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

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

1 Introduction

The mechanical disintegration of soil aggregates by use of ultrasonication following the method of Edwards and Bremner (1967a) 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, sonotrodes are applied to break down soil aggregates and separate the free and occluded light fractions (fLF and oLF) from the heavy fraction (HF). These operational fractions correspond largely to the free particulate organic matter (fPOM), the occluded particulate organic matter (oPOM) and the mineral-associated organic matter (MOM), which 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. 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 device type and settings, 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)

64   As an artifact of the method, ultrasonication is known to provide mechanical and thermal
65   stress strong enough to comminute mineral particles at energy levels >700 J ml^{-1} (Kaiser and
66   Berhe, 2014). Also, the destructive influence on POM was tested in different studies and
67   appears even at energy levels much lower than 700 J ml^{-1}. Without application of a solid
68   mineral matrix, Balesdent et al. (1991) found >60 % of the POM in suspension comminuted
69   after application of 300 J ml^{-1}. Amelung and Zech (1999) treated natural soils with
70   0 to 1500 J ml^{-1} and performed a separation into size fractions of <20 µm, 20 to 250 µm and
71   >250 µm. At ≥100 J ml^{-1} POM was transferred from the >250 µm to the <20 µm fraction. In a
72   similar manner, Yang et al. (2009) measured the mass and SOC content of sand, silt and clay
73   sized particle fractions in natural soils using an unconventional pulse/non-pulse
74   ultrasonication technique. The authors derived the comminution of POM at >600 J ml^{-1}. Oorts
75   et al. (2005) added ^{13}C-enriched straw to natural soils and could show that larger amounts of
76   POM were redistributed at 450 J ml^{-1} when its degree of decomposition was higher. In
77   conclusion, those studies consistently found a comminution of POM by ultrasonic treatment,
78   which appears, however, at very different energy levels and is likely affected by the
79   aggregation regime (suspended without mineral matrix, added as fPOM, occluded within
80   natural soils), direct or indirect quantification of POM and the type of POM.

81   The aim of this work was to test under standardized conditions how susceptible different
82   POMs are to comminution by ultrasonic treatment. We embedded three POMs (farm oPOM,
83   forest oPOM and pyrochar, applied as an analog for soil black carbon and biochar
84   amendments) and also six differently weathered microplastics (fresh and weathered low-
85   density polyethylene (LD-PE), polyethylene terephthalate (PET) as well as polybutylene
86   adipate terephthalate (PBAT), a common biodegradable material) into a fine sand matrix.
87   Then, we treated these mixtures with 0, 10, 50, 100 and 500 J ml^{-1}, re-extracted the organic
88   particles with density fractionation and measured their recovery rates and particle size
89   distributions. The sand matrix was used only to simulate the influence of pore space on
90   cavitation and, thus, our simplified approach excluded broadly varying POM–mineral
91   interactions resulting from aggregation processes in natural soil samples. We hypothesized
92   the strongest comminution in case of the two oPOMs, that already started to decomposed
93   within their former natural soil matrix, and we were curious about the effect of ultrasonication
94   and artificial weathering on the structural stability of microplastic, which has not been studied
95   before.

