Supplementary Information:

Rapid identification and quantification of microplastics in the environment by quantum cascade laser based hyperspectral infra-red chemical imaging

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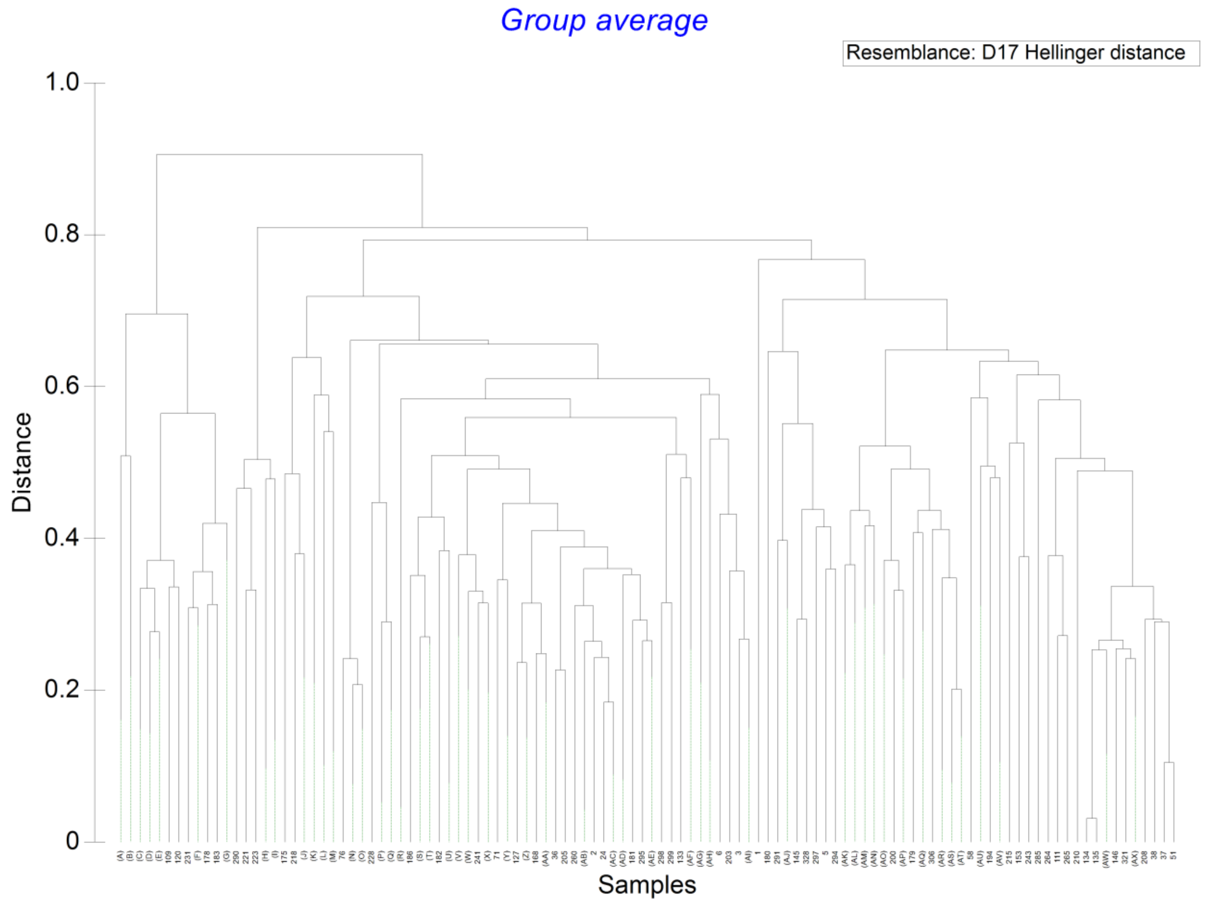

