥100 J ml^{-1} POM was transferred from the >250 µm to the <20 µm fraction. In a similar manner, Yang et al. (2009) measured the mass and SOC content of sand, silt and clay sized particle fractions in natural soils using an unconventional pulse/non-pulse ultrasonication technique. The authors derived the comminution of POM at >600 J ml^{-1}. Oorts et al. (2005) added ^{13}C-enriched straw to natural soils and could show that larger amounts of POM were redistributed at 450 J ml^{-1} when its degree of decomposition was higher. In conclusion, those studies consistently found a comminution of POM by ultrasonic treatment, which appears, however, at very different energy levels and is likely affected by the aggregation regime (suspended without mineral matrix, added as fPOM, occluded within natural soils), direct or indirect quantification of POM and the type of POM.

The aim of this work was to test how susceptible different POMs are to comminution by ultrasonic treatment under standardized conditions. We embedded three POMs (farm oPOM, forest oPOM and pyrochar, applied as an analog for soil black carbon and biochar amendments) and also six differently weathered microplastics (fresh and weathered low-density polyethylene (LD-PE), polyethylene terephthalate (PET) as well as polybutylene adipate terephthalate (PBAT), a common biodegradable material) into a fine sand matrix. Then, we treated these mixtures with 0, 10, 50, 100 and 500 J ml^{-1}, re-extracted the organic particles with density fractionation and measured their recovery rates and particle size distributions. The sand matrix was used only to simulate the influence of pore space on cavitation and, thus, our simplified approach excluded broadly varying POM–mineral interactions resulting from aggregation processes in natural soil samples.

[9]In advance to the treatment, the nine materials showed different mechanical stabilities. Unlike all six types of plastic particles, the occluded POMs and the pyrochar were easily to grind between two fingers and therefore assumed to be prone to ultrasonication. An examination of the recent literature on microplastic extraction from soils showed that the stability of microplastic in face of ultrasound has not been studied yet, neither with weathered nor juvenile material. Experiments with polymer-based adsorber resins indicated fractures on microbead surfaces after treatment with 100 J s^{-1} at 40 kHz for 70 minutes (Breitbach et al., 2002). When exposed to the environment, plastic undergoes weathering by UV radiation, mechanical comminution, microbial decay and chemical alteration (Kale et al., 2015; Andrady et al., 2017), which leads to embrittlement. We therefore hypothesized, that unweathered microplastic particles will be prone to ultrasonic treatment in a degree less than weathered microplastic and much less than pyrochar or natural oPOMs. ~~We hypothesized the strongest comminution in case of the two oPOMs, that already started to decomposed within their former natural soil matrix, and we were curious about the effect of ultrasonication and artificial weathering on the structural stability of microplastic, which has not been studied before.~~

2 Material and methods

2.1 Preparation of POM

The farm and forest oPOMs were extracted from air-dried soil aggregates of 630 to 2000 µm in diameter sampled in 10 to 20 cm depth from an organic horticulture near Oranienburg/Brandenburg (N 52° 46’ 54, E 13° 11’ 50, texture Ss, C_{org}=[6]~~4.9~~49.3 g kg^{-1}, pH 5.8) and a spruce/beech mixed forest near Bad Waldsee/Banden-Württemberg (N 47° 50' 59, E 9° 41' 30, texture Sl4, C_{org}=[6]~~7.3~~73.2 g kg^{-1}, pH 3.4). The extraction was performed by use of a density fractionation in 1.6 g cm^{-3} dense sodium polytungstate (SPT) solution: In 12-fold replication, 120 ml of SPT solution were added to 30 g of aggregates in a 200 ml PE bottle. The sample was stored for 1 h to allow the SPT solution to infiltrate the aggregates and was then centrifuged at 3500 G for 26 min. The floating free particulate organic matter (fPOM) was removed by use of a water jet pump and discarded. The remaining sample was refilled to 120 ml with SPT solution and sonicated for 30 sec (≈10 J ml^{-1}) by use of a sonotrode (Branson© Sonifier 250) in order to flaw the structure of macroaggregate (>250 µm). Then, centrifugation and removal of the oPOM were executed as for the fPOM. The gained oPOM was filtered off with an 0.45 µm cellulose acetate membrane filter, washed 3 to 5 times with 200 ml deionized water within the filter device until the rinse had an electrical conductivity of <50 µS cm^{-1}, removed from the filter by rinsing with deionized water, collected and gently dried for 48 h at 40°C. At the end, the oPOMs were sieved to 2000 µm, long-shaped residues were cut by a sharp knife, sieved again and pooled to one oPOM sample. The pyrogenic char sample (made from pine wood, pyrolysed at 850°C for 0.5 h by PYREG® GmbH) was dried for 24 h at 105°C, ground in a mortar and sieved to <630 µm. The microplastics (LD-PE, PET and PBAT) were made from plastic films by repeated milling (Fritsch Pulverisette 14) with liquid nitrogen and sieved to <500 µm. Then, half of each sample was weathered for 96 h at 38°C, 1000 W m^{-2} (solar spectrum, 280 to 3000 nm) and a relative air humidity of 50 % following DIN EN ISO 4892-2/3[11], which is the international industry standard for testing artificial weathering of polymere-based materials (Pickett, 2018).