2 Material and methods

2.1 Preparation of POM

98 The farm and forest oPOMs were extracted from air-dried soil aggregates of 630 to 2000 µm
99 in diameter sampled in 10 to 20 cm depth from an organic horticulture near
100 Oranienburg/Brandenburg (N 52° 46' 54, E 13° 11' 50, texture Ss, C_{org}=4.9 g kg^{-1}, pH 5.8)
101 and a spruce/beech mixed forest near Bad Waldsee/Banden-Württemberg (N 47° 50' 59,
102 E 9° 41' 30, texture Sl4, C_{org}=7.3 g kg^{-1}, pH 3.4), respectively. The extraction was performed
103 by use of a density fractionation in 1.6 g cm^{-3} dense sodium polytungstate (SPT) solution: In
104 12-fold replication, 120 ml of SPT solution were added to 30 g of aggregates in a 200 ml PE
105 bottle. The sample was stored for 1 h to allow the SPT solution to infiltrate the aggregates and
106 was then centrifuged at 3500 G for 26 min. The floating free particulate organic matter (fPOM)
107 was removed by use of a water jet pump and discarded. The remaining sample was refilled to
108 120 ml with SPT solution and sonicated for 30 sec (≈10 J ml^{-1}) by use of a sonotrode
109 (Branson© Sonifier 250) in order to flaw the structure of macroaggregate (>250 µm). Then,
110 centrifugation and removal of the oPOM were executed as for the fPOM. The gained oPOM
111 was filtered off with an 0.45 µm cellulose acetate membrane filter, washed 3 to 5 times with
112 200 ml deionized water within the filter device until the rinse had an electrical conductivity of
113 <50 µS cm^{-1}, removed from the filter by rinsing with deionized water, collected and gently
114 dried for 48 h at 40°C. At the end, the oPOMs were sieved to 2000 µm, long-shaped residues
115 were cut by a sharp knife, sieved again and pooled to one oPOM sample. The pyrogenic char
116 sample (made from pine wood, pyrolysed at 850°C for 0.5 h by PYREG® GmbH) was dried for
117 24 h at 105°C, ground in a mortar and sieved to <630 µm. The microplastics (LD-PE, PET
118 and PBAT) were made from plastic films by repeated milling (Fritsch Pulverisette 14) with
119 liquid nitrogen and sieved to <500 µm. Then, half of each sample was weathered for 96 h at
120 38°C, 1000 W m^{-2} (solar spectrum, 280 to 3000 nm) and a relative air humidity of 50 %
121 following DIN EN ISO 4892-2/3.

2.2 Mechanical stress treatment

123 In order to test their stability against ultrasonication, the nine POM types (farm and forest
124 oPOM and pyrochar as well as fresh and weathered LD-PE, PET and PBAT) were each
125 exposed in triplicates to different mechanical stress levels (0, 10, 50, 100 and 500 J ml^{-1}).
126 Therefore, 1 % w/w POM, and 0.5 % w/w in case of the oPOMs, were embedded into an acid-
127 washed and calcinated fine sand matrix, which simulates the soil mineral matrix. These
128 artificial soils (each 20 g) were stored in 100 ml of 1.6 g cm^{-3} dense SPT solution for 1 h in
129 200 ml PE bottles, that did not show measurable release of plastic fragments due to
130 sonication in preliminary tests with a pure fine sand matrix (data not shown). Mechanical
131 stress was applied by use of a sonotrode (Branson© Sonifier 250) as described by Büks and

132 Kaupenjohann (2016). The sonication times corresponding to 0, 10, 50, 100 and 500 J ml⁻¹
133 were determined by means of the sonotrode's energy output calculated following North
134 (1976). After the ultrasonic treatment, samples were centrifuged at 3500 G for 26 min. The
135 floated POM was removed by use of a water-jet pump, separated and cleaned by rinsing with
136 deionized water on a 0.45 µm cellulose acetate membrane filter until the electrical
137 conductivity of the rinse went below 50 µS cm⁻¹, and then lyophilized.

2.3 Determination of recovery rates

139 After lyophilization, the recovery rate $R = m_t\, m_0^{-1}$ was determined by weighing as the ratio of
140 the recovered POM mass after treatment (m_t) to the initial POM mass (m_0) for all POM types
141 and energy levels. The recovery rates for each replicate were plotted over the energy levels
142 to show initial rates at 0 J ml⁻¹ and the influence of the mechanical stress treatment increasing
143 to 500 J ml⁻¹ (Fig. 1). The recovery rate at a certain energy level is assumed significantly
144 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

