Particles under stress: Ultrasonication causes size and recovery rate artifacts with soil derived POM,

and recovery rate artifacts with soil
 but not with microplastics.

4 Frederick Büks¹, Gilles Kayser², Antonia Zieger¹, Friederike Lang², Martin Kaupenjohann¹

⁵ ¹Chair of Soil Science, Dept. of Ecology, Technische Universität Berlin, 10587 Berlin, Germany

6 ²Chair of Soil Ecology, University of Freiburg, 79085 Freiburg i.Br., Germany

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

Abstract. The breakdown of soil aggregates and the extraction of particulate organic matter 8 9 (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 10 11 soil samples. However, the investigation of POM physiochemical properties and ecological 12 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 13 anthropogenic influences, were tested for their structural stability in face of ultrasonication in a 14 range of 0 to 500 J ml⁻¹. The occluded particulate organic matter (oPOM) of an agricultural 15 16 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 17 of 21.7±10.7 % when being extracted. Our results imply that increasing ultrasonication causes 18 19 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 20 21 organic matter (MOM). This could e.g. lead to a false estimation of physical stabilization. In 22 contrast, neither fresh nor weathered polyethylene (PE), polyethylene terephthalate (PET) 23 and polybutylene adipate terephthalate (PBAT) microplastics showed a reduction of particle size or the recovery rate after application of ultrasound. We conclude that ultrasonication 24 25 applied to soils has no impact on microplastic size distribution and thus provides a valuable 26 tool for the assessment of microplastics in soils and soil aggregates.

27 **1 Introduction**

28 The mechanical disintegration of soil aggregates by use of ultrasonication following the 29 method of Edwards and Bremner (1967a) and subsequent density fractionation of particulate 30 organic matter is widely used in the assessment of soil organic matter (SOM) stability. This 31 includes characteristics such as aggregate composition and stability (Edwards and Bremner, 32 1967b), the constitution of SOM pools (Golchin et al., 1994), the stabilization of SOM in forest 33 ecosystems (Graf-Rosenfellner et al., 2016) and the occlusive strength of particulate organic 34 matter (POM) (Büks and Kaupenjohann, 2016). Ultrasonication is also applied to assess 35 quantities and qualities of anthropogenic contaminants such as microplastics (Zhang and Liu, 36 2018; Zhang et al., 2018).

37 In studies on soil carbon pools, ultrasound is applied to a soil slurry to break down soil 38 aggregates. 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 39 40 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 41 42 organic matter (oPOM) and the mineral-associated organic matter (MOM). This organic 43 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., 44 45 2007).

46 Furthermore, the extracted POM fractions may not only contain the natural but also anthropogenic components such as microplastic. Recent studies reported soil microplastic 47 concentrations between 1 mg kg⁻¹ dry soil at less contaminated sites and 2 to 4 orders of 48 magnitude above in samples from highly contaminated industrial areas (Fuller and Gautam, 49 2016; Rezaei et al., 2019). The agricultural application of sewage sludge, wastewater, 50 compost as well as plastic mulching and the input of road and tire wear are discussed as 51 52 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. 53 Zhang and Liu, 2018; Ding et al., 2020). In laboratory experiments, MP in the observed size 54 range was shown to influence soil biogeochemical properties such as water holding capacity, 55 soil structure, microbial activity and the health of soil biota, with strong dependence on the 56 57 size and shape of the applied particles (de Souza Machado et al., 2018; Büks et al., 2020). 58 Furthermore, the mobility within the soil pore space and preferencial flow channels, which is 59 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 60 a very topical task for both the impact assessment of given contaminations in landscapes and 61 62 the design of robust experimental setups to have extraction methods with high yield and a low 63 alteration of microplastic size and shape.

64 The common method of ultrasonication is carried out with a pieco-electric converter, that uses 65 electric energy to generate axial vibration of a sonotrode, which is dipped into a flask 66 containing a fluid and a submerged soil sample. The oscillating sonotrode emits acoustic 67 pulses within the fluid. In front of the shock-waves the medium is compressed, and the 68 increased pressure causes an increased gas solubility. Behind the wave the medium relaxes 69 and the pressure drops below the normal level leading to an explosive outgassing (Ince et 70 al., 2001). This so called cavitation effect produces lots of exploding micro-bubbles between 71 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 72 provokes the detachment of physiochemical bondings between soil primary particles and soil 73 aggregates and, thus, causes disaggregation. Depending on the type and settings of the 74 device, the vibration frequency can vary up to 10000 kHz, but low frequencies around 75 76 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., 77 78 2018).

