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of subsampling on polymer diversity**

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## 1 **Environmental Significance**

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6 2 Microplastics (MP) are a diverse contaminant suite and robust data on polymer profiles, particle  
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8 3 size, and morphology are needed to help inform the expected exposure to different positively  
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10 4 buoyant polymer types and the potential for treatment. Here, stormwater MP were studied to  
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12 5 provide guidance on the impact of subsampling on polymer profiles and the concentration of MP  
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14 6 determined. Smaller stormwater MP (63-250  $\mu\text{m}$ ) were present at one to two orders of  
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16 7 magnitude greater concentrations than larger MP (250-2000  $\mu\text{m}$ ) and had greater diversity of  
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18 8 polymer types. Many polymers had spectral signatures of surface oxidation indicative of  
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20 9 weathering. The data presented including polymer type, morphology, and size can help inform  
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22 10 exposure assessments for aquatic organisms or design of MP stormwater removal technologies.  
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# Urban stormwater microplastic size distribution and impact of subsampling on polymer diversity

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## **ABSTRACT**

Understanding not only microplastic (MP) concentration but also size distribution, morphology, and polymer profiles is desirable for stormwater, which is an important pathway of entry for MP into the aquatic environment. A challenge is that subsampling is often required for analysis of environmental samples and the impact of subsampling on the stormwater MP concentration determined and the polymer types identified is poorly characterized. To address this, MP were extracted from urban, suburban, and green infrastructure stormwater. Fourier Transform Infrared microscopy was performed to characterize MP, in addition particle dimensions and morphology were recorded. Varying the number of 63-250  $\mu\text{m}$  particles subsampled per sample demonstrated the coefficient of variation for concentration (standard deviation/mean) for most samples was  $<0.3$  when 20 particles (0.8-15% of total particles) or  $<0.2$  when 30 particles (1.2-24% of total particles) per sample were analyzed. MP concentrations in the 63-250  $\mu\text{m}$  size class ranged from 15 to 303 MP/L, one to two orders of magnitude greater than observed in previously reported paired samples from the 250-500 or 500-2000  $\mu\text{m}$  size classes. A total of 25 plastic polymer types were observed across samples, more than observed in the large size classes. Spectral signatures of surface oxidation indicative of weathering were observed on most

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3 polyethylene, polypropylene, and polystyrene particles, which were the most abundant polymer  
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5 types. Fragments were the dominant morphology with average maximum length of  $158 \pm 92 \mu\text{m}$ .  
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7 Overall, these results may help inform subsampling methods and be useful in future exposure  
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9 assessments for aquatic organisms or design of MP removal technologies for urban and suburban  
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11 stormwater.  
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15 **KEYWORDS:** FTIR microscopy; microplastic; subsampling; oxidized polymers  
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## 18 **Introduction**

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22 There is an urgent need to understand and control the pathways of entry for plastics into the  
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24 water environment given estimates that 4.8–12.7 MMT of plastic litter enters the ocean each  
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26 year.<sup>1</sup> Microplastics (MPs) are synthetic polymers <5 mm and the majority of aquatic MPs  
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28 observed in the environment result from the fragmentation of larger plastic debris. Physical and  
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30 chemical (e.g., UV-degradation, oxidation, and hydrolysis) processes can both contribute to  
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32 plastic fragmentation.<sup>2,3</sup> The resulting smaller size MPs may pose a greater threat to marine  
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34 biota due to the particles' resemblance to smaller size prey, increasing the chances of  
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36 ingestion.<sup>e.g.,4</sup> and translocation from the digestive tract to other tissues (for MP <83 $\mu\text{m}$ ,<sup>as reviewed</sup>  
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38 by<sup>5</sup>).  
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44 Stormwater is a significant pathway of entry for MP pollution into the freshwater environment.<sup>6-</sup>  
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46 <sup>11</sup> In addition to runoff, wet deposition of atmospheric MP can contribute to the number of MP  
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48 present in stormwater.<sup>12,13</sup> For example, -it was estimated that 62% of MPs in the Baltic Sea  
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50 originates from stormwater.<sup>14</sup> Also of concern are combined sewer overflows (CSOs) that carry  
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52 untreated wastewater in addition to stormwater in cities with outdated infrastructure.<sup>7,15,16</sup> One  
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54 sampling location for the present study is among the ~700 communities in the US that have  
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3 combined sewer infrastructure.<sup>17</sup> In recent years, MP observations in stormwater have been  
4 reported from around the world (Table 1) using a range of sample volumes, techniques for  
5 extraction, and analytical approaches. Cross-study comparison is complicated by the variety of  
6 approaches applied in these studies (Table 1). Nonetheless, these studies indicate that MPs are  
7 present in stormwater and retention basins and that green infrastructure may contribute to its  
8 removal. Also, differences in land use result in varying MP sources, MP concentrations, and  
9 polymer profiles, as recently reviewed.<sup>18</sup> The present study builds upon previous work by our  
10 team that (1) showed higher MP concentration in stormwater compared to wastewater effluent  
11 and hydraulically connected surface waters<sup>19</sup> and (2) the influence of rainfall depth/sampling site  
12 on inter-storm variation of MP concentrations in the 250–2000 µm particle size-class.<sup>20</sup>  
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27 Given that the number of particles remaining after extraction from many water matrices can  
28 exceed the quantity that can be readily analyzed particle-by-particle, many researchers perform  
29 subsampling using varying number of percentages of particles to provide a representative result  
30 (Table 1). Some researchers performed chemical confirmation of visually identified particles<sup>6, 7,</sup>  
31 <sup>16, 21-23</sup> representing an improvement for preventing false positives<sup>24</sup> over visual identification  
32 alone. Others report analysis of percentages ranging from 8-10% of a sample<sup>22, 25</sup> to <1-70% or  
33 more of filter area.<sup>10, 26</sup> Sub-sampling techniques have been explored indicating the need to  
34 consider extraction-specific characteristics (i.e., particle distribution over a filter when  
35 performing subsampling scans of a filter<sup>27</sup>) and matrix specific characteristics (i.e., matrix-to-  
36 matrix MP concentration differences, need for more subsampling when MP concentrations are a  
37 smaller proportion of total particles in a sample<sup>28, 29</sup>). Guidance based on analysis of the impact  
38 of the number/percentage of particles subsampled would be useful not only to understand MP  
39 concentration<sup>28</sup> but also polymer diversity; the latter is also explored here.  
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**Table 1** Summary of stormwater MP literature including location, methods (sampling, extraction, density separation, and analysis), target particle size range, and high and low MP concentrations reported. WPO= wet peroxide oxidation, VID=visually identified, nr=not reported. Citations grouped by continent the study was from (North America, Asia, Australia, Europe) then in alphabetical order by first author.