2.2 Mechanical stress treatment

In order to test their stability against ultrasonication, the nine POM types (farm and forest oPOM and pyrochar as well as fresh and weathered LD-PE, PET and PBAT) were each exposed in triplicates to different mechanical stress levels (0, 10, 50, 100 and 500 J ml^{-1}). [13]The treatment with 0 J ml^{-1} was used as a control with no mechanical agitation and 10 J ml^{-1} represents a gentle stimulation, which is suggested not to disaggregate soil structure (Kaiser and Berhe, 2014). Macroaggregates are prone to 50 J ml^{-1}, and 100 to 500 J ml^{-1} mark the range of microaggregate disaggregation, as many studies stated full disaggregation of soils after application of ~500 J ml^{-1} (Kaiser and Berhe, 2014). Larger values were ruled out, although some studies applied energy levels above 500 J ml^{-1}, like Pronk et al. (2011) who could show that silt-sized microaggregates were not dispersed at energy levels ≤800 J ml^{-1}. However, small microaggregates often contain little or no POM (Tisdall, 1996), and energies >710 J ml^{-1} cause physical damage on mineral particles (Kaiser and Berhe, 2014). Therefore we focus on the range of 0 to 500 J ml^{-1} as a safe space for the extraction of POM with no other known artifacts.

[15]We chose acid-washed and calcinated fine sand to simulate the soil mineral matrix. This texture can be easily suspended by ultrasonication (coarse sand cannot), has a low tendency to coat POM or coagulate (like clay does) and shows a fast sedimentation when the sample is centrifuged. Fine sand, moreover, represents soils that originated from Weichselian sanders or aeolian sand deposition. In this methodical paper, our aim, however, was not to simulate a set of soil textures, but to have a proof of concept to find out if natural or artificial POM is damaged by ultrasonication. Then, quantities of 1 % w/w POM, and 0.5 % w/w in case of the oPOMs, were embedded into the fine sand matrix.

These artificial soils (each 20 g) were stored in 100 ml of 1.6 g cm^{-3} dense SPT solution for 1 h in 200 ml PE bottles, that did not show measurable release of plastic fragments due to sonication in preliminary tests with a pure fine sand matrix (data not shown). Mechanical stress was applied by use of a sonotrode (Branson© Sonifier 250) as described by Büks and Kaupenjohann (2016). The sonication times corresponding to 0, 10, 50, 100 and 500 J ml^{-1} were determined by means of the sonotrode's energy output calculated following North (1976). After the ultrasonic treatment, samples were centrifuged at 3500 G for 26 min. The floated POM was removed by use of a water-jet pump, separated and cleaned by rinsing with deionized water on a 0.45 µm cellulose acetate membrane filter until the electrical conductivity of the rinse went below 50 µS cm^{-1}, and then lyophilized.

2.3 Determination of recovery rates

After lyophilization, the recovery rate $R=m_t m_0^{-1}$ was determined by weighing and described as ratio of the recovered POM mass after treatment (m_t) to the initial POM mass (m_0) for all POM types and energy levels. ~~The recovery rates for each replicate were plotted over the energy levelsto show initial rates at 0 J ml^{-1} and the influence of the mechanical stress treatment increasingto 500Jml-1 (Fig.1).~~ The recovery rate at a certain energy level is assumed significantly different to the 0 J ml^{-1} level, if a pairwise t-test results in a $p<0.05$ (Table 1).

2.4 Measurement of particle sizes

All samples continued to be used for particle sizing. After pre-trials have shown that mainly the hydrophobic particles (microplastics and pyrochar) coagulated in distilled water, aggregation was avoided by suspension in 0.1 % w/v Tween© 20 detergent solution and vortexing following Katija et al. (2017). [5]~~About 100 mg~~30 to 100 mg of POM were suspended in 500 ml 0.1 % Tween© 20 solution and size classified with a QICPIC image analysis device (Sympatec GmbH, Clausthal-Zellerfeld, Germany) using a modified method from Kayser et al. (2019). Counts were grouped into 34 size classes from <5.64 µm to 1200–1826.94 µm and plotted as cumulative histograms of each replicate and their mean values (Fig. 1a and 1b). As the primary criterion for the reduction in particle size, the first 10 % and 50 % quantile (median) values were compared by pairwise t-test between 0 J ml^{-1} and each other energy level, respectively. As particle size reduction could be significant but still marginal in case of a low variance between parallels and ~~and~~ a low grade of comminution at the same time, the averaged comminution factor (CF) was introduced. It is defined as

$$CF = \frac{\sum_i \left(\frac{x_{0,i}}{x_i}\right)}{i} \quad (1)$$

with i the number of parallels, $x_{0,i}$ the quantile value of the 0 J ml^{-1} energy level and x_i the value of the compared energy level. A sample is then assumed significantly different to the 0 J ml^{-1} control and not marginal, if the p-value given by the t-test is <0.05 and the comminution factor is >1.1 for the 10 % quantile, the median or both, while its standard deviation is sd<|CF-1|. (Table 2)

[2]2.5 Organic matter balance

A second set of triplicates of pyrochar and farm soil oPOM were treated similarly at 0 and 500 J ml^{-1} to balance the complement of the recovered POM. For this purpose, the C concentration within the lyophilized sediment was measured by use of a CNS analyzer and converted to POM mass by use of the C content (%) of the respective organic matter. In addition, the mass gain of the cellulose acetate filters was measured after rinsing the sample and drying the filter at 70°C for 24 hours. The DOC concentration of the filtrate was measured and converted to DOM by use of an assumed 50 % C content. The difference of these and the recovered fractions compared to the initial weight of organic particles is termed the balance loss during the extraction procedure. (Table 3)

3 Results

3.1 Resulting recovery rates

All microplastic samples (LD-PE, PET and PBAT) show a constantly high recovery rate of about 97.1±2.5 % in average over the whole range of applied energy levels. [16]In sharp contrast, all other samples were decreasingly recovered along with increasing energy levels. Farmland POM, forest POM and pyrochar showed significant differences to the 0 J ml^{-1} treatment at ≥10 J ml^{-1}, ≥100 J ml^{-1} and ≥ 100 J ml^{-1}, respectively. ~~In sharp contrast, all soil derived POMs (farmland, forest) and pyrochar were decreasingly recovered along with increasing energy levels and had significant differences to the 0 J ml^{-1} treatment at ≥10 J ml^{-1}, ≥100 J ml^{-1} and ≥100 J ml^{-1}, respectively.~~ (Table 1)

former Fig. 1 removed.

