Supplementary materials

The missing small microplastics: easily generated from weathered plastic pieces in labs but hardly detected in natural environments

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2. METHODS AND MATERIAL

2.3 Extraction of MPs from sediment samples

We compared the extracting efficiency of MPs and SMPs between saturated NaCl and NaBr (1.55 g/cm^3) solution. Firstly, the PS fluorescent microspheres (9.9 μm, G1000, Thermo, USA) and PP MPs fragmented from plastics were prepared as the interior standard. The PS original solution was prepared from 100 μL of PS latex into a glass bottle with 30 mL Milli-Q water. And we set three replicates with 200 μL of the solution. The solution was dropped on a circular silicon wafer and counted under a microscope (BX53, Olympus, Japan). The PP plastic piece was put into a glass tube with 30 mL Milli-Q water and then ultrasonicated for 1 min. The three replicates of circular silicon wafers with 200 μL of the solution was identified and counted with a Raman microscope (inVia Reflex, Renishaw, UK). Secondly, the sediment and 200 μL of PS and 1000 μL of PP MPs standard solution were put into a clean long glass tube; then the saturated solutions of 300 mL of NaCl/NaBr were added into glass tube. The method of extraction and filtration was as the same as that described in section

2.5. Identification and quantification of MPs using Raman microscope

The calculation abundance process of different sizes of plastics fragments produced from plastics pieces is s defined as:

$$Abundance_{size1} = average(\frac{DPn \times \frac{DA}{TA}}{WP})$$

Here, DPn represents the detected number of different sizes of plastic fragments, DA means detected area, TA is total area and WP is the weight of plastic pieces.

The calculation abundance process of different sizes of MPs/NPs in sediment is s defined as:

$$Abundance_{size2} = average(DPn \times \frac{DA}{TA} \times SPn)$$

Here, DPn represents the detected number of NPs/MPs, DA means detected area, TA is total area and SPn is the percentage of NPs/MPs with different sizes.

Legends of Supplementary Figures

Figure S1. Detected area using two different Raman. (Raman (Thermo Scientific™ DXR™ 3) with 20X magnification object and 785 nm laser was used to identify the large MPs (size > 50 μm); Raman (Renishaw, inVia Reflex, UK) with 50X magnification object and 785 nm laser was used to identify the small MPs (size of 10-50 μm)).

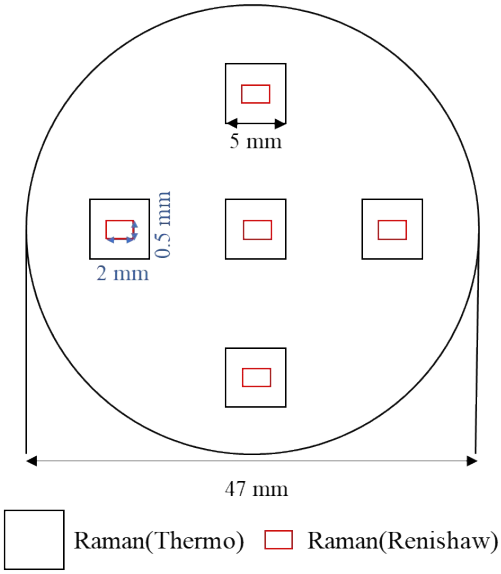
Figure S2. Morphological characteristics of plastic pieces from the environments. A1-C1 and A2-C2 are obtained using optical microscope; A3-C3 is obtained using SEM.

Fig. S3 Abundances and size distribution of six samples after treatments of ultrasonic and oscillator. Sample 1-5: polypropylene; Sample 6: polyethylene.

Figure S4. SEM images and Raman spectra of MPs/NPs on the SERS substrate and silicon wafer.