Citation number	Location	Volume sampled (L)	Extraction	Density separation	Analytical method, percent of particles on filter or number of particles analyzed	Particle size ( $\mu\text{m}$ )	MP conc. (MP/L) Min Max	
This work, <sup>19, 20</sup>	New Jersey, USA	5	WPO	NaCl	ATR-FTIR, all; microFTIR, 40 particles/sample	63-2000	14	354
Werbowski et al. <sup>11</sup>	San Francisco, CA, USA	25-295	nr	CaCl <sub>2</sub>	FTIR, Raman, Py-GC/MS, 895 particles total	125-1000	1.1	25
Grbic et al. <sup>22</sup>	Toronto, Canada	4	nr	CaCl <sub>2</sub>	Raman, 10% of particles	25 to ns	13	
Ross et al. <sup>23</sup>	Calgary, Canada	10	WPO	Nr	Raman, 10% of VID particles	37 to >1000	<1	204
Smyth et al. <sup>6</sup>	Vaughan, Canada	0.5-2.6	nr	CaCl <sub>2</sub>	FTIR, Raman, 10% VID particles	106 to >1000	22	705
Pinon-Colon et al. <sup>7</sup>	Tijuana, Mexico	3	nr	NaCl	ATR-FTIR, percent of VID particles		66	191
Cho et al. <sup>10</sup>	Gumi, South Korea	6	WPO	Li <sub>2</sub> WO <sub>4</sub>	microFTIR, <1% of filter area	20-5000	56	639
Sang et al. <sup>21</sup>	Wuhan, China	8	WPO	Nr	Raman, 96 VID particles	37-5000	2.7	195
Mak et al. <sup>30</sup>	Hong Kong	192	nr	Nr	FTIR, Raman	54-1000	1.2	6.8
Herath et al. <sup>31</sup>	New South Wales, Australia	30	WPO	NaCl	ATR-FTIR, all	48.5-5000	1.9	2.5
Monira et al. <sup>32</sup>	Melbourne, Australia	nr	WPO	Nr	ATR-FTIR, all	15-4600	15	33
Ziajahromi et al. <sup>16</sup>	Gold Coast, Australia	10	WPO	NaI	FTIR, >50% VID particles	25 to >500	<1	680
Lange et al. <sup>33</sup>	Vasternorrland, Sweden	<1	WPO	Nr	FTIR, microFTIR, all	20-100	<1	8580
Liu et al. <sup>25</sup>	North of Jutland, Denmark	722-1139	WPO	ZnCl <sub>2</sub>	FTIR, 8% sample volume	10-2000	<1	42
Treilles et al. <sup>26</sup>	Paris, France	82-103	WPO (selected)	NaI (selected)	ATR-FTIR, 4microFTIR, all or up to 70% of filter	25-5000	12	133

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3 The objectives of the present study were to (1) expand our understanding of the impact of the  
4 number/percentage of particles subsampled on stormwater MP concentration and polymer types  
5 and (2) to provide new data on stormwater MP concentration size, morphology, and polymer  
6 type for previously unanalyzed particles in the 63-250  $\mu\text{m}$  size class.<sup>19, 20</sup> FTIR microscopy was  
7 used to document the morphology, size, and polymer types of MP observed in stormwater.  
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10 Bootstrap analysis was applied to determine the impact of subsampling particles on the estimated  
11 MP concentration and number of total and synthetic polymer types observed. Results were then  
12 compared between sampling sites and storm events as well as to previously reported data from  
13 the larger size classes of MP.<sup>19, 20</sup> Overall, these results may help inform subsampling methods  
14 for urban stormwater and the data presented may be useful in future exposure assessments for  
15 aquatic organisms or design of MP removal technologies for urban and suburban stormwater.  
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## 18 **Materials & Methods**

### 19 *Stormwater collection and MP extraction*

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21 Composite stormwater sampling (5 L total, 1 L taken every 10 to 45 min) was conducted at  
22 various urban and suburban locations in New Jersey, USA (Fig. S1). The stormwater samples  
23 analyzed in this study include three described in Bailey et al.,<sup>19</sup> nine described in Boni et al.,<sup>20</sup>  
24 and one not previously reported (Table 2). Samples were stored in triple rinsed 1 L glass jars  
25 (Ball Corp. Broomfield, CO) at 4°C until sieving (63-250, 250-500, 500-2000  $\mu\text{m}$ ). A wet  
26 peroxide oxidation was performed (20 mL 30% hydrogen peroxide with 20 mL of 0.05 M  $\text{FeSO}_4$ ,  
27 heated on a hot plate at 65°C and stirred at 120 rpm for at least 30 minutes).<sup>31</sup> Then, samples  
28 were suspended in a saturated NaCl solution in glass funnels with surgical tubing clamped at the  
29 outlet. Funnels were topped with foil to prevent contamination, and allowed to sit overnight  
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3 after which settled particles were emptied through the surgical tubing.<sup>31</sup> Positively buoyant  
4 particles ( $\rho < 1.2 \text{ g/cm}^3$ ) were filtered onto stainless steel mesh (20  $\mu\text{m}$ , TWP, Berkeley, CA) and  
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6 stored in closed glass petri dishes until analysis. For quality assurance and control, field blanks  
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8 (two from Bailey et al.,<sup>19</sup> one from Boni et al.<sup>20</sup>) consisting of 1 L deionized or tap water were  
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10 analyzed in parallel with the field samples. Note, future studies should consider the now  
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12 available American Society for Testing and Materials (ASTM) methods<sup>34</sup> that recommend  
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14 sample volume, adding digestions to reduce non-plastic debris (e.g., cellulose degradation), and  
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16 facilitate capture of non-buoyant particles by not performing density separation (which would  
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18 include most tire-wear). Field blanks and matrix spike (MS) recoveries were performed as  
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20 described previously with MS recovery of  $97\% \pm 6\%$  for known quantities  
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22 of polyethylene extracted from a personal care product in the 250-2000  $\mu\text{m}$  size range.<sup>20</sup>  
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### 29 ***Subsampling***