79 As an artifact of the method, ultrasonication is known to provide mechanical and thermal 80 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 81 appears even at energy levels much lower than 700 J ml⁻¹. Without application of a solid 82 mineral matrix, Balesdent et al. (1991) found >60 % of the POM in suspension comminuted 83 84 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 85 >250 μ m. At >100 J ml⁻¹ POM was transferred from the >250 μ m to the <20 μ m fraction. In a 86 87 similar manner, Yang et al. (2009) measured the mass and SOC content of sand, silt and clay 88 particle fractions in natural soils using an unconventional pulse/non-pulse sized 89 ultrasonication technique. The authors derived the comminution of POM at >600 J ml⁻¹. Oorts 90 et al. (2005) added ¹³C-enriched straw to natural soils and could show that larger amounts of 91 POM were redistributed at 450 J ml⁻¹ when its degree of decomposition was higher. In 92 conclusion, those studies consistently found a comminution of POM by ultrasonic treatment. 93 which appears, however, at very different energy levels and is likely affected by the 94 aggregation regime (suspended without mineral matrix, added as fPOM, occluded within natural soils), direct or indirect quantification of POM and the type of POM. 95

96 The aim of this work was to test how susceptible different POMs are to comminution by 97 ultrasonic treatment under standardized conditions. We embedded three POMs (farm oPOM, 98 forest oPOM and pyrochar, applied as an analog for soil black carbon and biochar 99 amendments) and also six differently weathered microplastics (fresh and weathered low-100 density polyethylene (LD-PE), polyethylene terephthalate (PET) as well as polybutylene 101 adipate terephthalate (PBAT), a common biodegradable material) into a fine sand matrix.

3

102 Then, we treated these mixtures with 0, 10, 50, 100 and 500 J ml⁻¹, re-extracted the organic 103 particles with density fractionation and measured their recovery rates and particle size 104 distributions. The sand matrix was used only to simulate the influence of pore space on 105 cavitation and, thus, our simplified approach excluded broadly varying POM–mineral 106 interactions resulting from aggregation processes in natural soil samples.

107 In advance to the treatment, the nine materials showed different mechanical stabilities. Unlike 108 all six types of plastic particles, the occluded POMs and the pyrochar were easily to grind 109 between two fingers and therefore assumed to be prone to ultrasonication. An examination of 110 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 111 112 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., 113 2002). When exposed to the environment, plastic undergoes weathering by UV radiation, 114 mechanical comminution, microbial decay and chemical alteration (Kale et al., 2015; Andrady 115 et al., 2017), which leads to embrittlement. We therefore hypothesized, that unweathered 116 117 microplastic particles will be prone to ultrasonic treatment in a degree less than weathered microplastic and much less than pyrochar or natural oPOMs. 118

119 2 Material and methods

120 **2.1 Preparation of POM**

The farm and forest oPOMs were extracted from air-dried soil aggregates of 630 to 2000 µm 121 from an organic 122 in diameter sampled in 10 to 20 cm depth horticulture near Oranienburg/Brandenburg (N 52° 46' 54, E 13° 11' 50, texture Ss, C_{org} =49.3 g kg⁻¹, pH 5.8) 123 and a spruce/beech mixed forest near Bad Waldsee/Banden-Württemberg (N 47° 50' 59, 124 E 9° 41' 30, texture SI4, Cora=73.2 g kg⁻¹, pH 3.4). The extraction was performed by use of a 125 density fractionation in 1.6 g cm⁻³ dense sodium polytungstate (SPT) solution: In 12-fold 126 replication, 120 ml of SPT solution were added to 30 g of aggregates in a 200 ml PE bottle. 127 128 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 129 130 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 ($\approx 10 \text{ J ml}^{-1}$) by use of a sonotrode 131 132 (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 133 was filtered off with an 0.45 µm cellulose acetate membrane filter, washed 3 to 5 times with 134 200 ml deionized water within the filter device until the rinse had an electrical conductivity of 135 <50 µS cm⁻¹, removed from the filter by rinsing with deionized water, collected and gently 136 137 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 138 sample (made from pine wood, pyrolysed at 850°C for 0.5 h by PYREG[®] GmbH) was dried for 139 24 h at 105°C, ground in a mortar and sieved to <630 μ m. The microplastics (LD-PE, PET) 140 and PBAT) were made from plastic films by repeated milling (Fritsch Pulverisette 14) with 141 liquid nitrogen and sieved to $<500 \mu m$. Then, half of each sample was weathered for 96 h at 142 38°C, 1000 W m⁻² (solar spectrum, 280 to 3000 nm) and a relative air humidity of 50 % 143 144 following DIN EN ISO 4892-2/3, which is the international industry standard for testing 145 artificial weathering of polymere-based materials (Pickett, 2018).