Figure S1: Dendrogramm derived based on the Hellinger Distance and the manual evaluation of the combined spectra. The green dotted represent combined polymer IDs representing the polymer type clusters. A:14+226; B:70+72; C:52+54; D:117+272+273; E:53+12+13+219+278+276+277+224+80+319+116+123+225+270+300+320+118+119+86+87+88+124; F:249+108+325+143+248+247+252+229 +230+232+250; G:296+56+73; H:187+188; I:206+244; J:251+195+234; K:166+214+213+322+138+165; L:196+235; M:204+242; N:78+79; O:74+75; P:307+201+327; Q:202+107+263; R:258+259; S:23+18+20+21; T:275+274+44+45+43+170+261+164+163+185+173+189+171+139+216+217+174+323+227+172+136+98+99; U:141+142; V:33+4+35+32+318+95+197; W:303+305; X:140+304; Y:154+131+63+83+84; Z:257+256+284+282+283; AA:238+239+236+237; AB:65+66; AC:91+92; AD:211+212; AE:112+192+324+190+191+25+193; AF:130+17+129+184; AG:209+176+177; AH:67+288; AI:114+125; AJ:293+271+292+255+233+254+253+326+64+68; AK:40+301+94+106+245+246+96+93+104; AL:286+16+97; AM:7+207+10+8+9+81+121; AN*:329+333+330+332+331+334; AO:34+85+82+122+100+309+89+90+47+48+49+103+308+113+31+269; AP:102+128+69+101; AQ:39+59; AR:57+61; AS:198+199; AT:77+240; AU:55+60; AV:220+222; AW:302+144+289+310+317+314+316+30+50+268+62+15+132+287+315+26+29+311+312+11+115+27+28+126+313+266+267; AX:279+281+41+42+148+105+161+149+159+137+157+158+280+147+156+155+160+150+151+152; *: separated into 3 clusters but the separation could not be visualized (see ESM2.xlsx for details); ** 208 and 31 were merged to one cluster but could not be visualized.

Paragraph S1: Cluster analysis and data validation:

Similar to the FT-IR database, the clusters (see Figure S1) were ranked from high importance (1) to low importance (4) to allow a rapid testing and evaluation of the cluster performance on measured data. Starting with the clusters of high importance, a database was created using the marked spectra and tested against the environmental sample RefEnv1 as a first rapid screening. By carefully adding additional clusters to the list, the level of complexity was increased. During this process an additional cluster containing silicates measured via ATR-FT-IR was added. After the inclusion and evaluation of most cluster derived via the FT-IR database the process was amended. Instead of only the 36 files from environmental sample, a total of 310 files from the single polymers were analyzed prior to the environmental sample, to improve the data quality and check for cross identifications. Due to experience with different systems using a similar spectral range[1], the signal of water was added. Many materials on the filter, like, for example silicates, show a hydroscopic behavior, and allow quality control of the drying process by this cluster.

Once the maximum level of detail using the pure ATR-FT-IR spectra was reached (see Supplementary Figure 2) each individual cluster was supported by spectra from EC-QCL transmission measurements. It was found that an additional cluster was needed, containing spectra at maximal total absorbance, to improve the data quality and avoid false positives. Currently, the data of this cluster is removed for image analysis. The achieved database was than further validated using the EC-QCL measurement of RefEnv1 based on a manual reanalysis of the found hits for natural and synthetic polymers similar to previous studies[2, 3]. Here, for each polymer type 1000 spectra were randomly selected if available by assigning a random numbers from 0 to 1 to the datapoint in excel (10 times recalculated) and sorting by descending order. If a lower number was achieved, all spectra were selected for quality assurance. Within this process, each spectrum was manually compared with the assigned reference spectrum and evaluated by expert knowledge with a quality factor. This factor ranges from a perfect assignment (100), good assignment (75), satisfactory assignment (50), bad assignment (25)) down to a clear miss assignment (1). In most cases after the analysis of at least 100 spectra the resulting ratings showed clear a trend towards a threshold value, which was further tested with at least 100 additional spectra, if available. Due to time constrains the number of analyzed spectra ranged in average from 200-250 for each polymer type.

