

Environmental Toxicology

Insights into the in-situ degradation and fragmentation of macroplastics in a low-order riverine system

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Abstract

Inland riverine systems are major conduits of microplastics (MPs) to coastal environments. Plastic materials that pass through riverine systems are subjected to various degradation processes that facilitate their fragmentation into MPs. Low-order streams, a critical yet understudied part of river networks, significantly influence the fate and transport of MPs. Here, we investigate the in situ degradation of common macroplastic polymers (e.g., low-density polyethylene, polyethylene terephthalate, and polystyrene) and their fragmentation into MPs in urban and forested streams. We deployed macroplastic items and a natural biodegradable polymer (cellulose) into a stream habitat for 52 weeks. We found that regardless of stream type (forested or urban), macroplastic polymers produced MPs in 2 weeks, with polystyrene having the highest fragmentation rate (8 particles/week). We explored several degradation indices (carboxyl index, hydroxyl index, and vinyl index), which revealed that photooxidation played a role in macroplastic degradation over time. Another driver of degradation was biofilm formation observed on the surface of all items, mainly composed of diatoms. Finally, we found that field-aged macroplastics can leach plastic-derived dissolved organic. Our study narrows the knowledge gap regarding MP degradation and fragmentation in freshwater by providing real-time in situ data on the rate of polymer fragmentation in a low-order riverine system.

Keywords: plastic degradation, biofilm, oxidation, fragmentation, freshwater

Introduction

An estimated 1.7 million tons of plastics enter oceans each year from rivers (Organisation for Economic Co-operation and Development [OECD], 2022), contributing to the approximately 5.25 trillion pieces already reported in oceans by Eriksen et al. (2014). Of the plastics that enter oceans, 80% originate from rivers, which transport macro and microplastic (MP) particles (i.e., plastic items <5 mm in diameter) to coastal waters (OECD, 2022). Due to the ubiquity of MP pollution in water, its impact on aquatic organisms is a leading research priority (Ma et al., 2019; Nanthini Devi et al., 2022). Macroplastic fragmentation into MPs in aquatic systems results from multiple processes acting together (Weinstein et al., 2016). Photolysis (i.e., breaking chemical bonds via UV rays) can initially compromise the integrity of plastic (Cai et al., 2018), followed by physical forces (Hoellein et al., 2024) and microbial colonization (Kaiser et al., 2017) that can further degrade and fragment macroplastics (plastic items with a diameter ≥ 5 mm), resulting in secondary MP production (Dimassi et al., 2022). Secondary MPs, which form from the fragmentation of larger plastic debris, pose a greater threat to organisms than primary MPs (i.e., those manufactured for commercial products, like personal care items; Gray & Weinstein, 2017). The concentration of secondary MPs varies by region due to differences in local degradation processes (Boucher & Priot, 2017; Eriksen et al., 2014;

Ryan et al., 2019; Weinstein et al., 2016). As macroplastics degrade in aquatic systems, they can also leach plastic-derived dissolved organic carbon (DOC), a process that needs to be better characterized outside marine ecosystems or laboratory settings (Romera-Castillo et al., 2018, 2022a, 2022b). This poses an issue regarding biogeochemical cycling that has yet to be fully elucidated in freshwater ecosystems and highlights the need for quantifying the fragmentation rate of plastics (Zhu et al., 2021).

The rate at which macroplastics degrade varies by environment (Weinstein et al., 2020) and compared to oceans or coastal systems (Andrady, 2011; Weinstein et al., 2016), many aspects of plastic fate (degradation) are relatively unexplored in riverine systems, with the exceptions of a few studies (Liro et al., 2023, 2024). Low-order riverine systems have received limited attention regarding their role in capturing and producing MPs from larger plastic debris (Roy et al., 2024; van Emmerik et al., 2022, 2023). Understanding the degradation and fragmentation of MPs in low-order systems is important because they account for more than 70% of the total stream length in the continental United States (Leopold et al., 1964). Thompson et al. (2024) reviewed 20 years of plastic research and identified several existing knowledge gaps. One of the major knowledge gaps identified, which is also a novelty of the present study, is that fragmentation rates are not widely reported across literature, providing little insight into these dynamics in riverine systems with the exception of studies