146 All samples continued to be used for particle sizing. After pre-trials have shown that mainly
147 the hydrophobic particles (microplastics and pyrochar) coagulated in distilled water,
148 aggregation was avoided by suspension in 0.1 % w/v Tween© 20 detergent solution and
149 vortexing following Katija et al. (2017). About 100 mg of POM were suspended in 500 ml
150 0.1 % Tween© 20 solution and size classified with a QICPIC image analysis device
151 (Sympatec GmbH, Clausthal-Zellerfeld, Germany) using a modified method from Kayser et al.
152 (2019). Counts were grouped into 34 size classes from <5.64 µm to 1200–1826.94 µm and
153 plotted as cumulative histograms of each replicate and their mean values (Fig. 2a and 2b). As
154 the primary criterion for the reduction in particle size, the first 10 % and 50 % quantile
155 (median) values were compared by pairwise t-test between 0 J ml⁻¹ and each other energy
156 level, respectively. As particle size reduction could be significant but still marginal in case of a
157 low variance between parallels and and a low grade of comminution at the same time, the
158 averaged comminution factor (CF) was introduced. It is defined as

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

160 with i the number of parallels, $x_{0,i}$ the quantile value of the 0 J ml⁻¹ energy level and x_i the
161 value of the compared energy level. A sample is then assumed significantly different to the
162 0 J ml⁻¹ control and not marginal, if the p-value given by the t-test is <0.05 and the

163 comminution factor is >1.1 for the 10 % quantile, the median or both, while its standard
164 deviation is sd<|CF-1|. (Table 2)

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. 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. (Fig. 1 , Table 1)
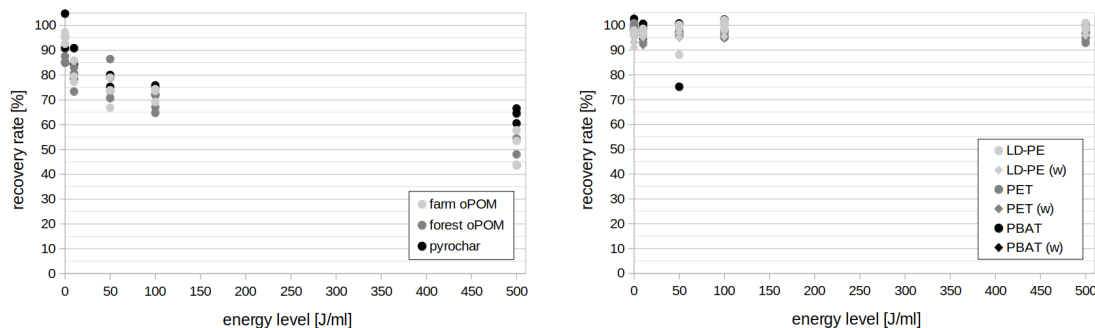
Figure 1: Recovery rates of natural POMs (left) and microplastics (right) after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3). The (w) marks weathered plastics.