Table 1: Recovery rates of natural POMs and microplastics from after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3). The (w) marks weathered plastics, mv mean value and sd standard deviation. Bold numbers are significantly different from the 0 J ml^{-1} treatment by $p<0.05$.

sample	recovery rate [%w/w]				
	0 J ml^{-1}	10 J ml^{-1}	50 J ml^{-1}	100 J ml^{-1}	500 J ml^{-1}
	mv sd	mv sd	mv sd	mv sd	mv sd
farm oPOM	95.0 ± 2.3	**80.8 ± 4.5**	**73.2 ± 6.1**	**72.3 ± 2.8**	**51.6 ± 7.2**
forest oPOM	89.3 ± 5.4	79.0 ± 5.1	76.9 ± 8.4	**67.8 ± 3.6**	**48.7 ± 5.4**
pyrochar	93.5 ± 10.1	84.6 ± 6.1	78.1 ± 2.5	**74.3 ± 1.9**	**63.8 ± 3.1**
LD-PE	96.9 ± 1.2	97.3 ± 1.0	95.8 ± 6.7	99.9 ± 1.9	99.2 ± 1.6
LD-PE (w)	93.9 ± 3.4	96.5 ± 1.2	96.6 ± 1.5	98.9 ± 3.0	97.8 ± 1.7
PET	98.6 ± 2.5	94.0 ± 1.6	98.7 ± 2.5	98.5 ± 2.0	94.3 ± 1.3
PET (w)	96.2 ± 2.5	95.4 ± 3.0	97.0 ± 2.0	95.5 ± 1.0	96.4 ± 3.3
PBAT	99.6 ± 2.5	99.5 ± 0.9	90.9 ± 13.8	98.3 ± 3.6	98.2 ± 0.9
PBAT (w)	97.7 ± 0.9	99.3 ± 1.9	96.8 ± 1.6	96.6 ± 1.7	99.3 ± 1.9

3.2 POM size distribution

None of the plastics shows a significant reduction of particle size due to ultrasonic treatment within the 10 % and 50 % quantile. In contrast, at ≥100 J ml^{-1} the particle size of farm and forest oPOM was significantly reduced compared to the 0 J ml^{-1} treatment in both quantiles. Ultrasonic treatment also causes a significant comminution of pyrochar, but of mainly the smaller fraction indicated by the 10 % quantile, which appeared at ≥50 J ml^{-1} and is only interrupted due to an outlier at 100 J ml^{-1}. The 50 % quantile data (median) remain insignificant. (Fig. 1a and 1b , Table 2)

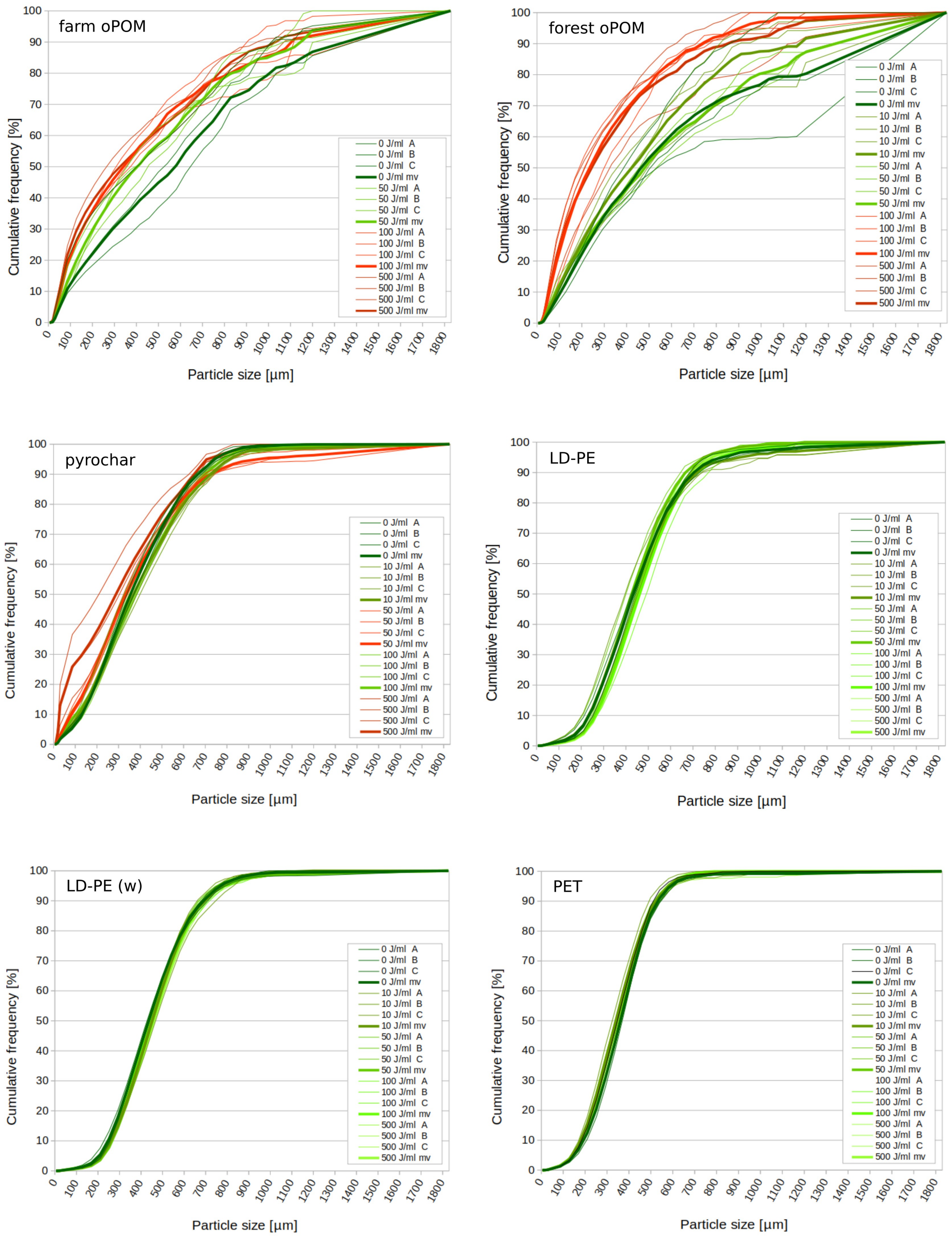

Figure 1a: Particle size distribution of natural POMs and microplastics after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3: [19]A, B, C). The (w) marks weathered plastics. Green graphs are similar to the 0 J ml^{-1} treatment, red graphs significantly different by $p<0.05$ and comminution factor >1.1. [19]Bold lines represent mean values (mv).