Table S1 The aging characters of six types of plastics

Table S2 The abundance and recycling rate of internal standard of PS spheres and PP fragments after extracting by two flotation agents (NaCl, NaBr



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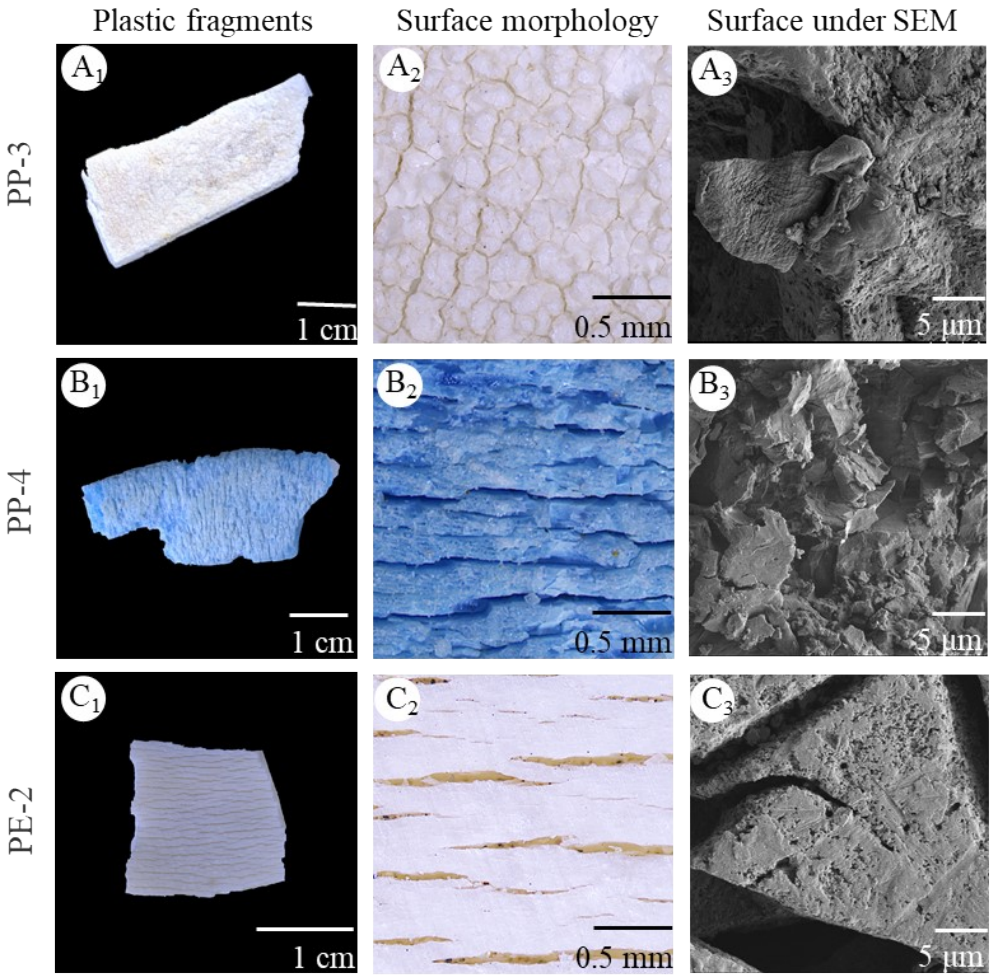

Fig. S2 Morphological characteristics of plastic pieces from the environments. A_1-C_1 and A_2-C_2 are obtained using optical microscope; A_3-C_3 is obtained using SEM.