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} mv sd	10 J ml^{-1} mv sd	50 J ml^{-1} mv sd	100 J ml^{-1} mv sd	500 J ml^{-1} 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. 2a and 2b , Table 2)
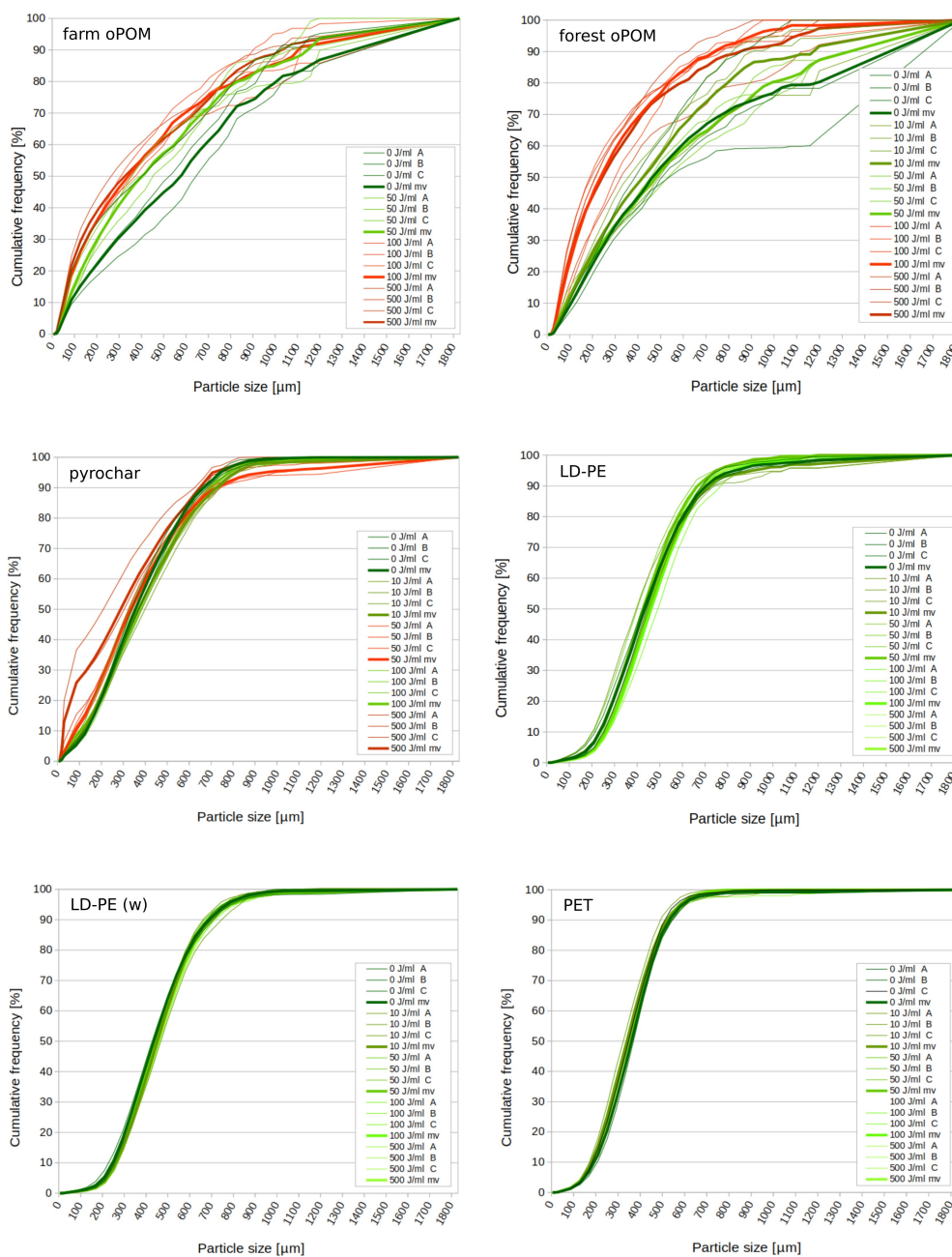
Figure 2a: Particle size distribution of natural POMs and microplastics after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3). The (w) marks weathered plastics. Green graphs are similar to the 0 J ml^{-1} treatment, red graphs significantly different by p<0.05 and comminution factor >1.1.
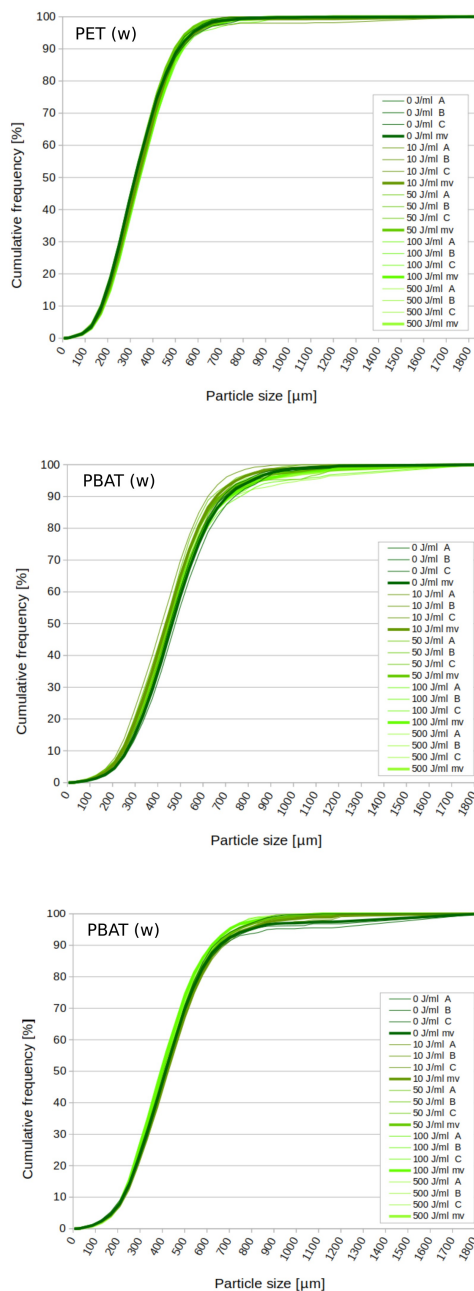
Figure 2b: Particle size distribution of microplastics after ultrasonic treatment with 0, 10, 50, 100 and 500 J ml^{-1} (n=3). The (w) marks weathered plastics. Green graphs are similar to the 0 J ml^{-1} treatment (p≥0.05 or comminution factor ≤1.1).

