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A simple method for the extraction and identification of microplastic from soil

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Abstract: This article introduces a simple and cost-saving method developed to extract, distinguish and quantify microplastics in soil. A floatation method using distilled water was used to extract light density polyethylene microplastics from soil samples. Microplastics and impurities were identified using a heating method (3-5 seconds at 130℃). The number and size of particles were determined using a camera (Leica DFC 425) connected to a microscope (Leica wild M3C, Type S, simple light, 6.4X). Quantification of the microplastics was conducted using a developed model. Results showed that the floatation method was effective in extracting microplastics from soils, with recovery rates of approximately 90%. After being exposed to heat, the microplastics in the soil samples melted and were transformed into circular transparent particles while other impurities, such as organic matter and silicates were not changed by the heat. Regression analysis of microplastic weight and particle volume after heating showed the best fit (R^2 =99%, p<0.001). Recovery rates based on the empirical model method were over 80%. Results from field samples collected from North-western China prove that our method of repetitive floatation and heating can be used to extract, distinguish and quantify light density polyethylene microplastics in soils. Microplastic mass can be evaluated using the empirical model.

Key words: Light density polyethylene microplastics; soil; extraction; identification;

1. Introduction:

Disposable plastic is heavily used world-wide but unfortunately, not always recycled (Hidalgo-Ruz et al., 2012; Steinmetz et al., 2016; Yan et al.). Generally, plastic, in its many forms, ends up as debris in the environment (Yan et al., 2015). It is widely deposited and dispersed in water, sediment and soil, which threatens the sustainable development of healthy ecosystems for generations (Cooper and Corcoran, 2010; Hidalgo-Ruz et al., 2012). Recently, many studies have focused on the adverse effects of plastics in the aquatic environment

(Hidalgo-Ruz et al., 2012; Wang et al., 2016b). Microplastics are small particles < 5 mm derived from plastic from different origin and composition such as polyethylene, polypropylene, polysterol etc. They are insoluble in water, nondegradable (according to standardized tests) and possess different physicochemical properties which are key aspects that determine its bioavailability to organisms (Leslie, 2014; Rocha-Santos and Duarte, 2015).

Many studies focus on the fate and effect of microplastics in the aquatic environment whereas there is a gap of knowledge on microplastics in the terrestrial environment. Plastics can enter into the terrestrial ecosystem by areal deposition, transport from landfills, sludge application to agricultural land and the application of light density polyethylene (LDPE) plastic film as mulch on agricultural land. Plastic mulch application to millions of ha worldwide with increasing tendency has let to plastic pollution of the terrestrial environment, however no monitoring on the occurrence of microplastics in the agricultural soils exists and no analytical techniques are described for their extraction from soils and identification. Their fate in the terrestrial environment is unknown and only few studies exist on the effect on soil organisms (Huerta et al. 2016, Huerta et al. 2017) and the accumulation in the terrestrial food chain (Huerta et al. 2017).

However, studies from the aquatic environment indicated that the external adsorption of microplastics could cause harm to algal species in water mainly due to the microplastics inhibiting the process of photosynthesis (Bhattacharya et al., 2010; Wright et al., 2013). Some studies found that microplastics are ingested by aquatic fauna, leading to their death and/or accumulate in the food chain possibly resulting in death (Hirai et al., 2011; (Hidalgo-Ruz et al., 2012), Lwanga et al., 2016; Van Cauwenberghe et al., 2015b; Welden and Cowie, 2016). Studies have also shown that organic pollutants can be attracted to microplastics (Brady and Weil, 2000; Frias et al., 2010; Gall and Thompson, 2015; Hirai et al., 2011; Leslie, 2014; Seltenrich; Yan et al.). In some cases, diethyl phthalate is added to plastic mulch during the production process (Zhang et al., 2014b), and thus the use of this mulch introduces potentially toxic substances directly into the environment.

It is a challenge to study the fate and effects of microplastics in the terrestrial environment, especially in agricultural soils where plastic mulch is applied.

It is obvious how important it is to quantify microplastic content and distribution, especially in agricultural soils where it could provide the crucial data needed for refining the

agricultural use of LDPE plastic mulch and cleaning up production processes (Leslie, 2014; Steinmetz et al., 2016).

In recent years, several methods have been developed to detect microplastics in water, focusing mainly on polyethylene (PE) and polypropylene Polymers (PP), and extract them from sediments (Hidalgo-Ruz et al., 2012; Wang et al., 2016b). A floating method using saturated solutions of NaCl, NaI, $ZnCl_2$, or sodium polytungstate (SPT) is usually used to extract plastics from the sediment of water bodies due to the differences in density(Hidalgo-Ruz et al., 2012; Imhof et al., 2012; Nuelle et al., 2014b). Microplastics are easily extracted from sediment containing few impurities and few organic matter by aa floatation using a high density solution (Hidalgo-Ruz et al., 2012; Wang et al., 2016b). However, microplastics could be strongly adsorbed onto soil particles (Brady and Weil, 2000), thus making extraction via flotation difficult. Low density PE and PP are widely used in agriculture to reduce evaporation and increase soil temperature (Lwanga et al., 2016; Steinmetz et al., 2016; Yan et al., 2015), and both, PE and PP densities are <1 g cm^{-3}, thus saturated solutions of saline can be replaced by distilled water (density is 1 g cm^{-3}) for agricultural soils. Generally, the particle size and quantity of microplastics can be determined using a microscope (e.g. TM3030, Hitachi, Japan)(Wang et al., 2016b) allowing microplastics to be classified as small microplastics (SMP) measuring <1 mm or large microplastics (LMP) measuring 1 mm to 5 mm (Imhof et al., 2012; Van Cauwenberghe et al., 2015b). Microplastics with a diameter > 1mm are easily detected whereas microplastics with a diameter < 1 mm are difficult to identify (Hidalgo-Ruz et al., 2012; Lenz et al., 2015). Mass determination is typically only possible for larger quantities of bigger, more visible microplastics. Thus, most studies reveal the total number of particles per volume of water or sediment (Hidalgo-Ruz et al., 2012). Infrared spectroscopy and Raman micro-spectroscopy are considered to be the most reliable yet most costly methods used to identify the properties of microplastics, but cannot identify the mass content of microplastics in soils (Frias et al., 2010; Hirai et al., 2011;; Nor and Obbard, 2014; Lenz et al., 2015; Wang et al., 2016b). Soils consist of many diverse components which have a wide range of densities due to differing concentrations of soil organic matter (SOM), sand, clay, silt and organic fibers. Some parts of SOM (density ranging from 0.5 to 0.8 g cm^{-3}) (Barrios et al., 1996; Perie and Ouimet, 2008; Castanha et al., 2012) are similar to microplastic density which leads to difficulties when trying to separate microplastics from soil (Hidalgo-Ruz et al., 2012). Furthermore, the presence of some soil components, such organic fibers and silicates, in the extracts makes the identification of

LDPE microplastics more difficult and a new detection/identification method should be developed and verified (Hidalgo-Ruz et al., 2012; Steinmetz et al., 2016). Thus, the identification of microplastics in soils needs to be developed and tested before assessing the microplastic content of different soils The quantification of microplastics in soil is a challenge and the analytical techniques required for this analysis still need to be innovated (Rillig, 2012; Steinmetz et al., 2016).