146 **2.2 Mechanical stress treatment**

In order to test their stability against ultrasonication, the nine POM types (farm and forest 147 oPOM and pyrochar as well as fresh and weathered LD-PE, PET and PBAT) were each 148 149 exposed in triplicates to different mechanical stress levels (0, 10, 50, 100 and 500 J ml⁻¹). The treatment with 0 J ml⁻¹ was used as a control with no mechanical agitation and 10 J ml⁻¹ 150 represents a gentle stimulation, which is suggested not to disaggregate soil structure (Kaiser 151 and Berhe, 2014). Macroaggregates are prone to 50 J ml⁻¹, and 100 to 500 J ml⁻¹ mark the 152 range of microaggregate disaggregation, as many studies stated full disaggregation of soils 153 154 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 \leq 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.

We chose acid-washed and calcinated fine sand to simulate the soil mineral matrix. This 161 162 texture can be easily suspended by ultrasonication (coarse sand cannot), has a low tendency 163 to coat POM or coagulate (like clay does) and shows a fast sedimentation when the sample is 164 centrifuged. Fine sand, moreover, represents soils that originated from Weichselian sanders 165 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 166 167 damaged by ultrasonication. Then, guantities of 1 % w/w POM, and 0.5 % w/w in case of the oPOMs, were embedded into the fine sand matrix. 168

These artificial soils (each 20 g) were stored in 100 ml of 1.6 g cm⁻³ dense SPT solution for 169 170 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 171 172 stress was applied by use of a sonotrode (Branson[©] Sonifier 250) as described by Büks and 173 Kaupenjohann (2016). The sonication times corresponding to 0, 10, 50, 100 and 500 J ml⁻¹ 174 were determined by means of the sonotrode's energy output calculated following North 175 (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 176 deionized water on a 0.45 µm cellulose acetate membrane filter until the electrical 177 conductivity of the rinse went below 50 μ S cm⁻¹, and then lyophilized. 178

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 (m₀) for all POM types and energy levels. The recovery rate of 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).

184 **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). 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 189 GmbH, Clausthal-Zellerfeld, Germany) using a modified method from Kayser et al. (2019). 190 Counts were grouped into 34 size classes from <5.64 µm to 1200–1826.94 µm and plotted as 191 192 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) 193 194 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 195 196 variance between parallels and a low grade of comminution at the same time, the averaged comminution factor (CF) was introduced. It is defined as 197

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

- x

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)

204 **2.5 Organic matter balance**

A second set of triplicates of pyrochar and farm soil oPOM were treated similarly at 0 and 205 500 J ml⁻¹ to balance the complement of the recovered POM. For this purpose, the C 206 207 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 208 addition, the mass gain of the cellulose acetate filters was measured after rinsing the sample 209 and drving the filter at 70°C for 24 hours. The DOC concentration of the filtrate was measured 210 and converted to DOM by use of an assumed 50 % C content. The difference of these and 211 212 the recovered fractions compared to the initial weight of organic particles is termed the 213 balance loss during the extraction procedure. (Table 3)

214 3 Results

215 **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. 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. (Table 1)

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.

	recovery rate [% w/w]							
sample	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			