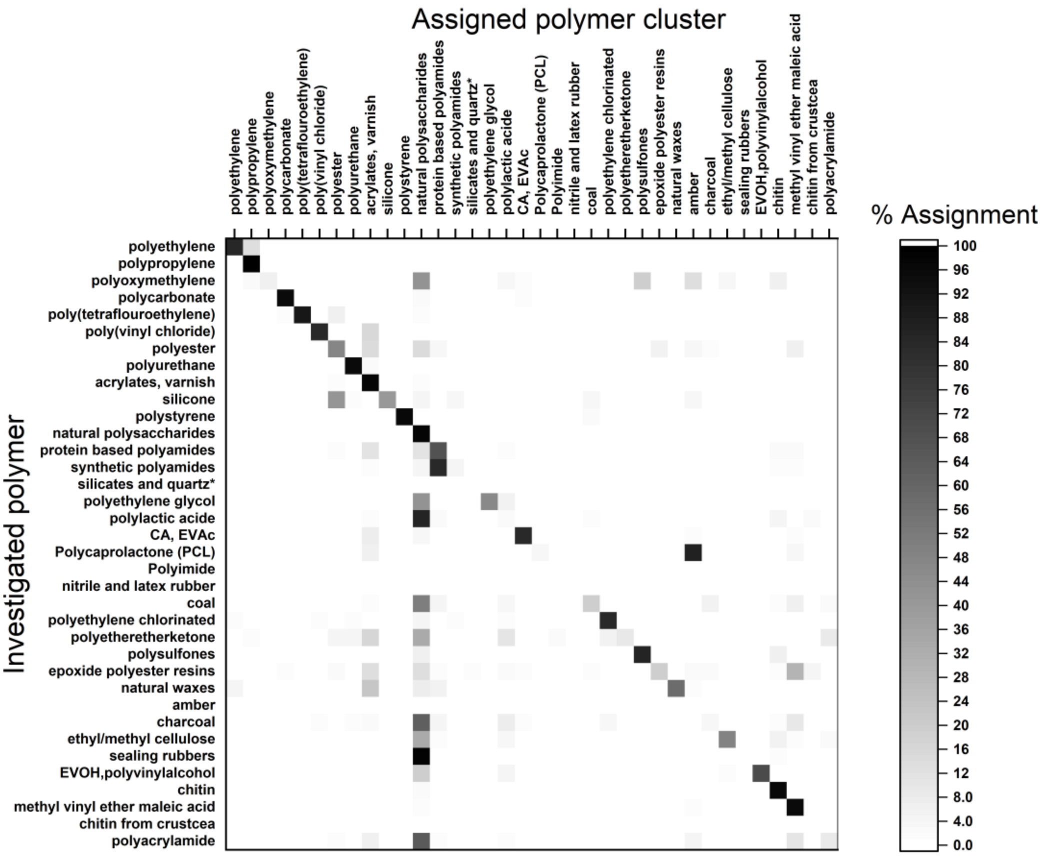

Figure S2: Cross-validation of polymer types separable with the range 1800 – 1084 cm^{-1} based on the hierarchical cluster analysis of attenuated total reflection (ATR) Fourier-transform infrared (FT-IR) spectra.

y,x	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6
6							1						
5							1						
4			1				1				1		
3				1	1	1	1	1	1	1			
2				1	1	1	1	1	1	1			
1				1	1	1	1	1	1	1			
0	1	1	1	1	1	1	1	1	1	1	1	1	1
-1				1	1	1	1	1	1	1			
-2				1	1	1	1	1	1	1			
-3				1	1	1	1	1	1	1			
-4			1				1				1		
-5							1						
-6							1						

Figure S3: The area around a target pixel (red) investigated for closing using the automated particle analysis (APA, green) and the added cross hair alike area (black) for the external cavity quantum cascade laser EC-QCL setup.

Table S1: Minimum thresholds value for positive identification for the hit quality index (ranging from a minimum of 600 to a maximum of 2000) for the derived polymer types together with an average density of mass calculation[4] based on the material data sheets and literature[5].

Cluster number	Threshold	Density for mass calculation	Polymer type name
1	600	0.95	polyethylene
2	600	0.95	polypropylene
3	1430	1.25	polyoxymethylene
4	1000	1.2	polycarbonate
5	1340	0	poly(tetraflouroethylene)
6	1230	1.44	poly(vinyl chloride)
7	1300	1.38	polyester
8	1100	1.297	polyurethane
9	1200	1.24	methacrylates, polycaprolactone, varnish
10	600	1.03	silicones
11	600	1.03	polystyrene
12	1100	1.55	natural cellulose
13	1325	1.15	natural polyamides
14	1325	1.09727273	synthetic polyamides
15	600	2.19	silicates and quartz
16	600	1.12	polyethylene glycol
17	1050	1.25	polylactic acide
18	1400	1.3	cellulose acetate, ethylene vinyl acetate/alcohol
19	1125	0.92	nitrile and latex rubber
20	1310	0.83	coal
21	1275	1.16	polyethylene chlorinated
22	1250	1.32	polyetheretherketone
23	1275	1.24	polysulfones
24	1300	0.961	natural waxes
25	1300	0.4	charcoal
26	600	0.99	sealing rubbers
27	1300	0.38	chitin
28	600	1	water
29	600	1.16	polyvinylalcohol
30	2000	0	baseline spectra