In this study, we aimed to develop an accurate, simple, cost-saving method to assess small microplastic particles of LDPE and PP measuring <1 mm in agricultural soils focusing on four main goals: (1) extraction, (2) validation, and (3) identification of microplastic particles in floatation separated from soil impurities (e.g. organic matter, organic fibers and silicates) as well as (4) quantification of the number and size of particles. We used different soil types to develop the method in hopes that the method could be widely used to determine the content and the particle size distribution of microplastics in soils. This new method will be helpful in evaluating the risks of microplastics in the soil, especially in farmland with long-term plastic mulching use.

2. Materials and Methods

The characteristics of LDPE and PP were considered during the development of the approach. Both LDPE and PP (Riblon, Ter Hell Plastic GmbH) were white and grounded into irregularly-shaped particles by the company. The sizes of the LDPE particles were < 150 μm and the PP particles were < 400 μm (Table 1, Figure 2). The densities for both kinds of plastic particles were less than 1g cm^{-3}. In order to avoid contamination during the experiments, the laboratory was thoroughly cleaned before the experiments were carried out and kept clean throughout the duration of our testing. Clothes made from plastic fibres were not allowed in the laboratory.

2.1 Incubation of soil samples

In this experiment, a floating method was used for extracting microplastic from different soil samples. Soil types such as clay soil (15.8% clay, 64. 6% silt, 19.6% sand, 3.23% OM and pH(KCl) 7.84), loess soil (10.4% clay, 76.3% silt,13.2% sand,4.20% OM and pH(KCl) 7.16) and sandy soil (3.97 % clay, 31.3 % silt, 64.7 % sand, 7.4 % OM and pH(KCl) 6.63) were used in this experiment. To homogenize , all soil samples were air-dried and sieved at 2 mm. LDPE and PP were added to soil samples at five concentration gradients (0.05%, 0.1%,

0.2%, 0.5% and 1.0%, w/w) with three replicates for each plastic. In each treatment, the microplastic particles were mixed homogenously with 10 g of soil which was adjusted to a soil moisture content of 20%. The mixture was mixed in an aluminium cup (100 ml) and sealed with plastic wrap. No debris dropping from the plastic wrap was verified before experiment, and this was also proved in the control treatment table 5). In order to incorporate the microplastics into the soil, the soil was incubated at 4℃ for the duration of a week (Hidalgo-Ruz et al., 2012; Paul and Clark, 1989; Reber, 1975).

2.2 Extraction of microplastic using a flotation method

The extraction procedures are illustrated in Figure 1A. Firstly, 50 ml of distilled water was added to an aluminium cup containing the soil sample (10 g) and a glass stick was used to stir the soil and water together manually in order to get a homogeneous suspension. Secondly, the glass stick was rinsed off using distilled water and the water was collected in the original cup. The cup was then refilled with water. Thirdly, the box was left over night in order to allow the soil particles to deposit on the bottom of the cups. The floating SOM, microplastic and other impurities (on the surface water) were poured off and filtered using filter paper (pore diameter < 3 μm). The same procedure was carried out at least four times for each sample until no materials were seen floating on the surface of the water or detected on the sides of the cups. The soil solutions were subjected to two hours of ultrasonic vibrations and then filtered again using the same filter paper. In order to reduce contamination during this process, all filter papers were covered by a light aluminium specimen box during the process of filtration.

2.3 Validation of extraction

Filters with floatation were dried to constant weight at 60℃ in an oven and then weighted (W_1). After the floatation was brushed off, the filters were weighted again (W_2). The total floatation (W_g) and the microplastics floatation (W_p) can be calculated by using the following equations: $W_g=W_1-W_2$, and $W_p=W_g-W_{control}$. $W_{control}$ is the floatation collected from the control treatment with no addition of microplastics. Recovery (R) was calculated by using the following equation: $RR= W_p/W_{added}$. The W_{added} is the initial weight of the PE or PP added to the soils.

2.4 Identification of microplastic under the microscope

The floatation included microplastic, organic matter, quartz, and fibres. The floatation was dried on the filter at 60℃. The dry floatation was placed on a glass slide and evenly

distributed over the slide using a small brush in order to avoid any overlapping or clusters. The bristles of the brush were tested before the sweeping procedure was performed and no plastic debris from the bristles was dislodged from the brush. After putting the slide under the microscope (Leica wild M3C, Type S, simple light) (6.4 X Zoom), a photo (Figure 2, before heating) was taken using a high resolution camera (Leica DFC 425) linked to a computer with image software (Leica Applicate Suite 4.8) in order to identify the number and size distribution of the particles. The glass slide was then heated in an oven for 3-5 seconds at 130℃ and a second photo (Figure 2, after heating) was taken. After heating, the microplastics on the glass slide were transformed from irregular particles into circular, transparent, shiny particles due to melting. Impurities such as organic matter, organic fibres and silicates did not change by heating. Comparing the photos from pre- and post-heating, the LDPE/PP particles can be clearly distinguished from the impurities and identified. Thus, the second photo was used to distinguish the microplastic from organic matter, quartz and organic fibres while the first photo was used to determine the number, sizes and shapes of the microplastic particles. The identification procedures are shown in Figure 1 B.

2.5 Mass estimation of microplastics using an empirical model

Due to the great variance in the shapes of microplastics in natural soils, it is difficult to quantify the volume of the microplastics. However, all of the microplastic particles in the soil samples were transformed into circular bubble-like shapes after heating, so it was easy to calculate the volume of the microplastics. In order to reduce any negative effects due to the impurities (organic matter and organic fibres), pure LDPE and PP were used to develop the models of microplastic weight and microplastic characteristics after heating. Six samples of pure LDPE and PP were weighed randomly on glass slides and the microplastic were scattered evenly across the slides. The weight of LDPE was 0.0010 g, 0.0017 g, 0.0031 g, 0.0015 g, 0.0011 g and 0.0020 g on the glass slides, respectively. The weight of PP was 0.0011 g, 0.0013 g, 0.0014 g, 0.0031 g, 0.0040 g and 0.0019 g on the glass slides, respectively. The LDPE and PP on the glass slides were heated at 130 ℃ for 3-5 seconds and then photos were taken under the microscope. The software of Image J (Schindelin et al., 2015) was used to calculate the number of microplastic and the area they covered after heating under the following conditions: (1) image type was 8bit; (2) colour and resolution of the image was adjusted to make sure all particles were included; (3) the redundant small colour pixels originating from glass slide and microscope were deleted. Heat actually changed the form of

187 microplastic so that microplastic volumes could be roughly calculated using hemisphere
188 measurements. The radius of each pellet was calculated using the vertical scanning area
189 (vertical angle of view) obtained by Image J (Figure S1). Regression analysis was carried out
190 on the following equations: the equations of weight (LDPE or PP) vs. counted number, weight
191 vs. area, and weight vs. volume, which were obtained after heating based on the area covered
192 by microplastics. Additionally, an empirical model was built to predict the mass of
193 microplastics in soils according to the best fitting model.
194