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32 The substantial number of particles present in the 63-250  $\mu\text{m}$  size class (Table 2) made analyzing  
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34 all particles using the methods applied here infeasible. Therefore, all particles were counted in  
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36 each sample and the subset of particles analyzed were scaled to estimate total MP concentrations  
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38 (Eq 1). For counting total particles, images were taken of the particles on the filters (47 mm  
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40 diameter) using a Moticam™ 580 microscope camera (Motic Asia, Hong Kong); particles were  
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42 manually counted using ImageJ™ (<https://imagej.nih.gov/ij/>). To aid the counting process, a  
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44 sample petri dish was placed on a pattern which consisted of 21 circular numbered segments  
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46 (Fig. S2). A 1951 USAF resolution test chart (Edmund Optics, Barrington, NJ) was used to  
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48 determine which particles were smaller than 62.50  $\mu\text{m}$  (in all dimensions, Fig. S3) and these  
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50 particles were excluded from the total particle count (as the focus was on the 63-250  $\mu\text{m}$  size  
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52 range particles).  
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3 To facilitate the subsampling of particles on a filter, given that particles appeared relatively  
4 evenly distributed across the filter (Fig. S4), a pie cut stencil was created with eight equal sectors  
5 that was then divided into twenty numbered sections (Fig. S5). Note, recommendations  
6 regarding the schema used for subsampling have been explored by other researchers and careful  
7 consideration of particle distribution patterns should be considered prior to subsampling.<sup>27, 28</sup> A  
8 random number generator was used to determine which of the twenty sections to sample from,  
9 with two particles in the center of the respective section selected for analysis followed by  
10 rotating the filter 45° counterclockwise to allow sampling from one of the twenty sections of the  
11 adjacent pie slice sector of the filter. The process was repeated until 40 particles per sample  
12 were collected for FTIR analysis. This number of particles was selected following bootstrap  
13 analysis of FTIR analysis results from 81 particles from Field P (10/12/2020), described below.  
14 Across the stormwater samples, a total of 521 particles were analyzed, representing 1.6-31% of  
15 total particles per sample (Table 2).  
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### 33 34 ***Vibrational spectroscopy*** 35

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37 Identification of stormwater MPs was conducted primarily via a Bruker LUMOS FTIR-  
38 microscope which utilizes a liquid nitrogen cooled MCT detector (Bruker Optics, Ettlingen,  
39 Germany). Due to their small size, selected particles were transferred onto an IR substrate using  
40 needles (Coats and Clark, Charlotte, NC). A visual image of the particle was taken, allowing for  
41 the length to be measured, morphology (fiber, rods, fragments, films, spheres) to be identified,  
42 and color to be recorded (examples of each morphology are provided in Fig. S7). For data  
43 collected in reflectance mode, particles were placed onto Mirr IR slides (Kevley Tech.,  
44 Chesterland, OH). For FTIR transmission data, particles were transferred onto a CaF<sub>2</sub> disc (size:  
45 32 mm × 3 mm) (International Crystal Laboratories, Garfield, New Jersey). An aperture of 10  
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3  $\mu\text{m} \times 10 \mu\text{m}$  for both background (64 scans of IR substrate) and sample scans (32-64 scans) and  
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5 a  $4 \text{ cm}^{-1}$  resolution provided acceptable spectra for this study. For black rubbery particles that  
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7 gave poor quality spectra on the LUMOS 1 microscope, analysis was repeated using an Alpha<sup>TM</sup>  
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9 FTIR-ATR Spectrometer (Bruker Optics, Billerica, MA) with a diamond IRE and Deuterated  
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11 Triglycine Sulfate (DTGS) detector. Background scans of air were collected (64 scans, to  
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13 provide the spectral background), and suspected rubber particles were transferred onto the IRE  
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15 crystal using metal tweezers. To be consistent with parameters used on the LUMOS, 32-64  
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17 sample scans were collected. If quality spectra could not be obtained via FTIR, analysis was  
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19 performed via Raman microscopy (XploRA Plus, Horiba, Piscataway, New Jersey). After  
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21 calibration with a silicon standard, particles were relocated onto a glass slide and placed on the  
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23 Raman stage. The 10x and 50x objectives were used to collect visual images and focus on the  
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25 surface of the sample and 50x and 100x were primarily used for spectral acquisition.  
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### 31 *Spectral Analysis*

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35 Spectra were interpreted by identifying major bands and by comparison with known polymer  
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37 spectra. Based on the wavenumber, relative intensity, and shape of the major bands, the identity  
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39 of each particle was determined. For simple spectra of well-known polymers, e.g., polyethylene,  
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41 polypropylene, or polystyrene with little to no weathering effects, no database search was  
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43 necessary, and the MP particle was identified as the respective polymer. Otherwise, the polymer  
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45 library from OPUS software (Version 7.2), siMPle<sup>TM</sup> (version 1.1.β),<sup>35-37</sup> and/or OpenSpecy<sup>TM</sup>  
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47 (<https://openanalysis.org/openspecy/>)<sup>38</sup> were searched for matches based upon hit quality.  
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51 Consistency of database matches was considered as well as visual inspection of spectral matches.  
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### 54 *Statistical Analysis*

The data collected from this study were evaluated using statistical analysis in Rstudio ([www.project-r.org](http://www.project-r.org)). To estimate the total 63-250  $\mu\text{m}$  microplastic concentration in each stormwater sample ( $[\text{MP}]_{\text{est}}$ , MP/L), the number of microplastics observed in each 40-particle subsample ( $N_{\text{MP}}$ ) was scaled assuming the percentage of MP in the subsample was similar to the total number of particles in the sample:

$$[\text{MP}]_{\text{est}} = \frac{N_{\text{MP}}}{40 \text{ particles}} \times \frac{\text{Total particles}}{\text{Volume of sample}} \text{ Eq 1}$$

A Shapiro-Wilk normality test confirmed the non-normality of the MP concentration data across sampling sites. Random forest (randomForest package) was used to evaluate the impact of factors (i.e., site, rainfall, antecedent dry days) on MP concentrations. To compare polymer profiles across collection sites and sieve size classes, polymer concentrations were log-transformed and a Bray-Curtis dissimilarity matrix was created for use in ordination and represented via non-metric multidimensional scaling (nMDS). An analysis of similarities (i.e., **Table 2** Details of sampling events: sampling site, date of sampling, cumulative rainfall measured in cm, antecedent dry days prior to storm event (data from <https://www.ncdc.noaa.gov/cdo-web/>), and total particles (MP and non-MP).