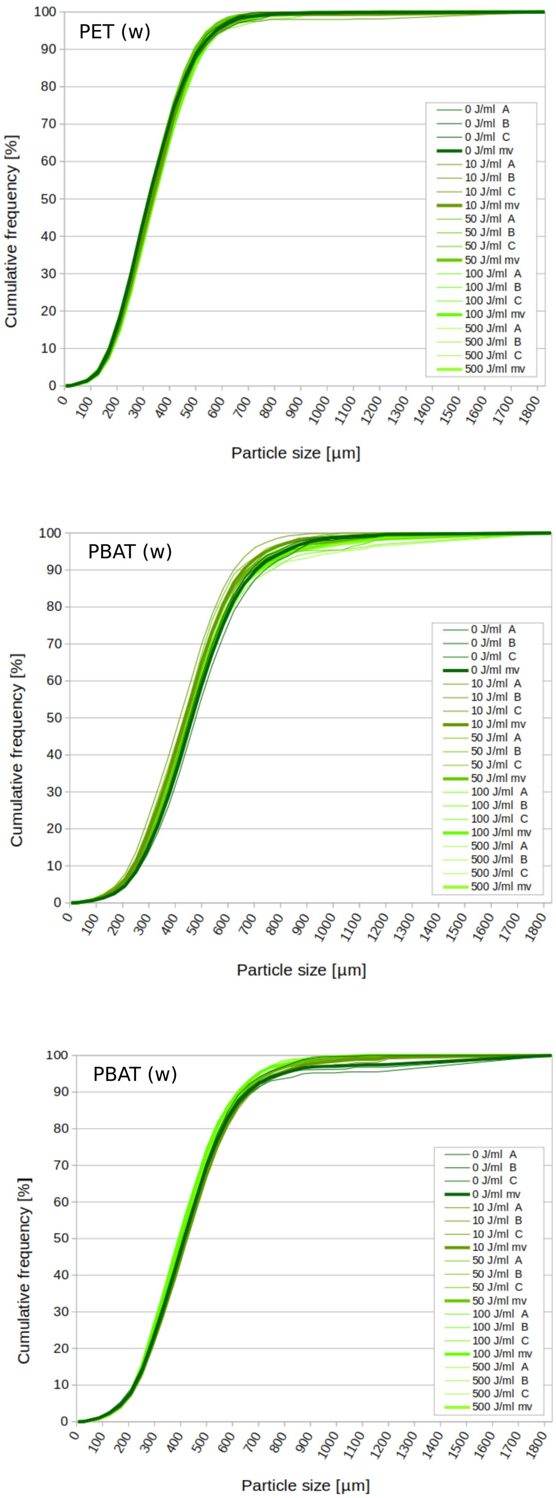

Figure 1b: Particle size distribution of microplastics after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3: [19]A, B, C). The (w) marks weathered plastics. Green graphs are similar to the 0 J ml^{-1} treatment (p≥0.05 or comminution factor ≤1.1). [19]Bold lines represent mean values (mv).

Table 2: Particle size distribution (10 % and 50 % quantile) and comminution factor of natural POMs and microplastics after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3). The (w) marks weathered plastics, mv mean value and sd standard deviation. Bold numbers are significantly different from the 0 J ml^{-1} treatment by p<0.05 and comminution factor >1.1.