195 2.6 Validation of the identification and empirical model

196 The density of PP is similar to that of LDPE (Table 1) whereas the size range of PP is
197 wider than LDPE. Thus, PP was used for validation. Nine samples of pure PP were randomly
198 weighed and then each sample was added to 10 g of clay soil (3 replicates), sandy soil (3
199 replicates) and loess soil (3 replicates). 10 g and 3 replicates of clay soil, sandy soil and loess
200 soil were weighed. All of the samples were subjected to the steps of incubation, floatation,
201 identification and calculation as mentioned above. The weight of the PP samples were 0.0014
202 g, 0.0013 g and 0.0010 g for clay soil; 0.0010 g, 0.0016 g and 0.0015 g for sandy soil; and
203 0.0015 g, 0.0013 g and 0.0014 g for loess soil. Measurements of the size distribution of the PP,
204 the recovery of the microplastic (W_{model}/W_{added}) and the relative error (RE, $|W_{model} - W_{added}|/W_{added}$)
205 of mass were all used to validate the methods of identification and the empirical
206 model. W_{model} was the mass calculated after heating using the experimental model.
207

208 2.6 Statistical analysis

209 Quantitative data of floatation collection, microplastic recovery, microplastic size,
210 microplastic number, and microplastic weight were described as mean±STD. Significant
211 differences ($p<0.05$) were tested via ANOVA and pothoc test Dunnet T3, since the data
212 followed a normal distribution tested by the KS test. The single linear regression analysis of
213 weight (LDPE/PP) vs. floatation and weight (LDPE/PP) vs. particle properties after heating
214 were all carried out using SPSS 17 (SPSS Institute Inc., 2007). Vector line figures were
215 drawn using Sigmaplot 10.0.

216 3.5 A case study to validate the technique − microplastic in soils of northwest China

This new developed method was used to identify the microplastic distribution in soils collected from the Loess plateau in Northwest China. Plastic mulch, containing mostly LDPE with a density of < 0.93 g cm^{-3}, has been used to reduce evaporation from agricultural fields for the past 20 years. From this area, we collected a total of sixty soil samples from the surface layer (0-10 cm, N=30) and a deeper layer (10-30 cm, N=30) in an agricultural field (N=20), an orchard (N=20) and a greenhouse (N=20) in August 2016.

3. Results

3.1 Extraction

A process of three time floatation or 2 h ultrasonic was necessary to extract the microplastics from the soil. A fourth flotation did not extract further microplastics, the water surface remained clean. The weight of floatation (microplastic with impurities) followed the following orden ($p<0.05$) for all LDPE and PP concentrations (Tab. 2): sandy soil> loess > clay > pure sand (Table 2). No microplastics were found in the control treatment.

3.2 Validation of extraction

The recovery rate based on the weight of LDPE ranged from 86.0%±0.8 to 102.7%±4.2 in loess, , 103.0% ±4.8 to 128.0% ±34.0 in sandy soil, 89.9% ±0.3 to 104.0% ±8.4 in clay soil and 87.9±31.1 to 112.7±22.0 in pure sand, respectively (Table 3).

For the PP, the recovery rate ranged from 97.5% ±4.0 to 126.7% ±15.4 in loess soil, from 80.0%±38.0 to 138.0%± 39.0 in sandy soil, from 97.0%±0.8 to 121.3%±8.8 in clay soil and from 91.2%±10.6 to 121.3%±27.7 in pure sand, respectively. It was obvious that the standard deviations were higher for the 0.05% concentration measurements than for the other concentrations for both LDPE and PP, especially in sandy soil and pure sand.

Concerning the weight of microplastic, the regression curves were estimated between the added weight of LDPE/PP and the recovered weight (Figure 3). The results showed that the regression coefficients (R^2) were all over 98.5% and most of them were even close to 99.9%.

3.3 Empirical model developing for microplastic quantification

The relationship between the weight of added microplastic and particle numbers and the area and volume after the heating treatment were regressed (Table 4).The results showed that the relationship between particle number and weight, especially for PP, was relatively low

(R^2=0.74). However, there was a good relationship between the added LDPE/PP weight vs. area/volumes (after heating) when these were regressed (R^2>0.99, p< 0.01). The real volumes of LDPE/PP can be calculated based on the weight of the LDPE/PP added and the real density of PE/PP (PP 0.92 g cm^{-3}, PE 0.91 g cm^{-3}). The volumes roughly calculated using hemisphere measurements were close to 4.5 times that of the actual volumes of PE/PP and were 1/9 of the volume of the spheres calculated using the radius of the vertical view area after heating by Image J. The equation of 1/9 volume of the sphere is shown below:

$$V = \frac{4}{27}\sum_{i=1}^{n}\sqrt{\frac{S_i^{\,3}}{\pi}} \qquad (1)$$

In the equation (1), V is the volume of the plastics after heating; n is the number counted, S is the vertical angle of the view area of the plastic after melting at 130 ℃ for3-5s.

Many reports have indicated that most of the microplastic found in agricultural fields are PE and PP and their densities are close to 0.90 g cm^{-3} (Hidalgo-Ruz et al., 2012; Liu et al., 2014a; Steinmetz et al., 2016). Thus, the mass of microplastic in the field could be roughly calculated based on the model shown below:

$$m = \frac{4}{27}\rho\sum_{i=1}^{n}\sqrt{\frac{S_i^{\,3}}{\pi}} \qquad (2)$$

In the equation (2), m is the weight of plastics, ρ is the density of plastics (0.90 g cm^{-3}), and n is the number counted.

3.4 Validation of empirical model

The size distribution of PP detected after floatation and before heating using ImageJ were close to the original proportions, especially for the particles measuring 100-250 μm and >250 μm in all three soil types (Table 5). The mass of PP calculated using the empirical equation was also close to the original (recovery was 81.9-98.6%; RE, 9.8-18.1%) and even more accurate when the density of 9.2 g cm^{-3} was used in the calculations (recovery was 83.7-100.8%; RE, 9.3-16.3%) in the soil types.

3.5 Application of the technique to the case study

The results indicate that both, the number and the weight of microplastics were higher in the deeper soil layers (10-30 cm) of the agricultural field, while they were lower in the deeper soil layers (10-30 cm) of the orchard (Table 6). Most of microplastic sizes were > 100 μm and the weights were <0.54 mg kg^{-1} in agricultural field, orchard field and green house. <icroplastics with a size < 50 μm were found only in the deeper soil layers of the greenhouse soil.

4. Discussion

4.1 Microplastic extraction from soil

Given the rapid increase in plastic production, contamination of the environment by small plastic fragments, referred to as microplastics, has become a hot topic especially with relation to aquatic organisms (Lusher et al., 2015; Van Cauwenberghe et al., 2015a) and water bodies (Cózar et al., 2015; Seltenrich, 2015). Admittedly, microplastics found in water bodies and solid medium, such as sediment from oceans or river beds, have been elutriated or floated, either by pure water or saturated NaCl and NaI, attributing to the density differences between microplastics (PE, PP and polyvinylchloride) and extraction solvents (Hidalgo-Ruz et al., 2012; Nuelle et al., 2014b). “White pollution”, derived from plastic mulching use in agriculture systems, has become the biggest issue for the surroundings and soil quality (Liu et al., 2014b).