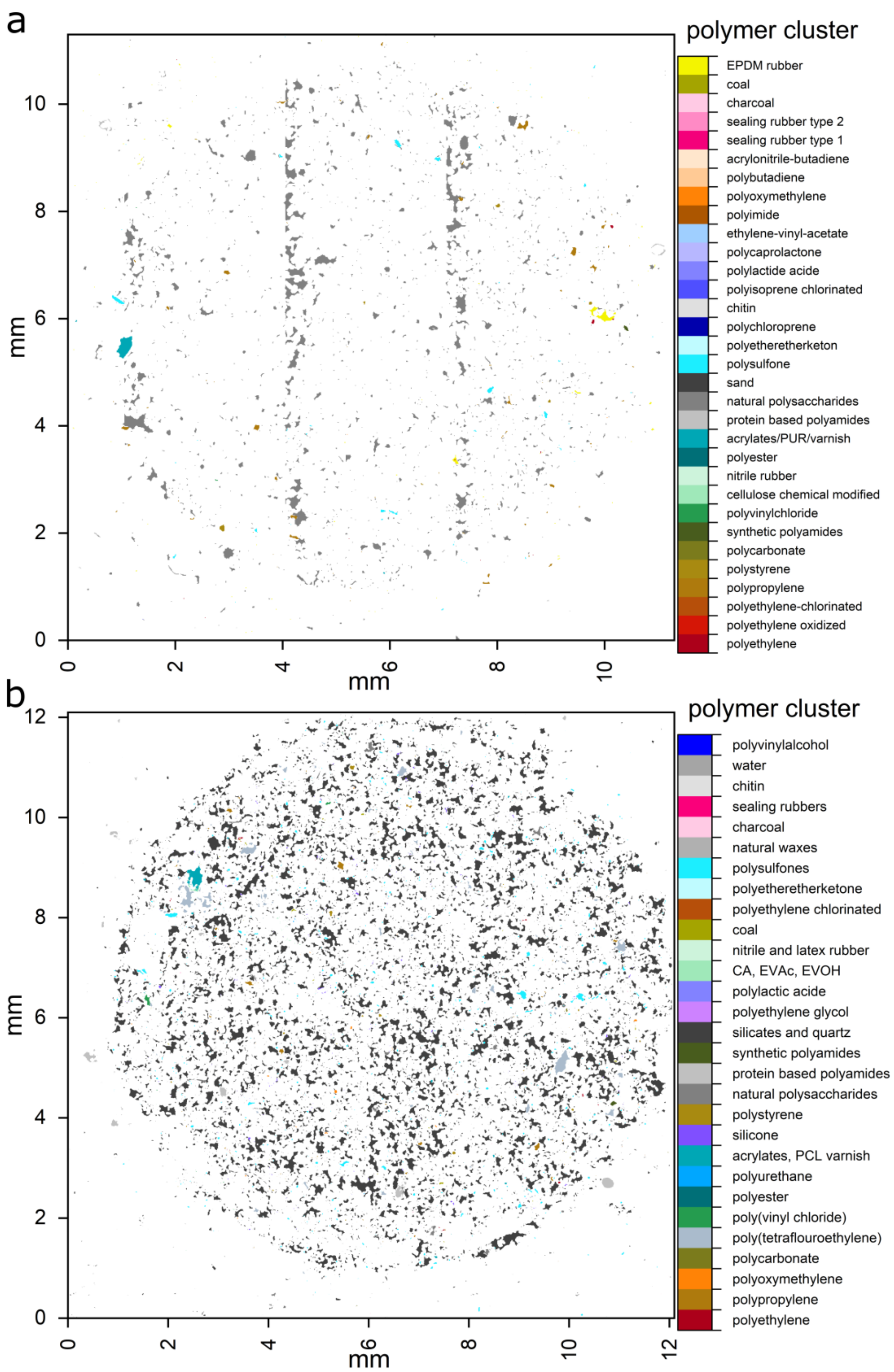

Figure S4: Polymer type dependent false color overview images of the sample surface of the sample RefEnv1 measured via a) hyperspectral Fourier-transform infrared (FT-IR) imaging and b) external cavity quantum cascade laser (EC-QCL) hyperspectral imaging. CA: cellulose acetate; EPDM: ethylene propropylene diene-monomer; EVAc: ethylene-vinyl-acetate; EVOH: ethylene-vinyl-alcohol; PCL: polycaprolactone; PUR: polyurethane.