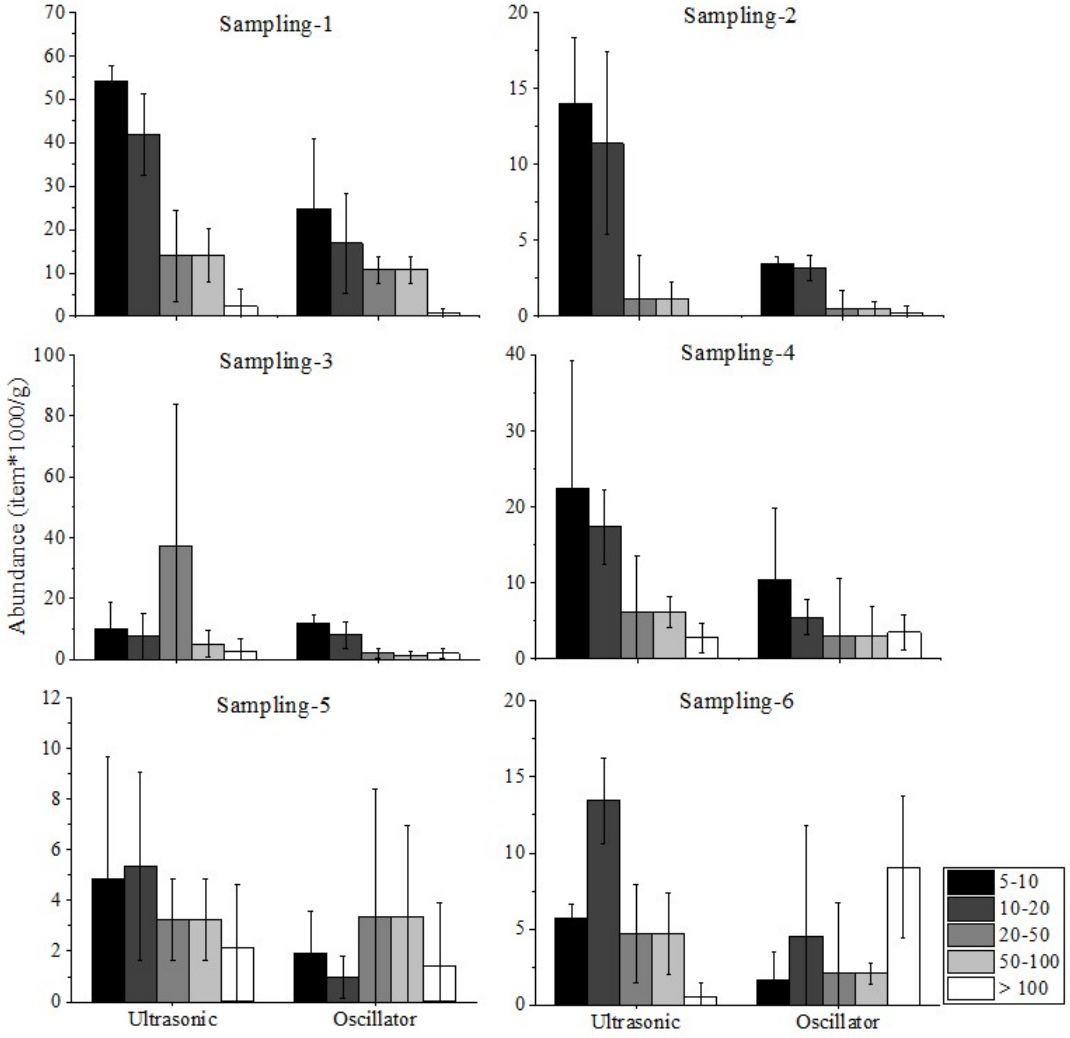

Fig. S3 Abundances and size distribution of six samples after treatments of ultrasonic and oscillator. Samples 1-5: polypropylene; Sample 6: polyethylene.