Most of the plastic mulching used for agriculture is made of LDPE and PP. Since the density of this kind of plastic is typically <1 g cm^{-3}, distilled water can be used to float the microplastic in the soil and can thus reduce clean-up costs as well as environment pollution (Lwanga et al., 2016; Steinmetz et al., 2016; Yan et al., 2015). However, other high-density solutions can also be used for microplastic extraction and flotation if the density of the microplastics in the soils is unknown. The process would be same except for the fact that the distilled water would be replaced by saturated solutions. Some studies have shown that the microplastic in soils can be efficiently extracted using a methanol and dichloromethane solution and can be weighted after evaporation to dryness under high temperatures(Fuller and Gautam, 2016). However, these extraction methods could overestimate microplastic concentration and contribute to the dissolution of soil organic matter at 180℃ (Schnitzer and Khan, 1998). In the present study, we developed a simple, low-cost method to extract microplastic from soils using distilled water. Although impurities influenced microplastic

collection, high regression coefficients were observed between the purified floatation and the microplastic that were initially added (Figure 3). It was obvious that most of the plastics and impurities were floated before the fourth flotation (Figure S2). One previous report indicated that ultrasonic waves could be beneficial when trying to separate particles from soils(Cooper and Corcoran, 2010; Hidalgo-Ruz et al., 2012). In this study, soil samples were shaken and exposed to ultrasonic waves for 2 hours after the fifth floatation but nothing was found on the surface of water (Figure S2). However, in order to make sure all of the microplastic could be floated on surface of the water, an ultrasonic step was also suggested after the last floating procedure. At the time of the extraction, the highest deviation errors in the collection and recoveries were observed for sandy soil (Table 3). This implies that the higher organic matter and the fine particles in the soil samples strongly influenced the microplastic recovery (Brady and Weil, 2000; Dekker and Ritsema, 1994; Zhang et al., 2014a). In addition, since the extraction and floating procedures took a long time for each sample, centrifugal and vacuum methods could be used for shorting the deposition and filtration times (Fossi et al., 2014; Steinmetz et al., 2016; Thompson et al., 2004).

4.2 Identification of microplastic

Microplastic in the environment, especially in water bodies (salt or fresh water) and sediment, can be detected, counted and weighed which can be attributed to either the large volume of samples and less impurities or the lager particles (1mm-5mm) being examined (Hidalgo-Ruz et al., 2012; Nuelle et al., 2014b; Wang et al., 2016b). However, soil heterogeneity is complex, especially when considering all of the components such as mineral soil, organic matter, chemical elements and plant/animal residues (Brady and Weil, 2000; Zhang et al., 2014a; Zhang et al., 2016). In our study, not only microplastic were extracted and floated, but organic matter and other residues were also floated in water which was similar to other studies (Brady and Weil, 2000; Dekker and Ritsema, 1994; Hidalgo-Ruz et al., 2012). All of the floatation needs to be identified, especially when distinguishing microplastic from other impurities under a microscope. There are two ways to separate microplastic from floatation. One way is to remove other impurities from the sample. In order to remove impurities from the microplastic, H_2O_2 was used to remove organic matter and plant residues in water sediments samples (Majewsky et al., 2016; Nuelle et al., 2014a; Wang et al., 2016a). However, H_2O_2 not only removed the impurities from the sample, but it also digested some of the microplastic (Claessens et al., 2013). Another way to remove impurities is to find some

advanced method to distinguish microplastic from impurities. In the present study, a heating method was used to identify microplastic in all of the floatation samples by comparing the shape change between pre- and post-heating. It was easy to distinguish between the impurities and the microplastic, which showed transparent, circular, and shiny properties, or had a big change of shapes, e.g. plastic fibre rolled up after heating(Figure2 and Figure S2). However, two points need to be taken into account: heating temperature and time. The melting point of LDPE and PP is 115-135 ℃ and 130-171℃, respectively. If the temperature is too high or the heating time is too long, the properties of the melted microplastic such as transparency, circular form and shine would not be observed. In this study, we found that the microplastic was transformed into transparent, circular and shiny areas after the floatation on the glass slide was heated for 3 to 5 seconds at a temperature of 130 ℃. The photo was then taken with a camera connected to the microscope. Previous research indicated that microplastic could be weighted after extraction but the limitation comes after trying to measure the size of the microplastic in the samples(Schnitzer and Khan, 1998). With the help of the microscope and Image J, particles size, shape and number could be determined using the photos taken before and after heating. Therefore, the heating method is strongly recommended when trying to identify microplastic collected from soil samples. Also, we found that the size distribution of microplastic after extraction from three the soil types proved that this identification method for microplastic is valid (Table 4). However, in this study, only the size of the microplastic >20 μm could be clearly detected based on the resolution of the microscope and the camera combination (16.4X). Concerning further testing, the limit of particle size detection could be smaller if a digital camera with a higher resolution was connected to the microscope. Furthermore, this method can't be used to distinguish the chemical components of microplastic, which can be detected using the method of thermal analysis, infrared spectroscopy or Raman micro-spectroscopy (Frias et al., 2010; Hirai et al., 2011; Lenz et al., 2015; Majewsky et al., 2016; Nor and Obbard, 2014; Wang et al., 2016b).

4.3 Quantification of microplastic with models

For microplastic, only the total number of particles per given volume of water or sediment were measured due to the small size and limited numbers of the microplastic and weighing difficulties(Hidalgo-Ruz et al., 2012; Wang et al., 2016b). In this study, we developed some models to predict the weight of microplastic in soils. After heating, the shape of the plastic changed to the form of a circle when using the vertical view in 2 dimensions and to nearly a

hemisphere in the 3 -dimensional view. The regression between weight and volume was the most accurate (R^2 >99%) (Table 4), and the 1/9 volume of sphere calculated by the radius of the vertical area after heating was close to the real volume of PE and PP. The empirical model for mass prediction was build according to the volume and the accuracy of the model was acceptable (recovery 82-99%) according to the validation using the steps of floatation by distilled water, identification under a microscope after heating method, and quantification based on an empirical model for three kinds of soil.

4.4 Case study: Microplastics in the soils of Northwest China

The plastic mulch used in fields in China are typically made of LDPE which has a density of less than 0.93 g cm^{-3}(Yan et al., 2015). In this case study, distilled water was used to extract microplastic from soils. The results indicated that the amount and weight of the microplastic found were different between the soil from the agricultural field and the soil of the orchard. This can be attributed to the fact that the microplastics deposited in the surface layers were easy washed away by runoff in the agricultural field. There was less runoff and soil loss in the orchard thus reducing the microplastic movement in the surface layer (Zhang et al., 2015). Light, small-sized microplastic can be easy carried away by runoff and soil erosion in agricultural fields and thus most of the larger microplastic found >100 μm remained in the deep soil layers. The weight of the microplastic found in each of the three land-use areas was less than 0.54 mg kg^{-1}. These results could be referenced in future research. Furthermore, the case study also proved that this method, used in conjunction with the floatation and heating techniques, can be used to investigate microplastic in soils.

5. Conclusions

The method of repetitive floatation can be used in extracting LDPE and PP microplastic from soils with high accuracy. The number of microplastic can be easily distinguished from impurities, and microplastic particles can be counted by comparing photos taken before and after heating under a microscope. Microplastic mass evaluated by empirical model method were acceptable.