Paragraph S2: Hyperspectral imaging with 1.4 µm pixel resolution:

Compared to the LowMag the wave number range is stronger limited by the Anodisc to 1800 to 1196 cm^{-1} indicating a strong loss of signal compared to the LowMag. This difference is probably caused by the higher numerical aperture of the HighMag lens (0.7 NA) compared to 0.3 NA with the LowMag objective, but could not be further investigated in this study. The same database as derived to the LowMag was used to investigate the potential of this measurement mode for future studies. It was rapidly found that a dataset of the full measurement (400 fields, ROI of 13.1 × 13.1 mm) was beyond the scope of most commercially available software tools for data visualization (limited to 90 million datapoints) while the 92.16 million spectra could be handled by the Python script. For data visualization, the dataset was reduced to 361 fields (ROI of 12.4 × 12.4 mm) and the derived polymer dependent false color image is depicted in Figure S5.

In this case for most polymer types both lenses achieved a similar composition (see Figure S6), while higher number of particles especially for small sized particles were determined via the HighMag. Additional assignments to PPSU and PP are indicated and PTFE is found in higher numbers in comparison. On a closer look (Figure S5), the resulting particles showed an increased fractioning caused by optical effects which might be caused by a measurement slightly out of focus or other measurement parameters. The impact of these on the result were not visible at an early stage of the measurement and the lack of certified small sized polymer reference materials made a further investigation impossible. This issue should be addressed in the future prior to application of this measurement mode. Despite this technical issue, the measurements using the HighMag show potential for rapid measurement, compared to Raman, of small sized microplastics, even <10 µm.