221 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 \geq 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 \geq 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: 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. 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: A, B, C). The (w) marks weathered plastics. Green graphs are similar to the 0 J ml⁻¹ treatment ($p \ge 0.05$ or comminution factor ≤ 1.1). 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% guantile	50% guantile	10% quantile	50% quantile	
POM type	J ml⁻¹	mv ± sd	mv ± sd	mv ± sd	mv ± sd	
	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	
farm oPOM	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	331.88 ± 69.03	1.76 ± 0.21	1.76 ± 0.23	
forest oPOM	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	
	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	
pyrochar	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	
	0	235.15 ± 19.46	433.21 ± 9.18	1.00 ± 0.00	1.00 ± 0.00	
LD-PE	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	
	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	
LD-PE (w)	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	
	0	193.66 ± 11.91	360.74 ± 11.96	1.00 ± 0.00	1.00 ± 0.00	
PET	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	
	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	
PBAT	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	
	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	
PBAT (w)	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	

229 3.3 Mass loss

The treatment of pyrochar triplicates with 500 J ml⁻¹ resulted in a recovery rate of 54.3 \pm 5.2 % after density fractionation. In turn, 34.9 \pm 3.7 % of the POM remained in the sediment, 0.6 \pm 0.1 % into the DOM fraction and <0.5 % onto the filter, leading to a balance loss of 10.2 \pm 2.1 % (Table 3). The respective data of farm oPOM are 54.6 \pm 1.9 %, 20.3 \pm 3.1 %, 5.1 \pm 0.2 %, <0.5 % and 20.0 \pm 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

237 4 Discussion

238 Our experiments indicate that soil derived oPOM and pyrochar embedded into a fine sand 239 matrix are prone to comminution by ultrasonic treatment at energy levels of \geq 50 J ml⁻¹. These 240 values are well below the 300 to 750 J ml⁻¹ given in the literature for the complete 241 disaggregation of various soils (Amelung and Zech, 1999; Oorts et al., 2006; Yang et al., 242 2009), namely in the range of values given for the destruction of macroaggregates (Amelung 243 and Zech, 1999; Kaiser and Berhe, 2014). This underpins the former implications by some 244 authors that ultrasonic treatment could lead to particle size artifacts. Microplastic, in contrast, 245 shows a constant particle size distribution over all energy levels and seems to resist 246 ultrasonication within the tested range of 0 to 500 J ml⁻¹. The recovery of microplastics also 247 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 248 249 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 guite parallel to observed size reduction. 250

251 The concurrent decrease of particle size and recovery rate of soil derived POMs and pyrochar 252 and its absence after ultrasonic treatment of microplastics might indicate a causal relationship 253 of these measures. The underlying process, however, has not been studied before. We 254 assume a mechanism that prevents POM from density fractionation. This effect appeared in 255 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 256 257 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 258 that there is no burning of organic matter due to ultrasound treatment. Also the formation of 259 large amounts of water-soluble molecules and colloids could be ruled out in our experiment. 260 261 The recovery rate decreases in the same degree as the retention in the sediment increases 262 when ultrasound is applied, while filter residues and lost DOM, which doubled on a low level, 263 play a minor role. Extreme thermal conditions occuring during ultrasoincation, however, may 264 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 265 major constituent of plant cell walls. One hour of treatment caused the formation of a high 266 molecular weight fraction of about 35% of the lignin content with molecular weights increased 267 by the 450-fold (Wells et al., 2013). This may also increase the density of lignin and ligninoid 268 269 fractions in soil POM towards the density of the fractionation medium and reduce their 270 recovery rate.

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 275 276 released oPOM remain within the sedimenting fraction, a carry-over artifact. This leads to an 277 underestimation of the extracted oPOM fractions and an overestimation of the mineral-278 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 279 280 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 281 282 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, 283 further research has to elucidate, if these results can be applied to natural soil samples. 284

285 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 286 287 of magnitude shorter then MOM, that endures hundreds of years. Malguantifications of these pools, such as counting POM to the MOM as implied by this work, would have influence on 288 289 e.g. the estimation of SOM decomposition and CO₂ emissions from land-use change. 290 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). In 291 292 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 293 294 specific energy cut-off for each natural soil in preliminary studies. Regarding the application of 295 higher energy levels, detailed investigation on the underlying mechanism are necessary to 296 give such recommendations.