Site	Description	Date (m/d/y)	Cumulative rainfall (cm)	Antecedent dry days	Total particles (63-20 $\mu\text{m}$ )	Citation (if previously reported)
City N	End of pipe, urban area and heavily trafficked highway	10/16/2019	3.05	9	275 (Field blank 41)	16
		8/4/2020	4.01	3	737	17
		10/12/2020	3.00	11	290	17
		10/29/2020	4.50	12	672	17
Field P	End of pipe, suburban college campus, adjacent to sports fields with artificial turf	10/16/2019	3.05	9	704 (Field Blank 160)	16
		8/4/2020	4.01	3	925	17
		10/12/2020	3.00	11	175	17
		10/29/2020	4.50	12	202	17
Bio-	Overflow pipe,	8/17/2020	1.50	4	2521	17

<b>retention P</b>	located on suburban college campus next to parking lot, road, and buildings	8/19/2020	1.88	1	2021 (Field blank 74)	17
<b>City B</b>	In-pipe, combined sewer*	10/16/2019	5.41	4	404	16
<b>City K</b>	End of pipe, urban area	12/10/2019	3.78	5	127	

*\*sampling conducted with an autosampler during CSO event; all other sampling by hand*

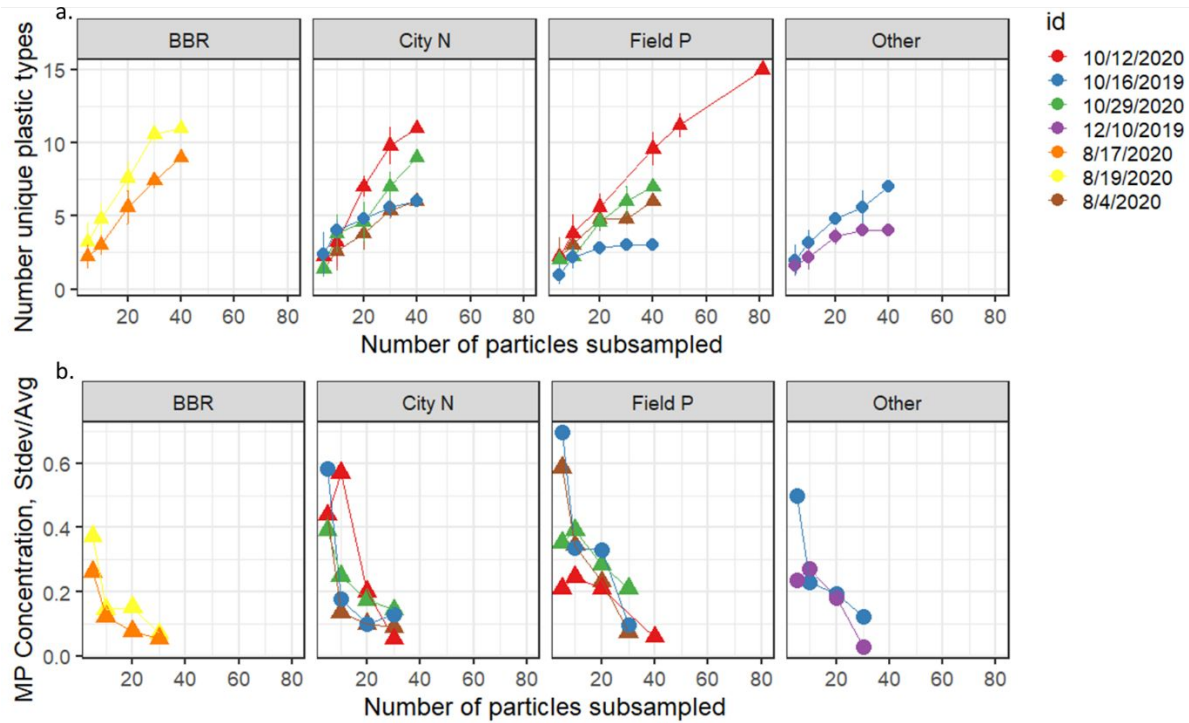
ANOSIM) test was performed on the dissimilarity matrix to understand if size class and - collection site were factors associated with the polymer profiles observed.

## Results

### *Particle count and subsampling*

Subsampling of particles prior to chemical analysis was required due to the large number of particles remaining after extraction and density separation. Total particles in 63-250  $\mu\text{m}$  size range after extraction had a median of 538 particles per 5L of stormwater sampled (Table 2). Field blanks had significantly less particles than the stormwater samples ( $p=0.0088$ , Wilcoxon rank sum), with a median of 74 particles per sample (Table 2). To determine the number of particles that can be subsampled and give reliable representation of the MP concentration, 81 particles were randomly subsampled from Field P 10/12/2020 (which had a total particle count of 175 particles). Out of the 81 randomly selected particles, 43 were identified to be MPs, the remaining particles were either non-plastic (e.g., plant matter, natural fibers) or spectra that could not be identified. Eighteen materials were identified of which 15 were plastics, out of the 81 particles analyzed. Bootstrap analysis indicated that subsampling 5, 10, 20, 40, or 50 particles and scaling (Eq 1) to estimate total MP concentration resulted in a relative percent difference from analyzing all 81 particles of  $18\pm 11$ ,  $19\pm 22$ ,  $20\pm 10$ ,  $4.7\pm 3.3\%$ ,  $3.6\pm 2.8\%$  RPD (N=5),

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3 respectively. Subsampling 5, 10, 20, 30, 40, or 50 particles resulted in  $4.0\pm 0.7$ ,  $6.8\pm 0.8$ ,  $9.0\pm 1.0$ ,  
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5  $13.6\pm 1.1$ , and  $15\pm 0.7$  unique polymer types, respectively, of which  $2.2\pm 0.4$ ,  $3.8\pm 1.3$ ,  $5.6\pm 0.9$ ,  
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7  $9.6\pm 1.1$ , and  $11\pm 0.8$  were plastics. Based on these results and practicality, subsampling of  
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9 stormwater samples was performed for 40 particles to reduce error on MP concentration  
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11 estimations and understand the diversity of polymer types in a given sample. Subsampling the  
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13 selected 40 particles/sample resulted in analysis of  $11.4\pm 9.3\%$  of particles/sample (range: 1.6 to  
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15  $31.5\%$  of total particles, total particle counts listed in Table 2). The bootstrap analysis was  
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17 repeated on the other samples subsampling 5, 10, 20, or 30 particles to create (1) covariance  
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19 plots for MP concentration (standard deviation/mean) at different sampling depths and (2)  
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21 polymer rarefaction curves (number of unique polymer types versus number of particles  
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23 subsampled). The latter provides visual representation of whether the sampling depth likely  
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25 captured most polymer types in a given sample (Fig. 1). This indicates that subsampling 40  
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27 particles underestimates the plastic diversity in some samples (e.g., BBR 8/17/2020, City N  
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29 10/16/2019) while in others, subsampling as few as 20 particles in our samples was sufficient to  
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31 capture the diversity observed by subsampling 40 particles (e.g., City K 12/10/2019, Field P  
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33 10/16/2019).  
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**Fig. 1 a.** Material rarefaction curves showing the number of unique materials versus the number of particles subsampled (top) and **b.** coefficient of variation (standard deviation/average) for extrapolated MP concentrations as a function of the number of particles subsampled (replication  $N=5$ ). The colors correspond to different storm events (date) and shape to sampling year (triangles for 2020, circles for 2019). City K and City B included under “Other.”