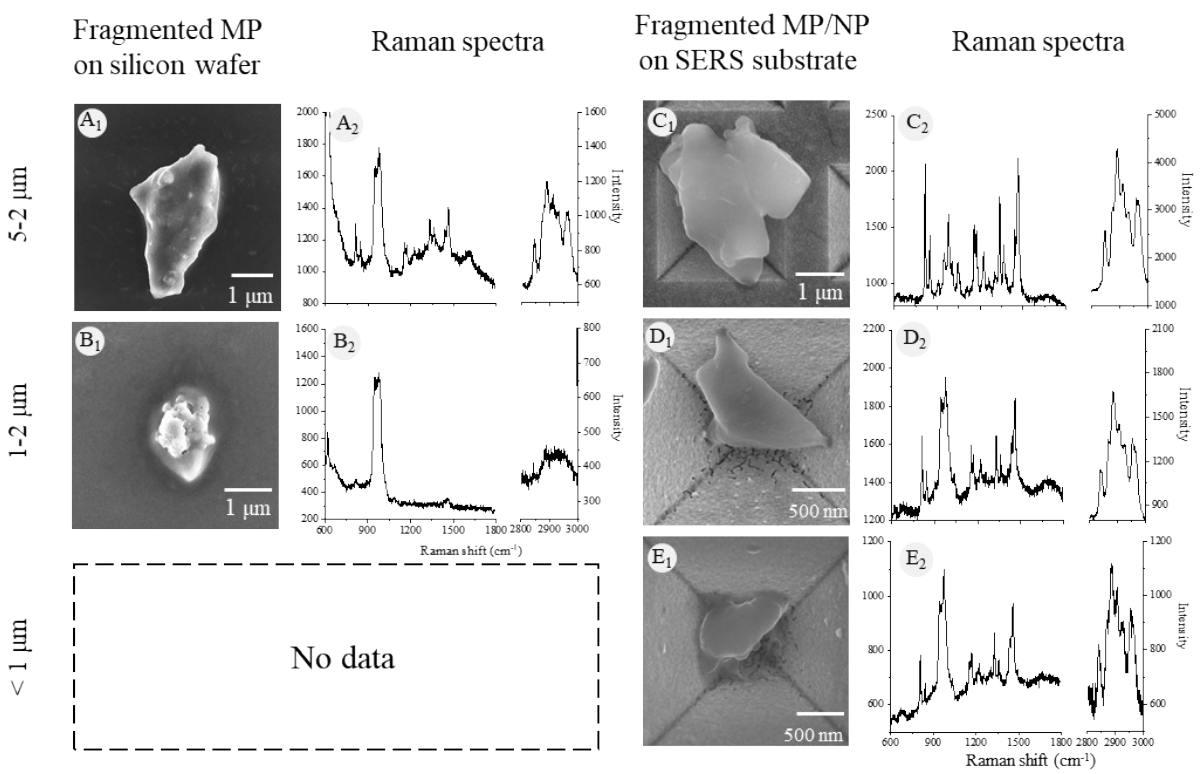

Fig. S4 SEM images and Raman spectra of MPs/NPs on the SERS substrate and silicon wafer

Table S1 The aging characters of six types of plastics

Sample number	Measurable parameters			Crack feature
	Line density	Surface loss	C*E（Contrast*Entropy）	
PP-1-1	19.000	34.685	0.189	
PP-1-2	13.033	29.647	0.141	Powdered
PP-1-3	18.138	26.616	0.158	
PP-2-1	13.029	29.741	0.202	
PP-2-2	7.934	26.779	0.162	Switch
PP-2-3	6.625	20.820	0.225	
PP-3-1	19.000	35.417	0.338	
PP-3-2	26.203	23.750	0.360	Polygon
PP-3-3	38.246	17.960	0.922	
PP-4-1	22.836	35.750	1.755	
PP-4-2	10.389	29.047	0.587	Long line
PP-4-3	11.212	32.146	0.505	
PE-1-1	5.826	61.210	3.101	
PE-1-2	4.924	18.067	3.401	Square
PE-1-3	4.716	45.186	3.369	
PE-2-1	6.038	9.543	0.177	
PE-2-2	25.744	15.968	1.087	Short line
PE-2-3	4.456	7.830	0.148	

* Line density: for measuring the morphological features of crack; Surface loss: using the color threshold for measurement; Contrast*Entropy: using gray-level co-occurrence matrix for measuring the morphological features of irregular patten.

Table S2 The abundance and recycling rate of internal standard of PS spheres and PP fragments after extracting by two flotation agents (NaCl, NaBr)

Sample	Abundance of PS spheres (items/100 g sediment)	Recycling rate of PS spheres	Abundance of PP fragments (items/100 g sediment)	Recycling rate of PP fragments
NaCl				
Sample-1	293±87	3.60%	369±373	26.61%
Sample-2	138±50	1.70%	724±678	52.23%
Sample-3	292±125	3.59%	609±179	43.91%
NaBr				
Sample-1	292±164	3.59%	355±346	25.61%
Sample-2	339±28	4.17%	369±366	26.61%
Sample-3	285±78	3.50%	253±391	18.30%