		size distribution				comminution factor			
		10% quantile		50% quantile		10% quantile		50% quantile	
POM type	J/ml	mv	sd	mv	sd	mv	sd	mv	sd
farm oLF	0	82.90	± 9.46	561.33	± 72.98	1.00	± 0.00	1.00	± 0.00
	10	N/A		N/A		N/A		N/A	
	50	72.31	± 15.39	**401.40**	**± 47.86**	**1.17**	**± 0.15**	1.17	± 0.34
	100	**53.40**	**± 2.61**	**344.64**	**± 33.40**	**1.56**	**± 0.26**	**1.56**	**± 0.23**
	500	**47.21**	**± 2.46**	**365.57**	**± 52.18**	**1.76**	**± 0.21**	**1.76**	**± 0.23**
forest oLF	0	108.08	± 17.40	476.26	± 79.01	1.00	± 0.00	1.00	± 0.00
	10	91.71	± 11.04	422.27	± 68.13	1.19	± 0.27	1.17	± 0.36
	50	84.92	± 16.97	485.08	± 41.44	**1.28**	**± 0.09**	0.98	± 0.14
	100	**60.48**	**± 16.40**	**233.11**	**± 58.78**	**1.87**	**± 0.55**	**2.18**	**± 0.80**
	500	**55.49**	**± 13.01**	**244.41**	**± 70.33**	**1.98**	**± 0.28**	**2.02**	**± 0.48**
pyrochar	0	130.33	± 6.33	355.79	± 16.19	1.00	± 0.00	1.00	± 0.00
	10	119.09	± 16.07	369.18	± 39.01	1.10	± 0.11	0.97	± 0.15
	50	**81.39**	**± 10.07**	333.41	± 9.59	**1.62**	**± 0.25**	1.07	± 0.08
	100	103.37	± 33.73	371.92	± 19.99	1.34	± 0.38	0.96	± 0.09
	500	**31.18**	**± 11.70**	284.35	± 67.85	**4.59**	**± 1.67**	**1.30**	**± 0.28**
LD-PE	0	235.15	± 19.46	433.21	± 9.18	1.00	± 0.00	1.00	± 0.00
	10	236.54	± 29.80	432.25	± 31.43	1.00	± 0.06	1.01	± 0.06
	50	237.80	± 28.51	425.20	± 26.47	1.01	± 0.20	1.02	± 0.08
	100	263.23	± 6.87	463.10	± 24.59	0.89	± 0.05	0.94	± 0.03
	500	266.29	± 5.32	454.22	± 9.98	0.88	± 0.06	0.95	± 0.01
LD-PE (w)	0	245.69	± 15.39	435.02	± 6.41	1.00	± 0.00	1.00	± 0.00
	10	260.20	± 5.64	451.72	± 16.36	0.94	± 0.04	0.96	± 0.03
	50	265.51	± 1.55	**451.20**	**± 6.71**	0.93	± 0.06	0.96	± 0.03
	100	253.61	± 7.67	442.70	± 3.57	0.97	± 0.08	0.98	± 0.02
	500	262.94	± 3.25	**458.59**	**± 4.03**	0.93	± 0.06	0.95	± 0.02
PET	0	193.66	± 11.91	360.74	± 11.96	1.00	± 0.00	1.00	± 0.00
	10	180.15	± 7.97	339.89	± 13.84	1.08	± 0.12	1.06	± 0.07
	50	179.69	± 5.09	344.78	± 7.76	1.08	± 0.09	1.05	± 0.06
	100	162.59	± 29.24	**341.00**	**± 1.94**	**1.21**	**± 0.19**	1.06	± 0.04
	500	181.14	± 7.12	344.70	± 6.93	1.07	± 0.08	1.05	± 0.04
PET (w)	0	171.89	± 5.20	321.46	± 4.19	1.00	± 0.00	1.00	± 0.00
	10	186.44	± 11.60	332.81	± 7.80	0.92	± 0.07	0.97	± 0.01
	50	172.80	± 7.98	324.73	± 7.55	1.00	± 0.08	0.99	± 0.04
	100	**182.74**	**± 0.80**	**340.28**	**± 7.11**	0.94	± 0.03	0.95	± 0.03
	500	157.67	± 25.54	331.51	± 9.52	1.11	± 0.18	0.97	± 0.04
PBAT	0	263.19	± 6.13	464.20	± 11.93	1.00	± 0.00	1.00	± 0.00
	10	243.05	± 15.60	437.71	± 18.57	1.09	± 0.08	1.06	± 0.04
	50	**240.26**	**± 6.80**	441.55	± 9.41	1.10	± 0.04	1.05	± 0.05
	100	246.75	± 5.27	**455.51**	**± 5.37**	1.07	± 0.02	1.02	± 0.04
	500	**242.52**	**± 3.78**	452.18	± 11.85	1.09	± 0.04	1.03	± 0.05
PBAT (w)	0	223.53	± 6.06	413.87	± 4.60	1.00	± 0.00	1.00	± 0.00
	10	225.56	± 6.97	**423.06**	**± 2.81**	0.99	± 0.06	0.98	± 0.02
	50	225.22	± 2.92	414.68	± 8.41	0.99	± 0.04	1.00	± 0.02
	100	220.13	± 1.97	**396.85**	**± 6.20**	1.02	± 0.03	1.04	± 0.03
	500	224.71	± 5.53	404.80	± 12.40	1.00	± 0.03	1.02	± 0.04

[3]3.3 Mass loss

The treatment of pyrochar triplicates with 500 J ml^{-1} resulted in a recovery rate of 54.3±5.2 % after density fractionation. In turn, 34.9±3.7 % of the POM remained in the sediment, 0.6±0.1 % into the DOM fraction and <0.5 % onto the filter, leading to a balance loss of 10.2±2.1 % (Table 3). The respective data of farm oPOM are 54.6±1.9 %, 20.3±3.1 %, 5.1±0.2 %, <0.5 % and 20.0±1.5 %. Samples treated with 0 J ml^{-1} instead showed a significantly higher recovery rate and lower retention compared to the 500 J ml^{-1} samples. In contrast, the balance loss remained constant between 0 and 500 J ml^{-1}.

Table 3: Mass balance that indicates the fate of OM fractions during the ultrasonication/density fractionation treatment. Bold numbers indicate differences with $p<0.05$ after t-test between the 0 and 500 J ml^{-1} variant (n=3).

POM (energy level)	recovery (%)	retention (%)	filter (%)	DOM (%)	mass loss (%)
pyrochar (0 J ml^{-1})	**79.6±3.6**	**8.7±0.3**	<0.5	**0.3±0.0**	11.4±3.4
pyrochar (500 J ml^{-1})	**54.3±5.2**	**34.9±3.7**	<0.5	**0.6±0.1**	10.2±2.1
farm oPOM (0 J ml^{-1})	64.8±6.9	**8.3±0.2**	<0.5	**2.7±0.0**	24.1±6.8
farm oPOM (500 J ml^{-1})	54.6±1.9	**20.3±3.1**	<0.5	**5.1±0.2**	20.0±1.5

4 Discussion

Our experiments indicate that soil derived oPOM and pyrochar embedded into a fine sand matrix are prone to comminution by ultrasonic treatment at energy levels of ≥50 J ml^{-1}. These values are well below the 300 to 750 J ml^{-1} given in the literature for the complete disaggregation of various soils (Amelung and Zech, 1999; Oorts et al., 2006; Yang et al., 2009), namely in the range of values given for the destruction of macroaggregates (Amelung and Zech, 1999; Kaiser and Berhe, 2014). [20]~~In consequence, particle size reduction will appear during most ultrasonic treatments aimed to extract oPOMs from soils.~~ This underpins the former implications by some authors that ultrasonic treatment could lead to particle size artifacts. Microplastic, in contrast, shows a constant particle size distribution over all energy levels and seems to resist ultrasonication within the tested range of 0 to 500 J ml^{-1}. The recovery of microplastics also shows a constantly high rate of nearly 100 %, which is not affected by the applied energy. In sharp contrast, the recovery rates of soil derived POMs and pyrochar decreased with increasing energies from 95.0 to 78.6 % to 63.8 to 35.8 %, which became significant at 50 to 100 J ml^{-1} and therefore is quite parallel to observed size reduction.