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Table 1 Size distribution of the original microplastics of PE and PP

	Density (g cm^{-3})	**Components of Particle sizes**			
Plastic Diameter		<50μm	50-100μm	100-150 μm	
PE	0.92	50%	22%	23%	
Plastic Diameter		<50μm	50-100μm	100-250μm	>250μm
PP	0.91	0.2%	1.5%	38.1%	58.4%

Table 2 The floatation collection by distilled water after LDPE and PP added, and the floatation collection by distilled water after PP added for a week.

	PE/PP added(mg)	Concentration (%, w/w)	Floatation collection (mg) (Mean±STD)			
			Loess	Sandy	Clay	Pure sand
LDPE	0	0.00	39.6±1.0b	121.7±14.2a	9.3±0.9c	0d
	5.0	0.05	44.7±1.8b	128.1±18.9a	14.6±1.2c	4.4±1.6c
	10.0	0.10	48.2±0.5b	133.7±30.4a	18.4±1.3c	11.3±2.2c
	20.0	0.20	62.2±3.1b	146.8±3.3a	29.0±2.0c	20.8±0.9d
	50.0	0.50	86.0±8.5b	173.2±11.8a	54.3±0.2c	50.5± 0.8c
	100.0	1.00	131.9±5.2b	244.7±3.0a	112.4±11.3c	89.6±9.4d
PP	0	0.00	39.6±1.0b	121.7±14.2a	9.3±0.9c	0d
	5.0	0.05	45.2 ±8.3b	125.7±16.1a	15.1±2.9c	6.1±1.4d
	10.0	0.10	52.2±6.5b	135.5±4.3a	19.1±0.2c	11.0±1.2d
	20.0	0.20	59.1±2.4b	139.8±14.7a	33.6±2.4c	19.7±1.5d
	50.0	0.50	89.5±1.3b	173.8±5.0a	67.0± 5.7c	45.6±5.3d
	100.0	1.00	141.8±3.7b	238.4± 22.7a	110.5± 5.8c	106.7± 9.0c

Values followed by the same letter within the same rows are not significantly different by LSD's multiple range test ($P \leq 0.05$).

Table 3 The recovery rate of LDPE and PP in different treatments

	Concentration (%, w/w)	**Recovery (%)(Mean±STD)**			
		Loess	Sandy	Clay	Pure sand
LDPE	0.05	102.7±4.2a	128.0±34.0a	104.0±8.4a	87.9±31.1a
	0.10	86.0±0.8a	120.0±16.0a	90.0±6.5a	112.7±22.0a
	0.20	113.3±5.7a	125.5±54.5a	98.3±6.9a	103.8±4.7a
	0.50	93.9±9.2a	103.0±4.8a	89.9±0.3a	101.0± 1.6a
	1.00	92.3±3.6a	123.0±11.2a	103.0±10.4a	89.6±9.4a
PP	0.05	112.0 ±20.5a	80.0±38.0a	114.7±22.1a	121.3±27.7a
	0.10	126.7±15.4a	138.0± 39.0a	97.0±0.8a	110.0±11.7a
	0.20	97.5±4.0a	90.5±2.5a	121.3±8.8a	98.5±7.4a
	0.50	99.9±1.5a	104.2± 18.4a	115.3± 9.7a	91.2±10.6a
	1.00	102.3± 2.6a	116.7± 8.4a	101.2± 5.3a	106.7± 9.0a

Values followed by the same letter within the same rows are not significantly different by LSD's multiple range test ($P \leq 0.05$).

Table 4 The regression of PE and PP between weight and number, weight and area, and weight and volume.

	Regression parameter	Equations*	R^2	*P*
PE	Number vs. weight (mg)	y =0.001x -0.35	0.89	<0.01
	Area (mm^2) vs. weight (mg)	y =0.05x + 0.89	0.97	<0.01
	Volume (mm^3) vs. weight (mg)	y = 1.14x + 0.46	0.99	<0.01
PP	Number vs. weight (mg)	y = 0.01x -0.74	0.74	<0.01
	Area (mm^2) vs. weight (mg)	y = 0.08x -0.53	0.98	<0.01
	Volume (mm^3) vs. weight (mg)	y = 0.67x +0.17	0.99	<0.01

*.Note: "y" is the weight of PE/PP. Area of PE/PP is the area from vertical angle of view under microscope after melt. Volume of PE/PP was calculated by equation (1).

Table 5 Validation of identification and experimental model for MPs

Soil types	N	PP size (%) (Mean±STD)			Recovery (%) (Mean±STD)		Mean RE of mass (Mean±STD)	
		<100 (nm)	100-250(nm)	>250(nm)	Model ρ=0.90 g cm^{-3}	Model ρ=0.92 g cm^{-3}	ρ=0.90 g cm^{-3}	ρ=0.92 g cm^{-3}
Clay	3	0	0	0	0	0	0	0
Loess	3	0	0	0	0	0	0	0
Sandy	3	0	0	0	0	0	0	0
Clay	3	5.9±2.4	34.8±7.1	59.3±6.2	87.5±6.4	89.4±8.2	12.8±7.2%	10.7±7.7%
Loess	3	7.1±7.0	34.8±3.3	58.2±9.9	81.9±8.9	83.7±9.1	18.1±8.9%	16.3±9.0%
Sandy	3	9.0±3.3	35.9±4.4	55.1±6.7	98.6±12.8	100.8±13.0	9.8±4.6%	9.3±6.5%

Table 6 Microplastics in soils of northwest China

Land use	N_1	Soil depth (cm)	Numbers (n kg^{-1})					Content (mg kg^{-1})	
			<50(μm)	50-100(μm)	>100(μm)	Mean±STD	CV	Mean±STD	CV_2
Agricultural field	10	0-10 cm	0	0	40	40±126	3.16	0.008±0.025	3.16
	10	10-30 cm	0	0	100	100±141	1.41	0.368±0.740	2.01
Fruit field	10	0-10 cm	0	40	280	320±329	1.03	0.540±0.603	1.12
	10	10-30 cm	0	20	100	120±169	1.41	0.460±0.735	1.60
Green house field	10	0-10 cm	0	20	80	100±254	2.54	0.130±0.307	2.84
	10	10-30 cm	40	0	40	80±193	2.42	0.024±0.051	2.11