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from Liro et al. (2024) and Liro et al. (2023). Liro et al. (2024) investigated this knowledge gap by utilizing mass loss of the plastic item over time and distance to calculate fragmentation. It is important to note that Liro et al. (2024) work was conducted in a mountain stream versus a lowland river system. Still, this underscores the need for more environmentally realistic assessments of macroplastic fragmentation in different freshwater environments, particularly lowland stream systems. Here, our research objective was to deploy and secure common macroplastic polymers (low-density polyethylene [LDPE], polyethylene terephthalate [PET], and polystyrene [PS]) in an urban and forested stream for a year to assess polymer degradation and fragmentation across time. We used Fourier-transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and other laboratory procedures to better understand in situ macroplastic degradation and fragmentation following deployment in freshwater without the use of accelerated aging under laboratory conditions. We hypothesized that over the course of a year, plastic degradation and fragmentation would differ due to differences in characteristics of urban versus forested stream types.

Materials and methods

Polymer identification

Macroplastics used in our study included PS peanuts (White Regular Loose Fill Shipping Packing Peanuts S-Shaped; 4.45 × 0.762 × 0.762 cm), LDPE sheeting (3 m × 30 m; Clear 4-mil Plastic Sheeting), and PET sheets (20 cm × 25 cm; Transparent Clear Flexible Plastic Sheet Panels). Polystyrene peanuts were left intact, and the polymer sheets were cut into strips (15.24 cm × 2.54 cm). Five strips of LDPE, five strips of PET, and three PS peanuts were placed into a glass fiber mesh bag (20.32 cm × 20.32 cm; Magzo Screen Mesh with 1 mm × 1 mm openings). Five strips of cellulose (15.24 cm × 2.54 cm, Total Home Earth Essentials Compostable Dessert Plate) were also added to the glass fiber mesh bag ($n = 6$ per location) to serve as a natural material comparison to understand fragmentation. Using cotton or cellulose strips is a common practice to understand degradation in stream habitats (Berg & McClaugherty, 2008; Tieggs et al., 2013; Walsh et al., 2005; Webb et al., 2019), because cellulose constitutes 10%–50% of plant leaf litter (Imberger et al., 2010). Details on polymer identification with Attenuated Total Reflectance (ATR)-FTIR can be found in [online supplementary material, Text S1](#).

Field deployment and sample collection

It is essential to consider the type of stream used for macroplastic waste because it can enter urban and forested stream systems directly or through transport, and the characteristics of these two habitat types can influence degradation and fragmentation differently. Urban streams have flashier hydrographs, elevated concentrations of nutrients and contaminants, altered channel morphology, and reduced biotic richness (Bell et al., 2012; Walsh et al., 2005). Non-disturbed or forested streams have unaltered hydrology (streamflow), diverse biological communities, diverse habitat features, and natural water chemistry (Bell et al., 2012). As macroplastic waste continues to be an environmental problem, it is critical to consider the contribution of stream ecosystems to the influence of MP occurrence, fate, and transport throughout the biosphere. Our study system was a third order stream that is influenced by urbanization and agriculture (O'Donnell & Hotchkiss, 2022). Details on our deployment of macroplastics and site characteristics can be found in [online supplementary material, Texts S2 and S3](#). A schematic overview

of the experimental design can be found in [online supplementary material, Figure S1](#).

Bag deployment and collection

Prior to deployment, macroplastics and cellulose used in our field study polymers were confirmed using ATR-FTIR. Details on the analysis can be found in [online supplementary material, Supplementary Information S1](#). Each stream location (i.e., forested and urban) had glass fiber mesh bags ($n = 6$ per location) that contained macroplastics (LDPE, PET, and PS) and a natural biodegradable cellulose item. Each bag was secured into the uppermost part of the sediment using metal spikes. Bags were collected from each location at 2, 4, 12, 24, 32, and 52 weeks (a map of the study locations can be found in [online supplementary material, Figure S1](#)). Bags were distributed along a 100 m reach and tagged with unique IDs.

Mass measurements and UV transmittance

Once collected from the field, macroplastics were weighed, and UV transmittance was measured compared to non-aged macroplastics. Percent transmittance was reported as a change or decrease in UV-A over time, similar to methods described by Weinstein et al. (2016). Details on sample mass and UV analysis can be found in [online supplementary material, Text S4](#).