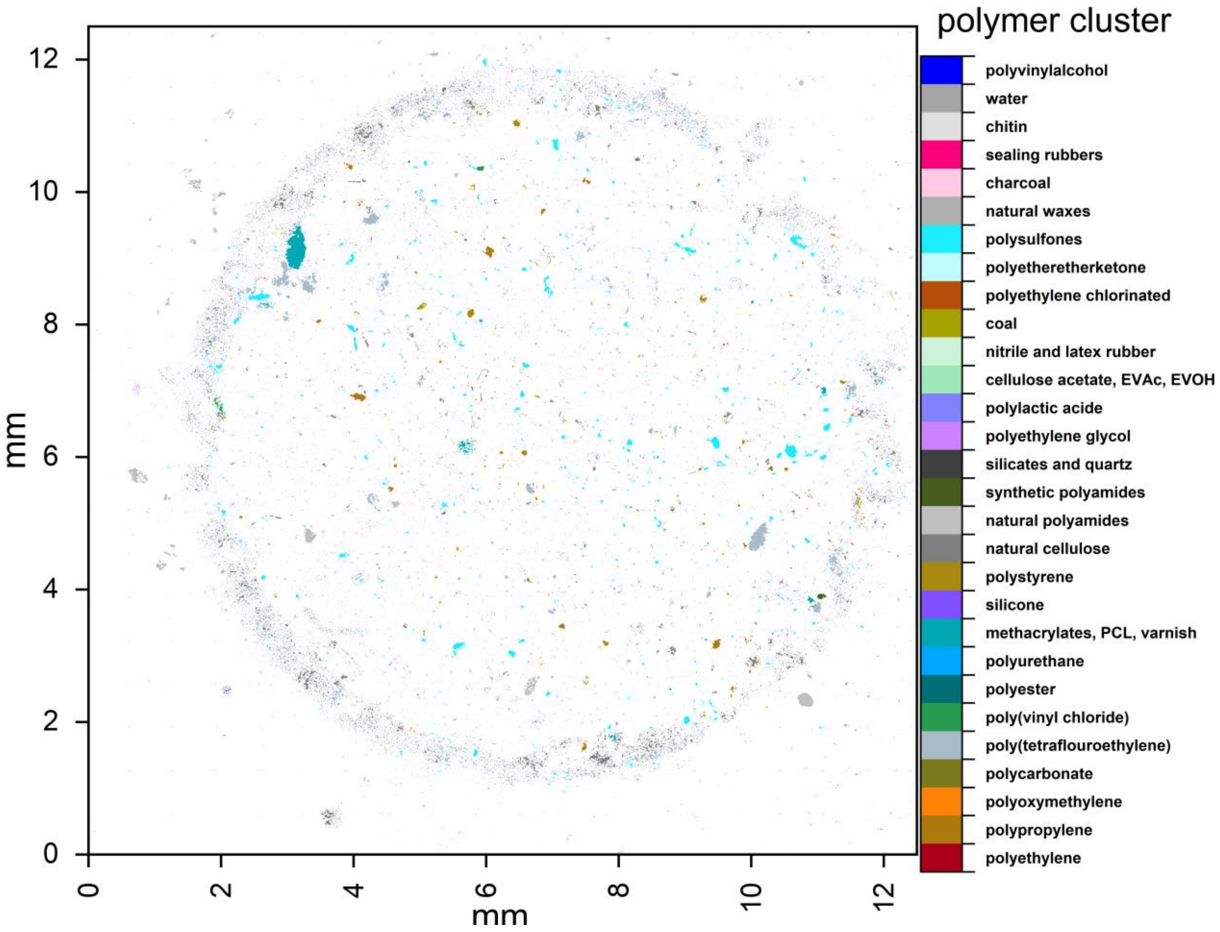

Figure S5: The sample RefEnv1 measured with external cavity quantum cascade laser (EC-QCL) at magnification of 12.5 with a resolution of 1.365 µm per pixel for a region of interest of 12.4 × 12.4 mm using derived database. No quality assurance was performed at this stage. PCL: polycaprolactone; EVAc: ethylene-vinyl-acetate; EVOH: ethylene-vinyl-alcohol.

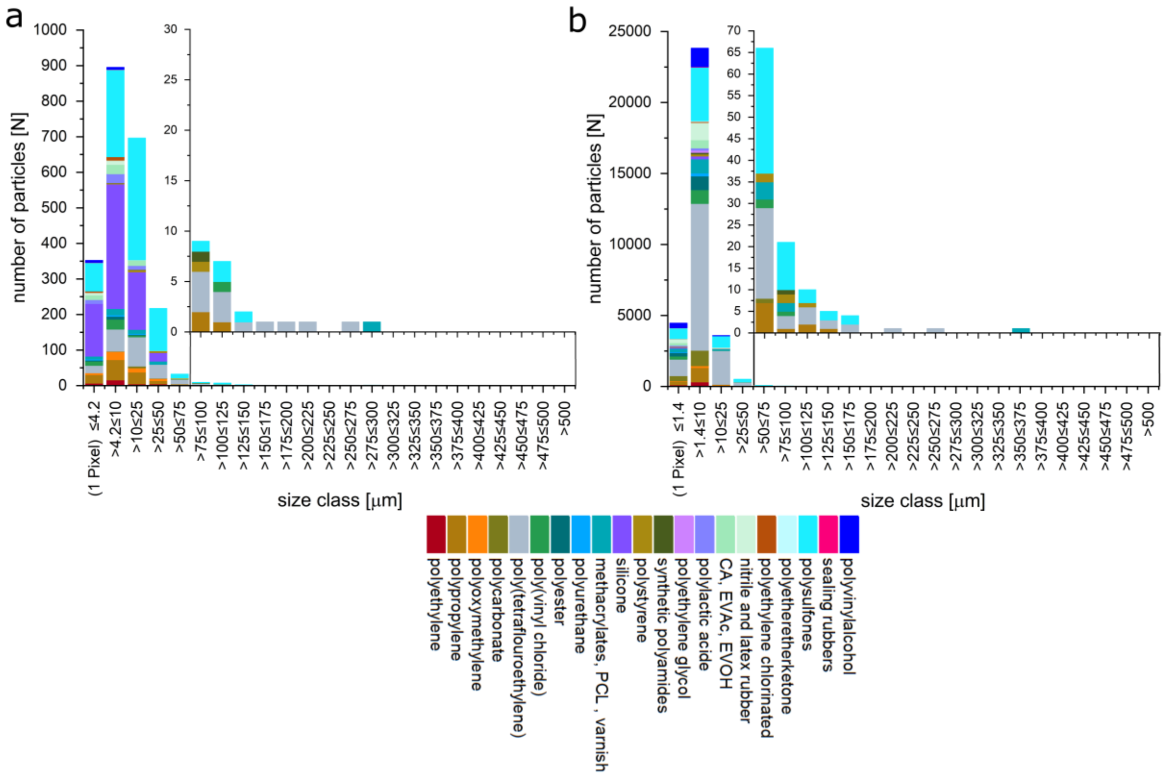

Figure S6: Polymer numbers derived from the sample RefEnv1 using a) the LowMag at a pixel resolution of 4.2 µm and b) the HiMag at a pixel resolution of 1.4 µm.