1.“*N*” is the numbers of soil samples
2. “*CV*” is the variable coefficient

Figure
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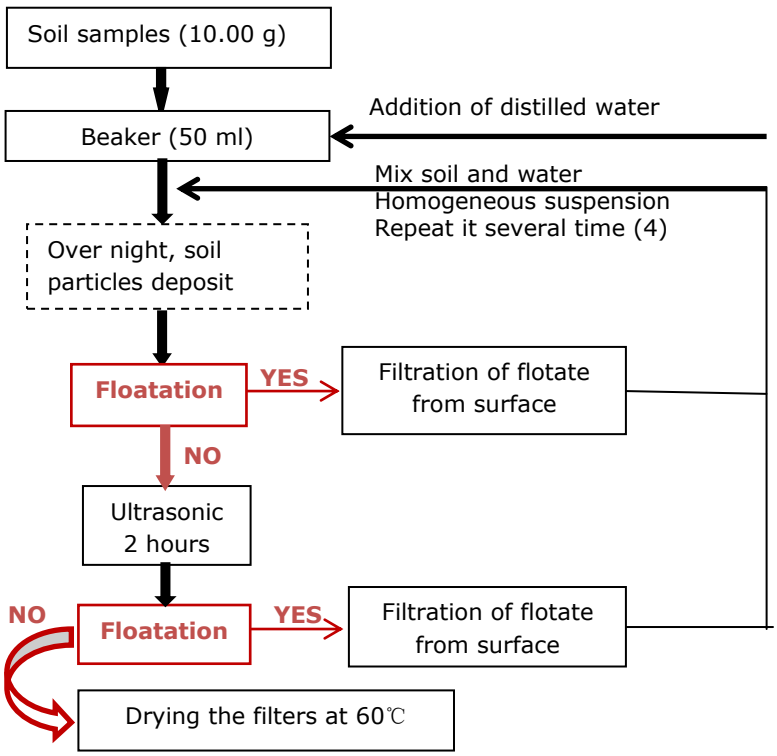
A: Extraction of MPs by flotation method



B: Identification of MP under microscope

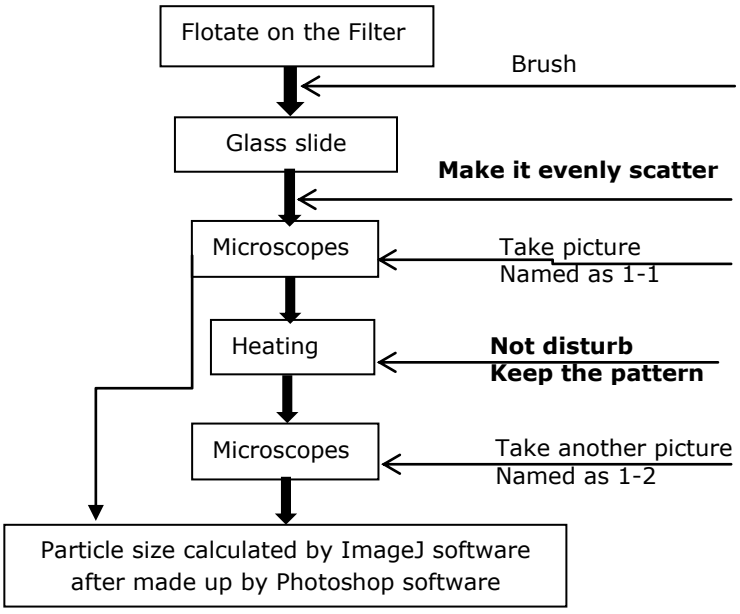


Figure 1 Framework of extraction and identification

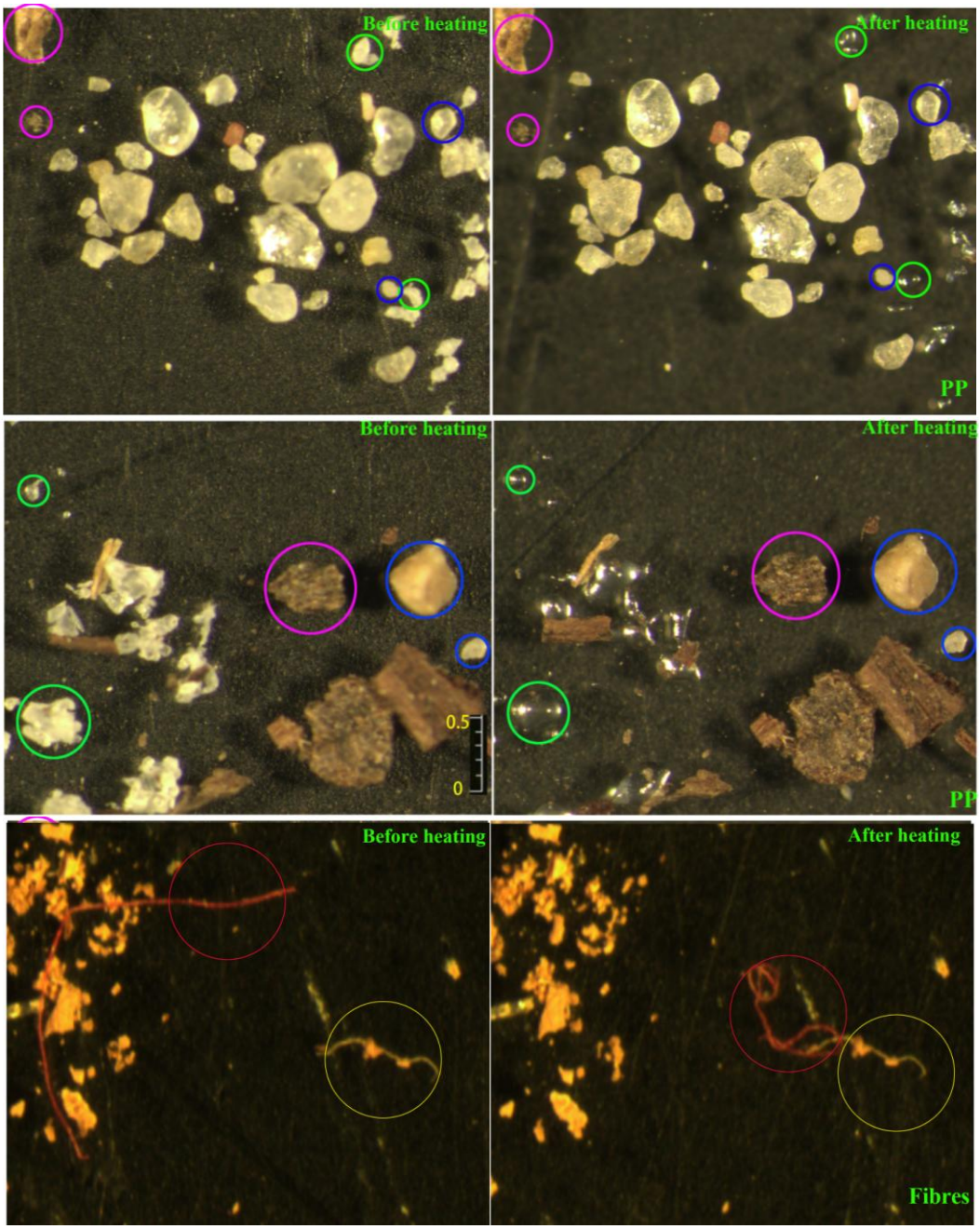


Figure 2 Definition of MPs from photos taken by camera (DFC 425) under microscope (6.4×). A selection of particles are marked to show the shapes of MP changed to circle and transparent after heating, while quarzs and organic matters were not changed. Plastics, sands and organic matters were circled by green colour, blue colour and purple colour, respectively. Wood fibre was not changed, while plastics fibre was rolled up. Wood fibre and plastics fibre were circled by yellow colour, red colour. MPs are easy connected after melt when the distance between particles are less than 10 um.