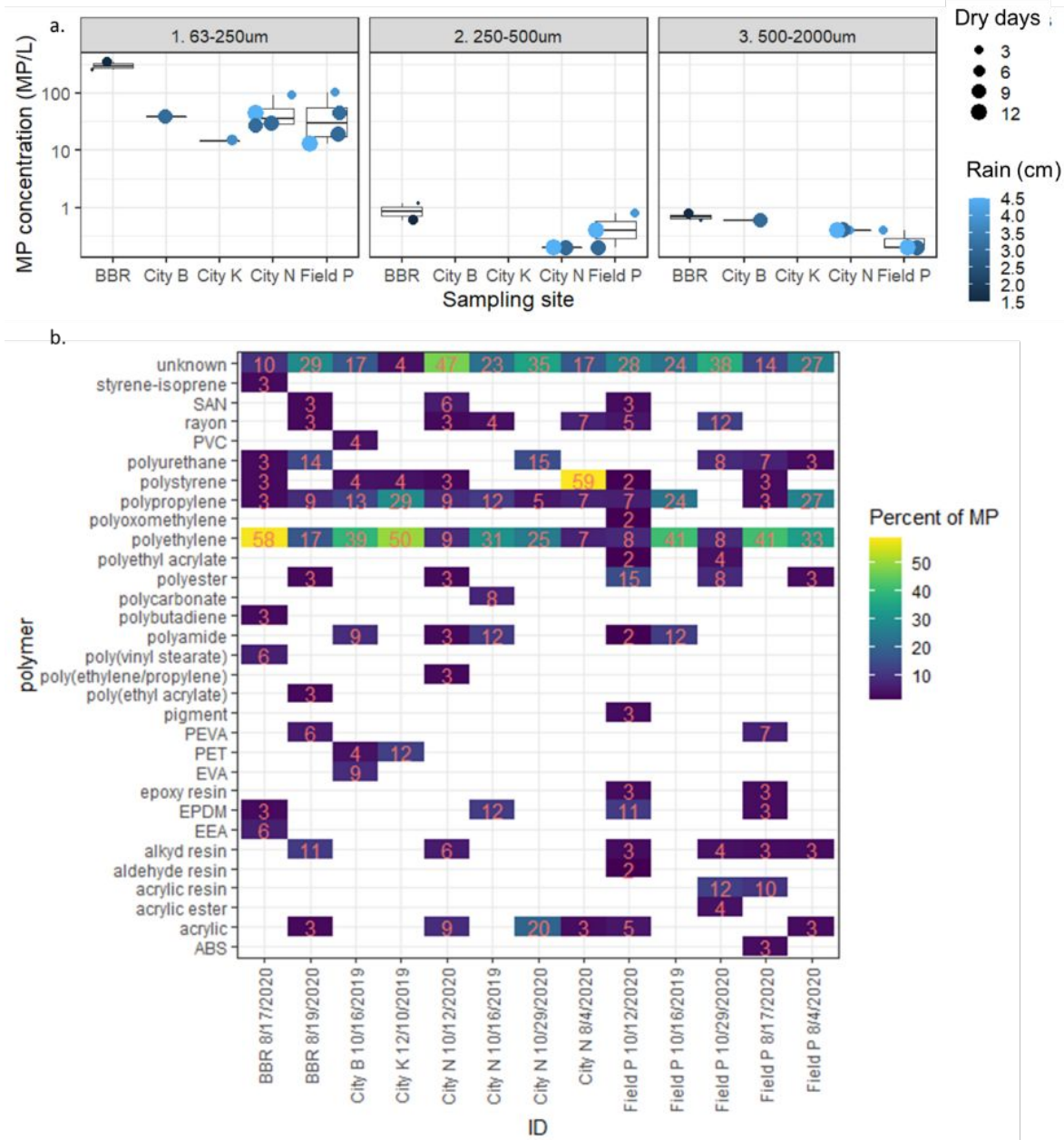
#### Microplastic polymer types, concentrations, morphologies, and size distributions

A total of 521 particles were analyzed via FTIR-microscopy. Microplastics were observed at each of the five sampling sites for the 63-250  $\mu\text{m}$  size class, resulting in observations of 24 plastic polymer types across the samples (Fig. 2). Major commercial polymers such as polyethylene (PE), polypropylene (PP), and polystyrene (PS) were detected at each sampling site. Other polymer types prevalent in stormwater were polyamide (PA), ethylene-propylene copolymer (EPDM), rayon, and acrylic MPs (example spectra are shown in Fig. S6).

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3 MP concentrations for the 63-250  $\mu\text{m}$  size class ranged from 15 MP/L at City K to 303 ( $\pm 50$ )  
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5 MP/L in Bioretention P effluent. MP concentrations in 63–250  $\mu\text{m}$  size class were one to two  
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7 orders of magnitude greater than those previously reported for 250-500  $\mu\text{m}$  and 500-2000  $\mu\text{m}$   
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9 size classes for these samples (both  $p=0.015$ , posthoc pairwise t-test with Bonferroni  
10  
11 correction).<sup>19, 20</sup> Random forest indicated that 60.8% of the variance in the concentration in the  
12  
13 63-250 $\mu\text{m}$  size class was explained by antecedent dry days (10.3% increase in mean square error  
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15 MSE) followed by site and rainfall depth (7.4 and 5.6% increase in MSE, respectively). Blanks  
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17 taken at Field P, City N, and City B had MP particles present: Field P blank had 56 MP, City N  
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19 had 8 MP, and City B had 30 MP. The reported stormwater MP concentrations were not  
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21 corrected for the contamination observed in the field blanks given that the field blanks were 1 L  
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23 water samples rather than 5 L as for the stormwater samples. The blanks (which only contained  
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25 PE and PP) had 6.3-57 times less MP per filter than storm samples ( $p= 0.0044$ , Wilcoxon rank  
26  
27 sum).