~~The concurrent decrease of particle size and recovery rate of soil derived POMs and pyrochar and their absence in microplastics indicate, that there is a causal relationship between recovery rate and sensitivity against mechanical stress.~~ [21]The concurrent decrease of particle size and recovery rate of soil derived POMs and pyrochar and its absence after ultrasonic treatment of microplastics might indicate a causal relationship of these measures. The underlying process, however, has not been studied before. ~~We assume a mechanism that retains particles at the mineral phase after comminution. Physical disruption of large and weak particles increases the number of smaller ones, coming along with an increase of surface area and, thus, surface forces (e.g. attraction through charges or hydrophobic interaction) compared to volumetric forces (such as inertial forces). This causes an increased adsorption of small POM to mineral surfaces immediately after the ultrasonic treatment and, in consequence, a stronger retention of those particles observable as a lower recovery rate. This effect appeared in our experiment from energies around 50 J ml^{-1} with the beginning destruction of oPOM [24]and might also occur with small-sized fPOM during density fractionation without application of mechanical stress.~~ [22]We assume a mechanism that prevents POM from density fractionation. This effect appeared in our experiment from energies around 50 J ml^{-1} with the beginning destruction of oPOM. [4]As mentioned in Ince et al. (2001) and confirmed in Kaiser and Berhe (2014), ultrasonication induced high temperature may reduce total C content due to oxidative reactions, but the balance loss, constant between 0 and 500 J ml^{-1} in both pyrochar and farm oPOM, implies that there is no burning of organic matter due to ultrasound treatment. Also the formation of large amounts of water-soluble molecules and colloids could be ruled out in our experiment. The recovery rate decreases in the same degree as the retention in the sediment increases when ultrasound is applied, while filter residues and lost DOM, which doubled on a low level, play a minor role. Extreme thermal conditions occuring during ultrasoincation, however, may explain the increased retention of POM within the sediment. Sparse data on molecular alteration of organic materials due to ultrasonication showed the transformation of lignin, a major constituent of plant cell walls. One hour of treatment caused the formation of a high molecular weight fraction of about 35% of the lignin content with molecular weights increased by the 450-fold (Wells et al., 2013). This may also increase the density of lignin and ligninoid fractions in soil POM towards the density of the fractionation medium and reduce their recovery rate.

~~No matter if the hypothesis on the underlying mechanism is valid, as a consequence of concurrent recovery rate and particle size reduction, farmland, forest and pyrochar POMs remain within the soil sample the more they are disrupted by stronger ultrasonic treatment. Thus, not only particle size artifacts are produced. With increasing energy level the extraction of occluded POM is increasingly hindered and, thus, parts of small POM are extracted with oPOM fractions at higher energy levels or remain within the heavy fraction – a carry-over artifact. This leads to an overestimation of the more strongly bound POM fractions or the mineral-associated organic matter (MOM), that natural part of the soil organic matter (SOM), which is adsorbed on mineral surfaces of the heavy fraction and mainly assumed to be molecular.~~

[25]As a consequence of the reduction of the recovery rate, farmland, forest and pyrochar POMs remain within a sandy matrix the stronger they are treated by ultrasound. If these findings are applied to ultrasonication/density fractionation of natural soils, not only an increasing number of particle size artifacts can be expected, but also the extraction of occluded POM is increasingly hindered at a certain energy level. After each extraction step, parts of the released oPOM remain within the sedimenting fraction, a carry-over artifact. This leads to an underestimation of the extracted oPOM fractions and an overestimation of the mineral-associated organic matter fraction (MOM), that natural part of the soil organic matter (SOM), which is adsorbed on mineral surfaces of the heavy fraction and mainly assumed to be molecular. According to our data, a reduction of recovery rates would appear at 10 J ml^{-1} in farmland soils and 100 J ml^{-1} in forest soils as well as at 100 J ml^{-1} when extracting pyrochar particles. Thus, the artifact would affect the extraction of oPOM from microaggregates of all samples and also the extraction of oPOM from macroaggregates in farmland soils. However, further research has to elucidate, if these results can be applied to natural soil samples.

An overestimation would have an impact e.g. on the assessment of operationally defined carbon pools within landscapes: POM is assigned to carbon pools with turnover times orders of magnitude shorter then MOM, that endures hundreds of years. Malquantifications of these pools, such as counting ~~up to around 36.2 to 64.2 % of~~ POM to the MOM as implied by this work, would have [26]~~phenomenal~~ influence on e.g. the estimation of SOM decomposition and CO_2 emissions from land-use change. Carrying-over SOM from little to highly decomposed fractions also could alienate genuine C:N ratios, which strongly differ between the functional carbon pools (Wagai et al., 2009). [27]In respect to coming experiments, comminution and reduced recovery rate of the oPOM can possibly be avoided by not exceeding the energy levels mentioned here – or by determining a specific energy cut-off for each natural soil in preliminary studies. Regarding the application of higher energy levels, detailed investigation on the underlying mechanism are necessary to give such recommendations.