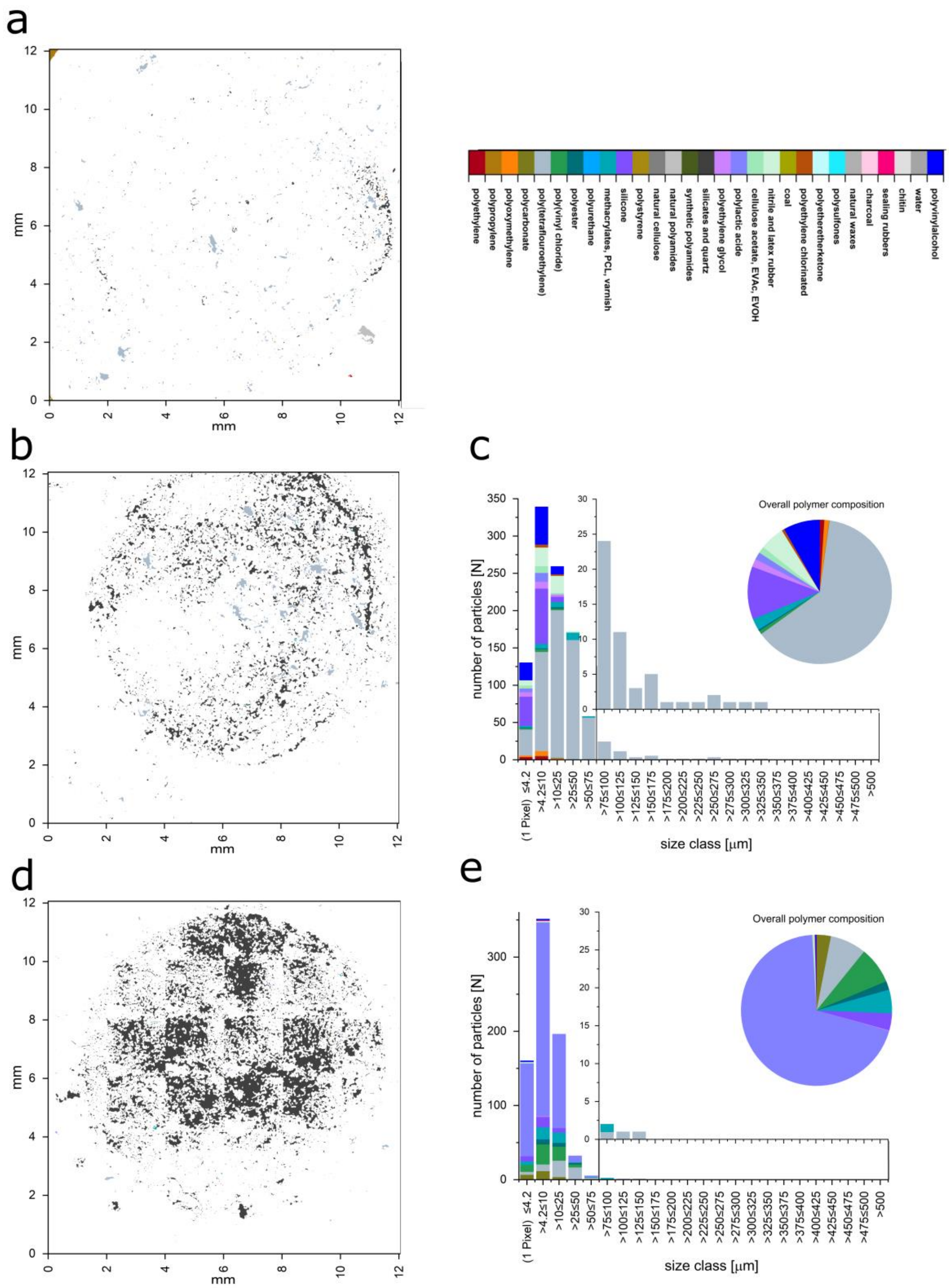

Figure S7: a) False color overview image of the Arctic deep sea sediment sample with the signal of the polypropylene support ring of the Anodisc present. Procedural blank samples analyzed by hyperspectral external cavity quantum cascade laser (EC-QCL) imaging for b,c) Arctic deep sea sediments and d,e) marine surface waters showing the determined polymer dependent false color overview images (b,d) and particle numbers and composition found per size class (d,e). In both cases high numbers of silicates were present. EVAc: ethylene-vinyl-acetate; EVOH: ethylene-vinyl-alcohol; PCL: polycaprolactone.