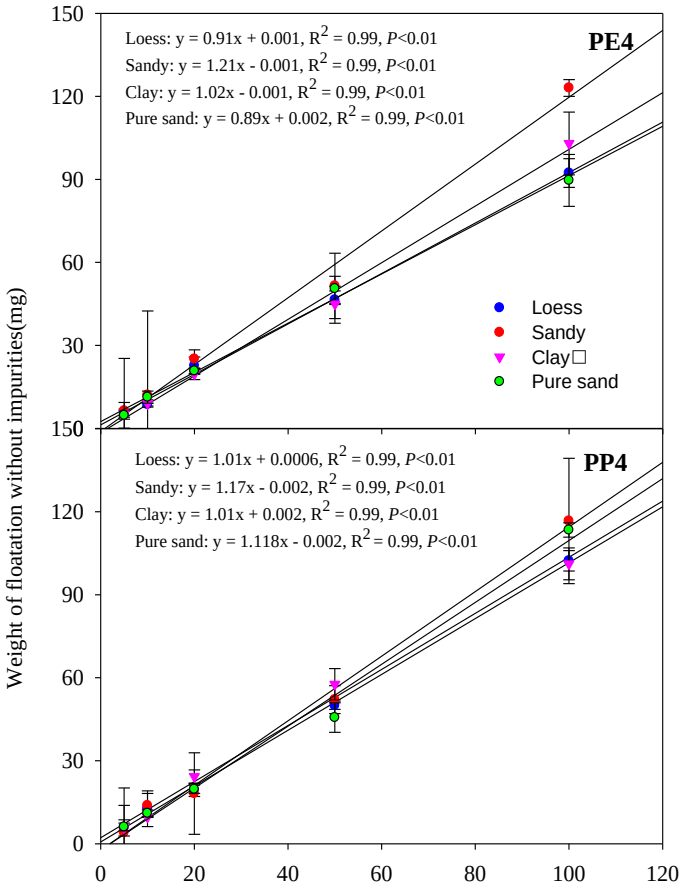


Figure 3 Recovery rate of incubation at 4℃ (PE4 and PP4)

Figure supplement
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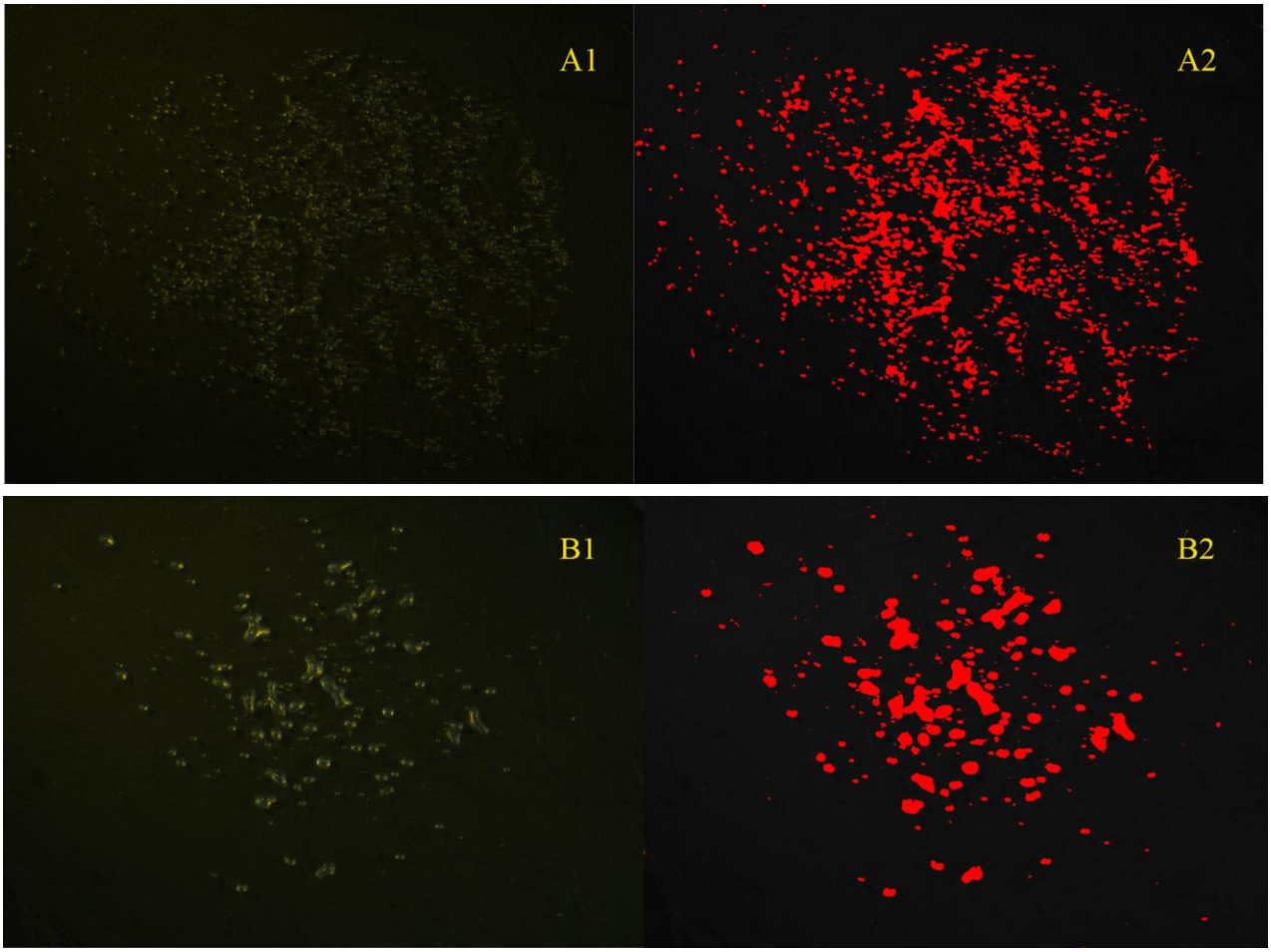


Figure S1 Photos of melted PE (A) and PP (B) under microscope (100×), and the numbers and shapes (A2 and B2) were calculated by Image J software.

Figure S2 No MPs or impurities floated on after four times' extraction by distilled water, then moved into the ultrasonic equipment, but MP was also not found on water surface under 2 hours' ultrasonic .