~~Plastic, in turn, is~~ [28]Microplastic particles, whether they are weathered following DIN ENISO4892-2/3 or pristine, are not prone to disruption by ultranonic treatment and its recovery rates are stable in a wide range of energy levels. We therefore assume that there will be ~~no~~ [29]significantly less carry-over of particles due to comminution when extracting microplastics from soils with ultrasonication/density fractionation. In consequence, the extractive performance is higher and subsequent particle size measurements give more valid information about the original particle size spectrum compared to the measurement of farmland, forest and pyrochar POM. This is a positive sign for research on soil microplastic, however, it does not mean that microplastic will be fully extracted from soils by this method. Soil microplastics appear within a wide range of sizes between some nanometers and its upper limit of 5 mm by definition. ~~Their smallest part, produced by physical, chemical and biological erosion within the soil, might also interact stronger with soil mineral surfaces than larger pieces causing enhanced retention onto the soil matrix.~~ [23]Their smallest part, fibers and microfragments produced by physical, chemical and biological erosion within the soil, might also be affected by chemical alteration due to both weathering and ultrasonication causing enhanced retention in the sedimenting fraction. Although we have introduced billions of tons of microplastics into ecosystems since the 1950s (Thompson et al., 2009; Geyer et al., 2017), there are still problems in producing microplastic fragments <100 µm on a laboratory scale with adequate use of time and material to perform experiments within this size range.

5 Conclusion

Unlike weathered and fresh PE, PET and PBAT microplastic, soil derived POMs like occluded POM from farm and forest soils and pyrochar concurrently show comminution and a reduced recovery rate after ultrasonication and subsequent extraction from a sandy matrix. ~~As comminution increases the retention,~~ Applied to natural soils, parts of the farmland, forest and pyrochar POM remain within [30]~~fractions only extractable with higher energy levels or were bound to~~ the ~~heavy~~ sedimenting fraction~~, so that they are misinterpreted as MOM~~ and can be misinterpreted as more strongly bound oPOM or MOM. An overestimation as shown in this study might lead to fundamentally different interpretations of physical protection of SOM, functional carbon pools and the expected mineralization rates in consequence of e.g. land-use change. On the contrary, the extraction of microplastics ~~does not~~ neither causes additional retention of particles [30]~~at the mineral phase~~ ~~and does not~~ nor alienates the particle size spectrum due to ultrasonic-driven comminution. We conclude that density fractionation in combination with ultrasonication is an appropriate tool for analyzing occlusion of microplastics within soil aggregates and studying the size distribution of particulate microplastics.

Author contribution

Frederick Büks developed the experimental concept, extracted all samples and prepared the manuscript. Gilles Kayser performed the particle size analysis. Antonia Zieger supported the development of the experimental concept. Martin Kaupenjohann and Friederike Lang supervised the whole study.

Acknowledgement

Many thanks to Zoltán Mátra, who kindly helped us to conduct the QICPIC analysis.

Competing interests

The authors declare that they have no conflict of interest.

References

Amelung, W. and Zech, W.: Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons, Geoderma, 92(1-2), 73–85, https://doi.org/10.1016/S0016-7061(99)00023-3, 1999.

Andrady, A.L.: The plastic in microplastics: a review, Mar. Pollut. Bull., 119, 12–22, https://doi.org/10.1016/j.marpolbul.2017.01.082, 2017

Balesdent, J., Pétraud, J. P. and Feller, C.: Effets des ultrasons sur la distribution granulométrique des matières organiques des sols, Science du sol, 29(2), 95–106, 1991.

Bläsing, M. and Amelung, W.: Plastics in soil: Analytical methods and possible sources, Science of The Total Environment, 612, 422–435, https://doi.org/10.1016/j.scitotenv.2017.08.086, 2018.

Breitbach, M., Bathen, D., Schmidt-Traub, H. and Ebener, H.: Stability of adsorber resins under mechanical compression and ultrasonication, Polymers for Advanced Technologies, 13(5), 391-400, https://doi.org/10.1002/pat.203, 2002.

Büks, F. and Kaupenjohann, M.: Enzymatic biofilm digestion in soil aggregates facilitates the release of particulate organic matter by sonication, Soil, 2(4), 499, https://doi.org/10.5194/soil-2-499-2016, 2016.

Büks, F., Loes van Schaik, N., and Kaupenjohann, M.: What do we know about how the terrestrial multicellular soil fauna reacts to microplastic?, SOIL, 6, 245–267, https://doi.org/10.5194/soil-6-245-2020, 2020.

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, Environmental Science & Technology, 52, 17, 9656–9665, https://doi.org/10.1021/acs.est.8b02212, 2018.

Ding, L., Zhang, S., Wang, X., Yang, X., Zhang, C., Qi, Y. and Guo, X.: The occurrence and distribution characteristics of microplastics in the agricultural soils of Shaanxi Province, in north-western China, Science of The Total Environment, 137525, https://doi.org/10.1016/j.scitotenv.2020.137525, 2020.

Edwards, A. and Bremner, J.: Dispersion of Soil Particles by Sonic Vibration, Journal of Soil Science, 18, 47–63, https://doi.org/10.1111/j.1365-2389.1967.tb01487.x, 1967a.

Edwards, A. P. and Bremner, J.: Microaggregates in Soils, Journal of Soil Science, 18, 64–73, https://doi.org/10.1111/j.1365-2389.1967.tb01488.x, 1967b.

Fuller, S. and Gautam, A.: A procedure for measuring microplastics using pressurized fluid extraction, Environ. Sci. Technol., 50, 5774–5780, https://doi.org/10.1021/acs.est.6b00816, 2016.

Geyer R., Jambeck J. R. and Law K. L.: Production, use, and fate of all plastics ever made, Science Advances, 3, e1700782, https://doi.org/10.1126/sciadv.1700782, 2017.

Golchin, A., Oades, J. M., Skjemstad, J. O. and Clarke, P.: Study of free and occluded particulate organic matter in soils by solid state ^{13}C CP/MAS NMR spectroscopy and scanning electron microscopy, Soil Research, 32(2), 285–309, https://doi.org/10.1071/SR9940285, 1994.