Microplastic particles, whether they are weathered following DIN ENISO4892-2/3 or pristine, 297 298 are not prone to disruption by ultranonic treatment and its recovery rates are stable in a wide 299 range of energy levels. We therefore assume that there will be significantly less carry-over of 300 particles due to comminution when extracting microplastics from soils with ultrasonication/density fractionation. In consequence, the extractive performance is higher 301 302 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. 303 304 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 305 wide range of sizes between some nanometers and its upper limit of 5 mm by definition. Their 306 307 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 308 and ultrasonication causing enhanced retention in the sedimenting fraction. Although we have 309 introduced billions of tons of microplastics into ecosystems since the 1950s (Thompson et al., 310 311 2009; Gever et al., 2017), there are still problems in producing microplastic fragments

- <100 μ m on a laboratory scale with adequate use of time and material to perform experiments
- 313 within this size range.

314 **5 Conclusion**

315 Unlike weathered and fresh PE, PET and PBAT microplastic, soil derived POMs like occluded 316 POM from farm and forest soils and pyrochar concurrently show comminution and a reduced 317 recovery rate after ultrasonication and subsequent extraction from a sandy matrix. Applied to 318 natural soils, parts of the farmland, forest and pyrochar POM remain within the sedimenting 319 fraction and can be misinterpreted as more strongly bound oPOM or MOM. An overestimation 320 as shown in this study might lead to fundamentally different interpretations of physical 321 protection of SOM, functional carbon pools and the expected mineralization rates in 322 consequence of e.g. land-use change. On the contrary, the extraction of microplastics neither causes additional retention of particles nor alienates the particle size spectrum due to 323 324 ultrasonic-driven comminution. We conclude that density fractionation in combination with ultrasonication is an appropriate tool for analyzing occlusion of microplastics within soil 325 326 aggregates and studying the size distribution of particulate microplastics.

327 Author contribution

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

332 Acknowledgement

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

334 Competing interests

335 The authors declare that they have no conflict of interest.

336 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

342 Balesdent, J., Pétraud, J. P. and Feller, C.: Effets des ultrasons sur la distribution 343 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.

347 Breitbach, M., Bathen, D., Schmidt-Traub, H. and Ebener, H.: Stability of adsorber resins 348 under mechanical compression and ultrasonication, Polymers for Advanced Technologies, 349 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 ¹³C 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.

387 Kaiser, M. and Berhe, A. A.: How does sonication affect the mineral and organic constituents aggregates?—A 388 of soil review. J. Plant Nutr. Soil Sci., 177, 479-495. https://doi.org/10.1002/jpln.201300339, 2014. 389

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.

402 North, P.: Towards an absolute measurement of soil structural stability using ultrasound,
403 Journal of Soil Science, 27, 451–459, https://doi.org/10.1111/j.1365-2389.1976.tb02014.x,
404 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.

408 Oorts, K., Vanlauwe, B., Recous, S. and Merckx, R.: Redistribution of particulate organic 409 matter during ultrasonic dispersion of highly weathered soils, European Journal of Soil 410 Science, 56(1), 77–91, https://doi.org/10.1111/j.1351-0754.2004.00654.x, 2005.

- 411 Pickett, J. E. (2018). Weathering of plastics. In *Handbook of Environmental Degradation of*412 *Materials* (pp. 163-184). William Andrew Publishing.
- 413 Pronk, G. J., Heister, K., and Kögel-Knabner, I.: Iron Oxides as Major Available Interface
 414 Component in Loamy Arable Topsoils, Soil Science Society of America Journal, 75(6), 2158,
 415 https://doi.org/10.2136/sssaj2010.0455, 2011.
- 416 Rezaei, M., Riksen, M. J., Sirjani, E., Sameni, A., and Geissen, V.: Wind erosion as a driver 417 for transport of light density microplastics, Sci. Total Environ., 669, 273–281, 418 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.

438Zubris, K. A. V., and Richards, B. K.: Synthetic fibers as an indicator of land application of439sludge,EnvironmentalPollution,138(2),201–211,

440 https://doi.org/10.1016/j.envpol.2005.04.013, 2005.