ATR-FTIR characterization and degradation indices

We collected spectra of non-field-aged macroplastics (before deployment) and of field-aged plastics following field deployment at 24 and 52 weeks to profile the change over time (4,000–650 cm^{-1} , 8 cm^{-1} spectral resolution, 64 co-added scans (32 background scans and 32 sample scans). Spectra were compared to those available through the OpenSpecy software; we set our cutoff at 0.6 Pearson's R to confirm polymers. Data was visualized using Spectragraphy 1.2, and peaks were compared to those established in the literature. More details can be found in the [online supplementary material, Texts S1, S5, and online supplementary material, Table S1](#). Degradation or oxidation indices (carbonyl index, hydroxyl index, and vinyl index) were derived from the absorbance ratio of the index peak to a relatively consistent reference peak as described (Alimi et al., 2023; Almond et al., 2020; Babaghayou et al., 2016; Celina et al., 2021; Cheung & Not, 2024; Fotopoulou & Karapanagioti, 2015; Khalaf & Awad, 2016; Martínez-Romo et al., 2018; Ruoko, 2012). Because we assessed three different polymers, we used different peaks for carbonyl (Alimi et al., 2023; Almond et al., 2020; Celina et al., 2021), hydroxyl (Khalaf & Awad, 2016; Martínez-Romo et al., 2018; Ruoko, 2012), and vinyl indexes (Babaghayou et al., 2016; Cheung & Not, 2024; Fotopoulou & Karapanagioti, 2015).

SEM

To visually observe the changes in the surface of non-field-aged and field-aged macroplastics, we used an SEM (JEOL IT500 and FEI Quanta 600 FEG) to depict the changes. Week 24 was selected because it was the time when the mature biofilm was evident (vibrant green coloration) on the item's surfaces. Three approximately 1 mm × 1 mm pieces (representative of the size classification for MPs) were then sputter coated (10 nm of gold/palladium) to image the surface of items before placement in the field (non-field aged) after biofilm formation and when biofilm was removed. Chemical analysis of the surface was conducted using energy-dispersive spectroscopy (EDS). Details on SEM imaging and EDS characterization can be found in [online supplementary material, Supplementary Information S6](#).

Laboratory macroplastic fragmentation test

To determine fragmentation, macroplastic items and cellulose collected from the field (sediment particles brushed off but not cleaned of biofilm) were subjected to a laboratory fragmentation test. Items were placed in 1 L of filtered (0.7 μm GF/C, Whatman). Ultrapure water (Type 1; 18.2 M Ω cm) was rotated gently (120 RPM) for 24 hr. Following 24 hr, the macroplastic item or cellulose was removed, and the ultrapure water was poured through 63- μm sieves. Particles retained on sieves were enumerated and characterized using a dissecting scope (Leica S9 Stereoscope). Filtered hydrogen peroxide (0.7 μm GF/C, Whatman) was added to any collected particle solution with residual biological or organic materials (Weinstein et al., 2016). Suspected MPs were identified using the guide developed by Markley et al. (2024). We took a subset of particles ($n = 42$) to profile the size and shape of suspected MPs with ImageJ Software. A subset of particles from each polymer was analyzed with ATR-FTIR as described in the online supplementary material, Text S1 to confirm that suspected particles matched their parent polymer or macroplastic. Details on laboratory fragmentation tests can be found in online supplementary material, Text S7.

To compare our fragmentation work with others, we took our samples at 3 months to further corroborate any fragmentation observed following our laboratory test. Any loss or decline in mass is assumed to be due to fragmentation. We placed our samples through a cleaning procedure (described in the following section) to remove any residue. We then dried samples in an oven at 45 °C for 6 hr, as described by Liro et al. (2024) and Gerritse et al. (2020). Following this, we compared the mass before deployment and after cleaning to calculate mass loss from our samples. We compared the mass after cleaning to the initial mass to determine the mass percent loss, similar to the methods described by Gerritse et al. (2020).

Plastic-derived organic carbon leaching experiment

To assess whether the aged macroplastics could leach measurable levels of DOC, we used items collected at 24 weeks, within the same time frame that biofilm was observed to be well established. Following biofilm removal (Romera-Castillo et al., 2022a, 2022b), we cut items into 1 mm \times 1 mm MPs and conducted a leachate experiment in our laboratory with samples taken every 24 hr for 72 hr. Dissolved organic carbon was determined by either heated persulfate digestion or high-temperature combustion followed by infrared absorbance (American Public Health Association, 2017). More details on DOC analysis can be found in online supplementary material, Text S8.