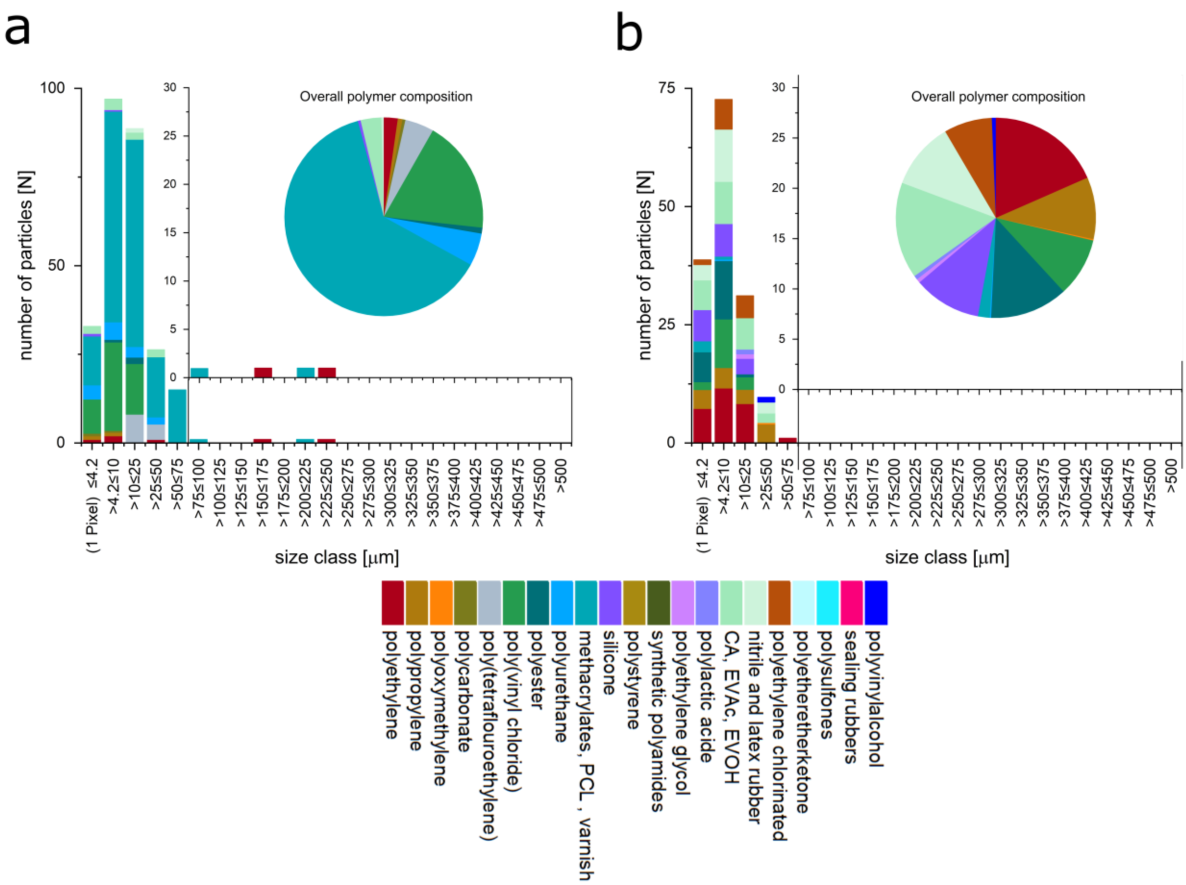

Figure S8: a) Synthetic polymers identified in marine surface water after blank correction with the respective particle numbers from the procedural blank. b) Synthetic polymers identified in deep sea sediments after removal of the PTFE findings and blank correction with the respective particle numbers from the procedural blank.

References

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