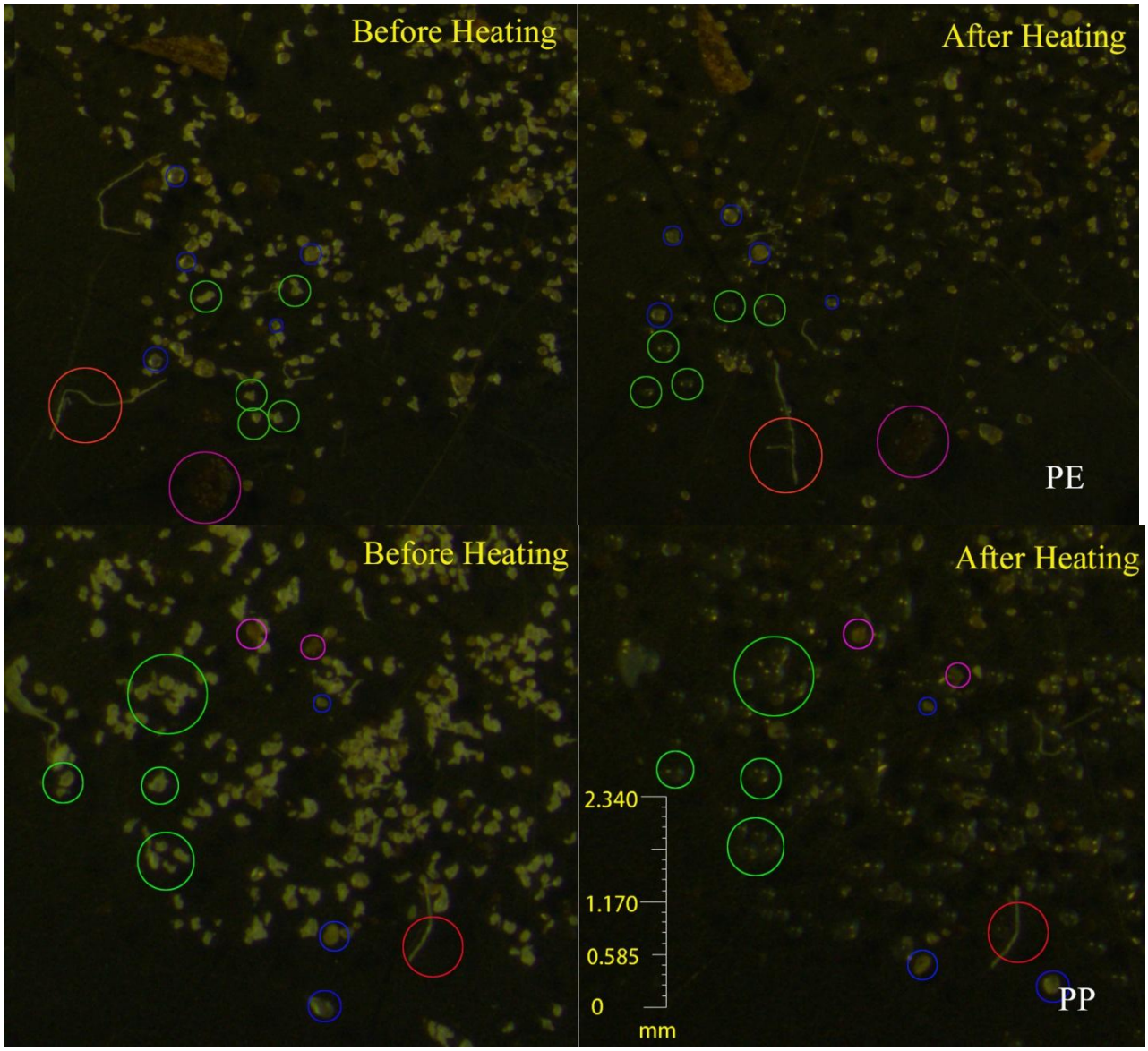
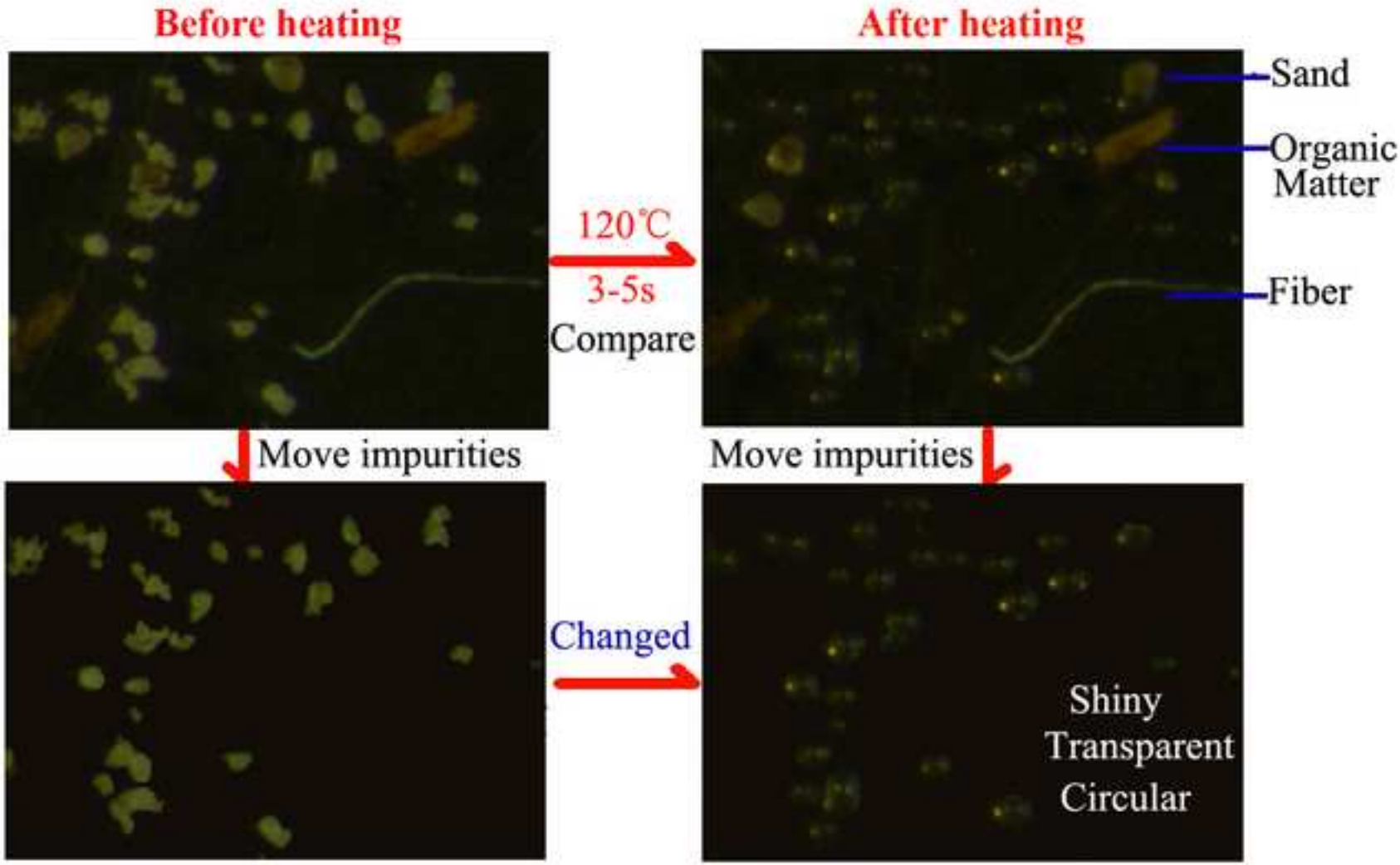


Figure S3 Identification of MPs under microscope (100×). A selection of particles were marked to show how the shapes of MPs changed to transparent circular forms after heating, while quartz, organic matter and wood fibres were not changed. Plastics, sands, organic matter and wood fibres were circled using green, blue, purple and red colours, respectively. MPs were easy connected together after melting when the distance between particles was less than 10 um. The numbers were 186 and 155 in the pictures of PE and PP, respectively.

*Graphical Abstract



Highlights

- A low cost-saving method were developed to extract, distinguish and quantify MP from soils.
- Floatation method was efficient in extracting MP from soils.
- After heating, MPs were transformed into circular, transparent and shiny particles.
- Regression analysis of MP weight and particle volume after heating showed the best fit.