Graf-Rosenfellner, M., Cierjacks, A., Kleinschmit, B. and Lang, F.: Soil formation and its implications for stabilization of soil organic matter in the riparian zone, Catena, 139, 9–18, https://doi.org/10.1016/j.catena.2015.11.010, 2016.

Graf-Rosenfellner, M., Kayser, G., Guggenberger, G., Kaiser, K., Büks, F., Kaiser, M., Mueller, C. W., Schrumpf, M., Rennert, T., Welp, G. and Lang, F.: Replicability of aggregate disruption by sonication—an inter-laboratory test using three different soils from Germany, Journal of Plant Nutrition and Soil Science, 181(6), 894–*904,* https://doi.org/10.1002/jpln.201800152, 2018.

Ince, N., Tezcanli, G., Belen, R. and Apikyan, I. G.: Ultrasound as a catalyzer of aqueous reaction systems: the state of the art and environmental applications, Applied Catalysis B: Environmental, 29, 167–176, https://doi.org/10.1016/S0926-3373(00)00224-1, 2001.

Kaiser, M. and Berhe, A. A.: How does sonication affect the mineral and organic constituents of soil aggregates?—A review, J. Plant Nutr. Soil Sci., 177, 479–495, https://doi.org/10.1002/jpln.201300339, 2014.

Kale, S. K., Deshmukh, A. G., Dudhare, M. S., and Patil, V. B.: Microbial degradation of plastic: a review, J. Biochem. Technol., 6, 952–961, 2015.

Katija, K., Choy, C. A., Sherlock, R. E., Sherman, A. D. and Robison, B. H.: From the surface to the seafloor: How giant larvaceans transport microplastics into the deep sea, Science advances, 3(8), e1700715, https://doi.org/10.1126/sciadv.1700715, 2017.

Kayser, G., Graf-Rosenfellner, M., Schack-Kirchner, H. and Lang, F.: Dynamic imaging provides novel insight into the shape and stability of soil aggregates, *European Journal of Soil Science*, *70*(3), 454–465, https://doi.org/10.1111/ejss.12796, 2019.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., and Marschner, B.: SOM fractionation methods: relevance to functional pools and to stabilization mechanisms, Soil Biology and Biochemistry, 39(9), 2183–2207, https://doi.org/10.1016/j.soilbio.2007.03.007, 2007.

North, P.: Towards an absolute measurement of soil structural stability using ultrasound, Journal of Soil Science, 27, 451–459, https://doi.org/10.1111/j.1365-2389.1976.tb02014.x, 1976.

O'Connor, D., Pan, S., Shen, Z., Song, Y., Jin, Y., Wu, W. M. and Hou, D.: Microplastics undergo accelerated vertical migration in sand soil due to small size and wet-dry cycles, *Environmental pollution*, *249*, 527–534, https://doi.org/10.1016/j.envpol.2019.03.092, 2019.

Oorts, K., Vanlauwe, B., Recous, S. and Merckx, R.: Redistribution of particulate organic matter during ultrasonic dispersion of highly weathered soils, European Journal of Soil Science, 56(1), 77–91, https://doi.org/10.1111/j.1351-0754.2004.00654.x, 2005.

Pickett, J. E. (2018). Weathering of plastics. In *Handbook of Environmental Degradation of Materials* (pp. 163-184). William Andrew Publishing.

Pronk, G. J., Heister, K., and Kögel-Knabner, I.: Iron Oxides as Major Available Interface Component in Loamy Arable Topsoils, Soil Science Society of America Journal, 75(6), 2158, https://doi.org/10.2136/sssaj2010.0455, 2011.

Rezaei, M., Riksen, M. J., Sirjani, E., Sameni, A., and Geissen, V.: Wind erosion as a driver for transport of light density microplastics, Sci. Total Environ., 669, 273–281, https://doi.org/10.1016/j.scitotenv.2019.02.382, 2019.

Thompson R. C., Swan S. H., Moore C. J. and Vom Saal F. S.: Our plastic age, https://doi.org/10.1098/rstb.2009.0054, 2009.

Tisdall, J.: Formation of soil aggregates and accumulation of soil organic matter, Structure and organic matter storage in agricultural soils, 57-96, 1996.

Wagai, R., Mayer, L. M. and Kitayama, K.: Nature of the “occluded” low-density fraction in soil organic matter studies: a critical review, Soil Science and Plant Nutrition, 55(1), 13–25, https://doi.org/10.1111/j.1747-0765.2008.00356.x, 2009.

Wells, T., Kosa, M., & Ragauskas, A. J.: Polymerization of Kraft lignin via ultrasonication for high-molecular-weight applications, Ultrasonics Sonochemistry, 20(6), 1463–1469, https://doi.org/10.1016/j.ultsonch.2013.05.001, 2013.

Yang, X. M., Drury, C. F., Reynolds, W. D. and MacTavish, D. C.: Use of sonication to determine the size distributions of soil particles and organic matter, Canadian journal of soil science, 89(4), 413–419, https://doi.org/10.4141/cjss08063, 2009.

Zhang, G. S. and Liu, Y. F.: The distribution of microplastics in soil aggregate fractions in southwestern China. *Science of the Total Environment*, *642*, 12–20, https://doi.org/10.1016/j.scitotenv.2018.06.004, 2018.

Zhang, S., Yang, X., Gertsen, H., Peters, P., Salánki, T., and Geissen, V.: A simple method for the extraction and identification of light density microplastics from soil, Science of the Total Environment, 616, 1056–1065, https://doi.org/10.1016/j.scitotenv.2017.10.213, 2018.

Zubris, K. A. V., and Richards, B. K.: Synthetic fibers as an indicator of land application of sludge, Environmental Pollution, 138(2), 201–211, https://doi.org/10.1016/j.envpol.2005.04.013, 2005.