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	

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). 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 95.0–78.6 % to 63.8–35.8 %, 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. 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 and might also occur with small-sized fPOM during density fractionation without application of mechanical stress.

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.

218 An overestimation would have an impact e.g. on the assessment of operationally defined
219 carbon pools within landscapes: POM is assigned to carbon pools with turnover times orders
220 of magnitude shorter then MOM, that endures hundreds of years. Malquantifications of these
221 pools, such as counting up to around 36.2 to 64.2 % of POM to the MOM as implied by this
222 work, would have phenomenal influence on e.g. the estimation of SOM decomposition and
223 CO_2 emissions from land-use change. Carrying-over SOM from little to highly decomposed
224 fractions also could alienate genuine C:N ratios, which strongly differ between the functional
225 carbon pools (Wagai et al., 2009).

226 Plastic, in turn, is not prone to disruption by ultranonic treatment and its recovery rates are
227 stable in a wide range of energy levels. We therefore assume that there will be no carry-over
228 of particles due to comminution when extracting microplastics from soils with
229 ultrasonication/density fractionation. In consequence, the extractive performance is higher
230 and subsequent particle size measurements give more valid information about the original
231 particle size spectrum compared to the measurement of farmland, forest and pyrochar POM.
232 This is a positive sign for research on soil microplastic, however, it does not mean that
233 microplastic will be fully extracted from soils by this method. Soil microplastics appear within a
234 wide range of sizes between some nanometers and its upper limit of 5 mm by definition. Their
235 smallest part, produced by physical, chemical and biological erosion within the soil, might also
236 interact stronger with soil mineral surfaces than larger pieces causing enhanced retention
237 onto the soil matrix. Although we have introduced billions of tons of microplastics into
238 ecosystems since the 1950s (Thompson et al., 2009; Geyer et al., 2017), there are still
239 problems in producing microplastic fragments <100 µm on a laboratory scale with adequate
240 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, parts of the farmland, forest and pyrochar POM remain within fractions only extractable with higher energy levels or were bound to the heavy fraction, so that they are misinterpreted as 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 do not cause additional retention of particles at the mineral phase and do not alienate the particle size spectrum by 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

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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.

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Competing interests

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The authors declare that they have no conflict of interest.

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