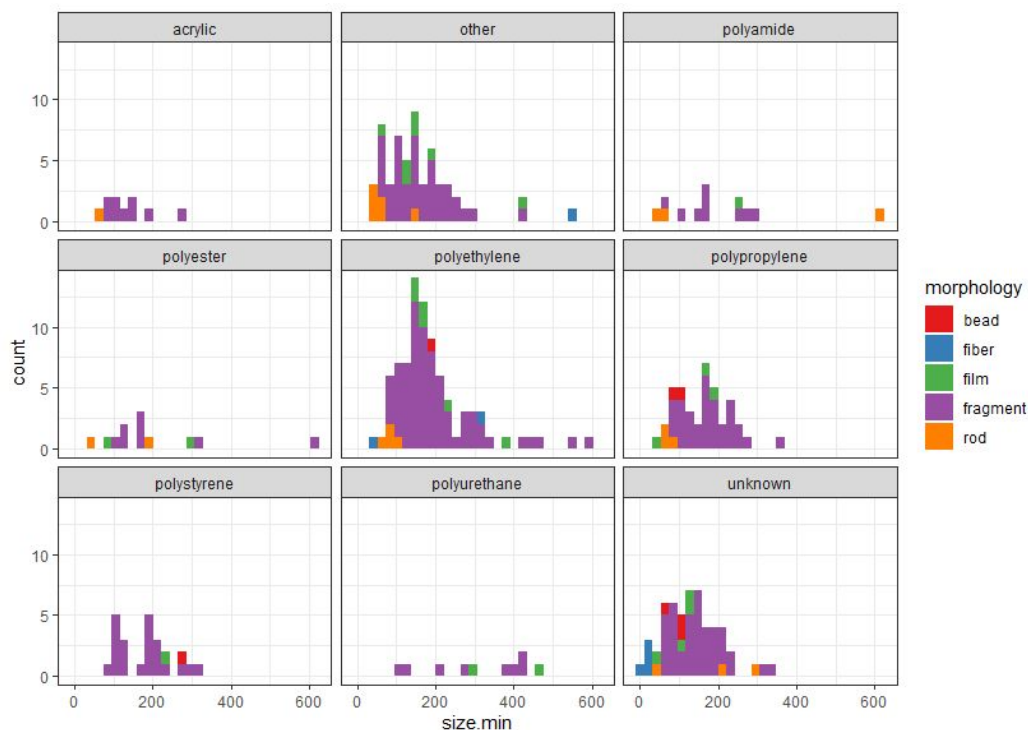
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34 Fragments were the most commonly observed morphology for MP for all polymer types (Fig. 3)  
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36 and sampling sites. Film and rods were the next most observed morphologies while beads were  
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38 rarely observed in these samples (examples are shown in Fig. S7). Examples of all morphologies  
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40 observed are shown in Fig. S7. Histograms of the minimum measured dimension across particle  
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42 morphologies indicated that data were generally right skewed, and the tail illustrated that some  
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44 particles larger than expected for this sieve size were observed. These minimum dimensions  
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46 measured however do not capture all three dimensions and for example the PA rod's diameter  
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48 likely allowed it to pass through the 250  $\mu\text{m}$  sieve opening.  
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Polymer profiles differed by size class rather than by sampling site ( $p=0.001$  and  $0.55$ , respectively, ANOSIM) when including the data collected in this study and the previously reported larger MP. Some clustering within the  $63 - 250 \mu\text{m}$  size class was observed by sample site, with Field P separated from BBR and City N, and City N but with overlap for all with City N (Fig. 4). A similar pattern is seen for the larger two MP size classes within their cluster.

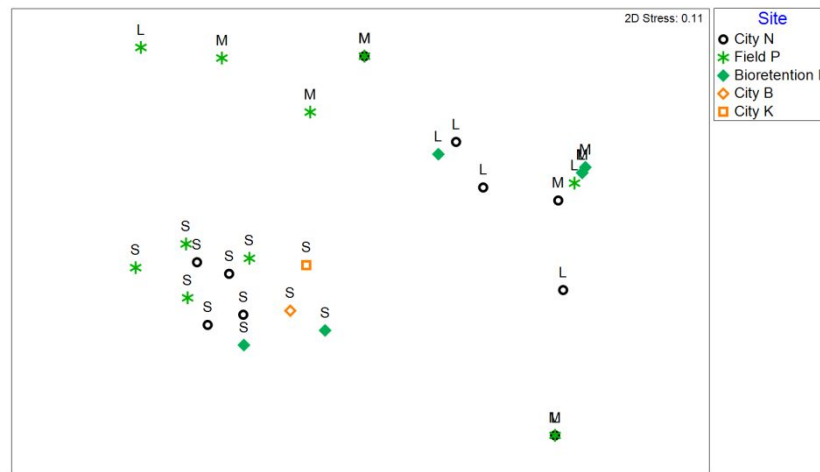




**Figure 2 a.** Boxplots of MP concentrations (MP particles per liter of water) observed in the bioretention basin and stormwater at each sampling site: City N, City B, City K, Bioretention Basin (BBR), and Field P. For each respective size class 63–250  $\mu\text{m}$ , 250–500  $\mu\text{m}$  and 500–2000  $\mu\text{m}$ . Circle color represents cumulative rainfall in cm and size represents antecedent dry days. The two larger size classes show data from Boni et al. and Bailey et al. **b.** Heatmap of polymer profile distribution observed across sampling sites and events. BBR represents Bioretention P. Polymer abbreviations: SAN is Styrene-Acrylonitrile copolymer, PVC is Polyvinyl Chloride, PU is Polyurethane, PS is Polystyrene, PP is Polypropylene, PEVA is Polyethylene Vinyl Acetate, PET is Polyethylene Terephthalate, PEA is Polyethyl Acrylate, PE is Polyethylene, PB is Polybutadiene, PA is Polyamide, EPDM is a Poly(Ethylene-Propylene) copolymer, EEA is Ethylene-Ethyl-Acrylate copolymer, ASA is Acrylonitrile Styrene Acrylate copolymer, and ABS represents Acrylonitrile Butadiene Styrene copolymer. (Example spectra are available in the Appendix, note butadiene polymer spectra were obtained with ATR-FTIR.)



**Figure 3:** Histograms of measured particle length (minimum dimension measured) as a function of polymer material with color corresponding to the observed particle morphology. Other includes microplastics present at lower prevalence in the samples and does not include unknown or natural materials observed.



**Fig. 4** nMDS of log-10 transformed polymer profiles. Symbols correspond to different sampling locations (site). The letter above each symbol indicates the size range: S for 63-250 μm, M for 250-500 μm, and L for 500-2000 μm.

## Discussion

### Subsampling

The sample matrix/volume sampled and extraction methods applied here (wet peroxide oxidation and density separation) resulted in too many particles to analyze in the 63-250 μm size range via manual FTIR microscopy. This was partly due to the presence of non-plastic particles including materials identified via FTIR as natural fibers, plant matter, clay, sand, and cellulose. Notably, applying a cellulose digestion (e.g., using modified Schweizer's reagent via ASTM Method

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3 8333-20<sup>34</sup> or enzyme digestion via cellulase<sup>39</sup>) could help reduce the number of non-plastic  
4 particles remaining identified as cellulose. Cellulose was observed in nine of the twelve samples  
5 analyzed and when observed it accounted for  $7.3 \pm 6.1\%$  (range of 2.5 to 20%) of particles  
6 analyzed.  
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13 The percentage of particles to analyze was explored given that there is not a consensus in the  
14 literature for subsampling stormwater (Table 1). Random sampling rather than analysis of only  
15 visually identified particles was pursued given reports that visual identification can over- or  
16 underestimate microplastic concentration,<sup>24</sup> as particles without vibrant colors may be missed.  
17 Here, the subsampling pattern was selected after visual inspection of the filters, as others have  
18 suggested the particle distribution should be considered prior to subsampling for methods with  
19 scans of filter area.<sup>27</sup> Subsampling patterns with randomly selected particles were not previously  
20 shown to vary by the sampling pattern selected (cross, spiral, etc.).<sup>28</sup>  
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33 The number of subsampled particles to accurately estimate MP concentrations has been  
34 investigated, however the sample matrix and preparation should be considered when comparing  
35 across studies. For example, a study<sup>27</sup> of surface water, wastewater, and rainwater particles  
36 washed with ethanol reported that subsampling 50% of particles would result in error of 20%.  
37 However, those authors note decreasing error for subsampling with increasing MP content, and  
38 the oxidation or density separation performed in the present study helps reduce debris prior to  
39 filtration. Another team recommended subsampling at least 30 particles  $>100 \mu\text{m}$  extracted from  
40 surface water, stormwater, and sediment to accurately estimate MP concentrations (i.e., error  
41  $<20\%$ ), noting that the portion of MP in a sample was more important than the total particle  
42 count.<sup>29</sup> The selection of 40 particles/sample here represents up to 31.5% of sample particles and  
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3 given the focus on particles in a smaller size range is not surprisingly higher than previously  
4 suggested.  
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9 Further subsampling of Field P 10/12/2020 is expected to result in more plastic types being  
10 observed given that the polymer rarefaction curves have not reached asymptote even at 50%  
11 sampling depth (N=81 particles). However, the leveling of the polymer rarefaction curves for  
12 the same site when sampled on 10/16/2019 indicates temporal variation in the subsampling  
13 required. Of course, these analyses are based upon the polymer classes reported here, which in  
14 themselves contain spectra with varying amounts of oxidation. Researchers should also consider  
15 the study aim when subsampling is needed for analysis because the subsampling coverage for  
16 consistent estimates of total MP concentration may be insufficient for describing polymer  
17 diversity. Reporting the concentration of specific polymer types may be desirable rather than  
18 total MP knowing that the polymers and their associated plasticizers, additives, and dyes have  
19 varying properties<sup>40</sup> and the ecological impacts could be polymer-target specific (e.g.,  
20 demonstrated impact of tire rubber (leaching of anti-oxidant) to coho salmon<sup>41</sup>).  
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### 37 *Observed MP concentrations, sizes, and morphologies*

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41 MP concentrations for the 63-250  $\mu\text{m}$  size class were significantly larger when compared to  
42 concentrations for 250-500  $\mu\text{m}$  and 500-2000  $\mu\text{m}$  for paired samples.<sup>19, 20</sup> Higher concentrations  
43 of MP in smaller size classes are consistently reported across water matrices,<sup>42</sup> likely due to  
44 particle counts increasing as plastics fragment. Direct comparisons to other studies are  
45 complicated by the varying sample volumes, extraction techniques, analytical techniques, target  
46 size ranges<sup>18, 42</sup> as described in the introduction, in addition to likely geographic and temporal  
47 differences. Nonetheless, the maximum concentration observed here were within an order of  
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3 magnitude of the maximum concentrations reported in several studies.<sup>6, 7, 21, 26, 43</sup> One order of  
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5 magnitude lower maximum concentrations were reported by some studies, potentially due to  
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7 those researchers looking at smaller size ranges<sup>11, 22, 30</sup> or differences in sample volume.<sup>30, 44, 45</sup>  
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10 Researchers in Sweden that reported an order of higher maximum MP concentration in  
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12 stormwater notably included particle sizes smaller than examined here, down to 20  $\mu\text{m}$ .<sup>33</sup>  
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14 Including smaller particle sizes is recommended in future studies but is impractical to do using  
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16 the methods applied here given the labor required and the analytical limitations.  
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20 Antecedent dry days followed by rainfall depth and sampling site were factors associated with  
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22 MP concentrations here, similar to our previous study of the larger MP which indicated these  
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24 same factors; rainfall and sampling site exhibited greater mean square error than antecedent dry  
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26 days.<sup>20</sup> Similarly, a relationship between antecedent dry days and MP concentrations was  
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28 reported from a study in Mexico, albeit with longer dry periods (c.a. months) before storm  
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30 events.<sup>7</sup> Site-to-site differences were expected due to differences in land use and land cover, also  
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32 reported in other catchments,<sup>10, 23</sup> as well as sewer type. For example, City B samples were  
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34 collected during a storm from combined sewers, whereas City N has separate sanitary sewers.  
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39 Polymer morphologies were primarily fragments, similar to other stormwater studies that  
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41 reported morphologies.<sup>11, 21, 23</sup> Fibers are also often abundant in stormwater including that from  
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43 CSOs,<sup>7, 11</sup> but this morphology was not especially prevalent for the MP observed here including  
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45 from the CSO event sampled for City B.  
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50 Polymer minimum dimensions provided here demonstrated observations of some particles not  
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52 within the expected size class based on the two dimensions measured. This is likely partly due to  
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54 the presence, for example, of rods with small enough diameter to pass through the sieves  
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3 (diameter dimension was not measured) or films whose flexibility may allow passage. Other  
4 factors may include irregularities in the sieves or contamination which is more likely for smaller  
5 particles (notably, PE and PP were the only MP observed in blanks). Reporting actual particle  
6 size in addition to size classes can aid in cross-study comparison. The recommendation to  
7 measure particle size is being incorporated into an emerging standard method<sup>46</sup> and remains of  
8 interest given that different standard methods are suggesting different sieve sizes.<sup>34, 46</sup>  
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10 Understanding particle size and morphology may aid in design of treatment systems (if removal  
11 of MP is desirable) and inform ecological exposure assessment, as different particle sizes and  
12 morphologies may appeal to different aquatic organisms. Likewise, different morphologies have  
13 different translocation/stress induction potential<sup>as reviewed by 5</sup> and fate given the different bivalve  
14 egestion rates for spheres versus fibers.<sup>47</sup>  
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29 Observations of MP present in the field blanks may be due to atmospheric deposition (field  
30 blanks were left open during sampling) and/or cross contamination in the lab. Others have  
31 reported wet deposition of MP during rainfall events.<sup>12, 13, 48</sup> Negligible blank contamination is  
32 commonly reported, particularly for studies looking for smaller MP: of the 14 stormwater studies  
33 included in Table 1, eleven included field and/or lab blanks in their protocols<sup>6, 7, 11, 16, 22, 23, 25, 30,</sup>  
34 <sup>33, 44</sup> and eight reported some contamination in the blanks. If not from atmospheric deposition  
35 during sampling, cross contamination in the lab or during sampling processing is possible, hence  
36 recommendations to not wear synthetic materials while sampling, working in HEPA filtered  
37 areas, as recently outlined.<sup>46</sup>  
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51 *MP polymer profiles in stormwater*  
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3 The smaller size MPs analyzed here showed a greater variety of polymer types, resulting in  
4 certain polymer species found in this study that were not previously noted in the >250  $\mu\text{m}$  size  
5 classes (for which only PE, PP, PS, PETE, and ABS were observed).<sup>19, 20</sup> Examples of polymers  
6 only observed in the smaller size class (63-250  $\mu\text{m}$ ) include acrylic copolymers, styrene-isoprene  
7 copolymers, epoxy resins, and EPDM copolymers. The greater number of polymer types in the  
8 smaller size range may be associated with (a) the larger number of total particles such that larger  
9 sample volumes would be needed to observe these particles in the larger size ranges, (b) the  
10 greater likelihood of certain polymers to fragment into smaller sizes, and/or (c) differences in  
11 transport via stormwater and/or deposition as a function of particle size. The most prevalent  
12 polymer types (PE, PP, PS, polyamides, and acrylics) have an array of applications and are  
13 commonly observed across water matrices<sup>49</sup> including stormwater.<sup>40-42</sup> Several other polymers  
14 are used in bitumen applications such as roofs and pavement (e.g., styrene isoprene copolymer,  
15 polyvinyl stearate, and EEA copolymer) or tires (e.g., polybutadiene). While butadiene rubbers  
16 often do not provide good spectra via FTIR, there have been ATR-FTIR studies of ABS that  
17 were able to document spectral changes associated with photodegradation.<sup>50</sup> Here the two  
18 observed particles were analyzed via ATR-FTIR (see example spectra Fig. S6i) where we  
19 hypothesize that with these smaller particles when compressed had sufficient contact with the  
20 IRE crystal to provide quality spectra. (Notably this is likely an underestimate of ABS  
21 prevalence in our samples given that a density separation was performed during extraction, and  
22 others report rubbers to be prevalent in stormwater particularly from road runoff<sup>11</sup>). Further  
23 study would be needed to confirm the potential sources of the plastics observed, including  
24 perhaps through more extensive spectral analysis to possibly identify unique dyes, or specific co-  
25 polymers, or other additives<sup>e.g.,51</sup> that may be more use specific.

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3 Interestingly, some polymers (PVC and PC) were not expected to be observed in this study due  
4 to having higher densities than the saturated NaCl solution used for density separation ( $>1.202$   
5 g/mL). Newer standard methods no longer suggest density separation in order to facilitate  
6 observation of denser polymers<sup>34</sup> or researchers may apply denser salt solutions (Table 1). These  
7 denser particles may have been attached to less dense particles allowing them to bypass density  
8 separation. It is believed to be unlikely that contamination was the source of these particles  
9 because no PVC or PC polymer was found/identified in the field blanks (only PE and PP) and  
10 the samples were not exposed to PVC or PC during processing.  
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22 Challenges with the spectral analysis are noted, including the influence of surface oxidation (Fig.  
23 S6) and particle thickness. In some samples this limited the ability to initially collect quality  
24 spectra in transmission mode; multiple locations per particle were analyzed to obtain spectra  
25 which could be identified as a specific polymer. Matches from spectral databases often required  
26 additional manual interpretation. Different researchers have reported using various proprietary  
27 and open spectral libraries and a range of match scores to accept and/or inspect spectra. Here,  
28 this included analyzing if there was consistency in the list of top hits as well as manual  
29 inspection of major bands to confirm identity, rather than accepting any spectral match above a  
30 certain database match score.  
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## 44 **Conclusion**

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47 Results of the present study indicate that MP concentrations were one to two orders of magnitude  
48 greater in the 63-250  $\mu\text{m}$  particle size class compared to 250-500  $\mu\text{m}$  or 500-2000  $\mu\text{m}$ ,  
49 previously reported.<sup>19, 20</sup> A greater number of polymer types were observed in the smaller size  
50 range, information that may be useful in future exposure assessments for aquatic organisms.  
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3 Future studies are recommended that target a wider range of particle densities and include  
4 cellulose degradation to help remove non-plastic debris. Likewise, including particles in smaller  
5 size range than was feasible here is also recommended, but is impractical to do so quantitatively  
6 using the methods applied in this study and likely requires higher throughput methods that may  
7 provide less robust particle size/morphology data. Subsampling analyses indicated that a greater  
8 diversity of polymer types is expected to be observed in samples from the same site if more  
9 particles were analyzed, again underscoring the need for subsampling designs that are study  
10 specific<sup>27, 28</sup> or analyses implementable with higher throughput, for which FPA and Raman  
11 scanning methods have been proposed.<sup>35, 36, 52</sup> However, higher throughput should not sacrifice  
12 the ability to accurately interpret results, as here the need for manual interpretation of many  
13 spectra from the environmental samples was noted. The polymer profiles' spatial and temporal  
14 variation at stormwater outfalls in the urban/suburban environment as well as a bioretention  
15 basin was demonstrated within the same region. These results may help inform the expected  
16 exposure to different positively buoyant polymer types in stormwater and the potential for  
17 treatment.

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