Quality assurance/quality control

We utilized established laboratory protocols, as reported in Gray et al. (2024), to minimize contamination across samples. All solutions used in our methods were filtered through 0.7 μm GF/C filters (Whatman) to reduce any possible MPs introduced into samples. High efficiency particulate air filters are kept in each laboratory room, including the main lab space and adjacent microscope room (~1,200 sq ft). All lab materials used in the study were rinsed 3 \times with filtered deionized (DI) water and covered with aluminum foil. Apart from DI wash bottles (100% LDPE, Fisher Scientific), plastic materials were avoided at all study steps, from field deployment to extraction. Light blue lab coats (100% cotton, Fisher Scientific) and nitrile gloves (Fisher Scientific) were worn in the laboratory. Filters were stored in glass petri dishes to reduce atmospheric deposition. Blanks were

used to assess contamination from the lab, whether atmospherically or procedurally. Our procedural blanks had an average particle count of 1–1.25 \pm 0.22 (Gray et al., 2024). Details in online supplementary material, Text S7 outline steps taken to discount MPs from other sources besides our field deployed items.

Statistical analysis

We obtained data specific to changes in UV transmittance, sample mass changes, MP fragmentation counts and rates across time, and the rate of DOC leaching. Due to the non-normal distribution of the data and not meeting assumptions for the parametric test, we employed an aligned-rank transformation to determine differences for each analysis. The aligned rank transformation is a pre-processing step that aligns data before applying averaged ranks, followed by analysis of variance procedure application to the analysis (Leys & Schumann, 2010; Wobbrock et al., 2011). Graphs were created using R Studio (3.5.2) ggplot2 package and GraphPad Prism Ver. 10.2.3.; additional visuals were created using BioRender. All results are expressed as mean \pm standard error (SE) unless stated otherwise (Wickham, 2016).

Results and discussion

Changes in sample mass and UV transmittance

Aside from cellulose (UV transmittance was reduced by almost 100% by Week 2 across locations), we found that the longer the items were left in the field at each site, the greater the decrease in UV transmittance and increase in mass (due to biofouling; Figure 1A and B). Ultraviolet transmittance had decreased by 94% compared to the controls for PS items by 2 weeks (Figure 1A and B). The PET and LDPE items by 12 weeks had declined as much as 50% in transmittance across sites, with a greater than 90% decrease in transmittance by the end of the study compared to the controls (non-field aged; Figure 1A and B). The reduction in UV transmittance corresponds with increases in sample mass. The percent mass increase reflects biofouling or biofilm formation, a process wherein a complex community of microorganisms can occupy the surface of a substrate, consisting of microorganisms and extracellular polysaccharides. The increase in mass due to biofouling was also demonstrated by Weinstein et al. (2016). The observed and recorded increases in sample mass over time differed between stream locations ($F_{1,58} = 6.95$, $p = 0.01$). However, this was largely driven by site differences between forested and urban stream for PET where percent change in sample mass for PET was significantly higher at the urban site (9.0 ± 1.9) versus the forested (5.0 ± 1.5 ; Figure 1C). Low-density polyethylene and PS did not differ in sample mass, suggesting regardless of site, they accumulated relatively similar increases in sample mass across the study (Figure 1C). Changes in items can be seen visually in online supplementary material, Figure S6.

ATR-FTIR and degradation indices

Polymers in the present study had different types of backbone polymer chemistry. Polystyrene and LDPE are carbon-carbon (C-C) backbones, and PET is an ester or C-O backbone that can be easily hydrolyzed and degraded through thermal or photo-oxidation (Ali et al., 2021; Schmidt et al., 2017). Krueger et al. (2015) reported that C-C backbone polymers are resistant to hydrolysis and biodegradation and susceptible to thermal oxidation. Polyethylene terephthalate, a heteroatomic polymer, has been shown to be processed through various degradation processes such as photo-oxidation, hydrolysis, and biological degradation (Gewert et al., 2015). We employed FTIR to analyze the changes in the functional groups and determine if the changes

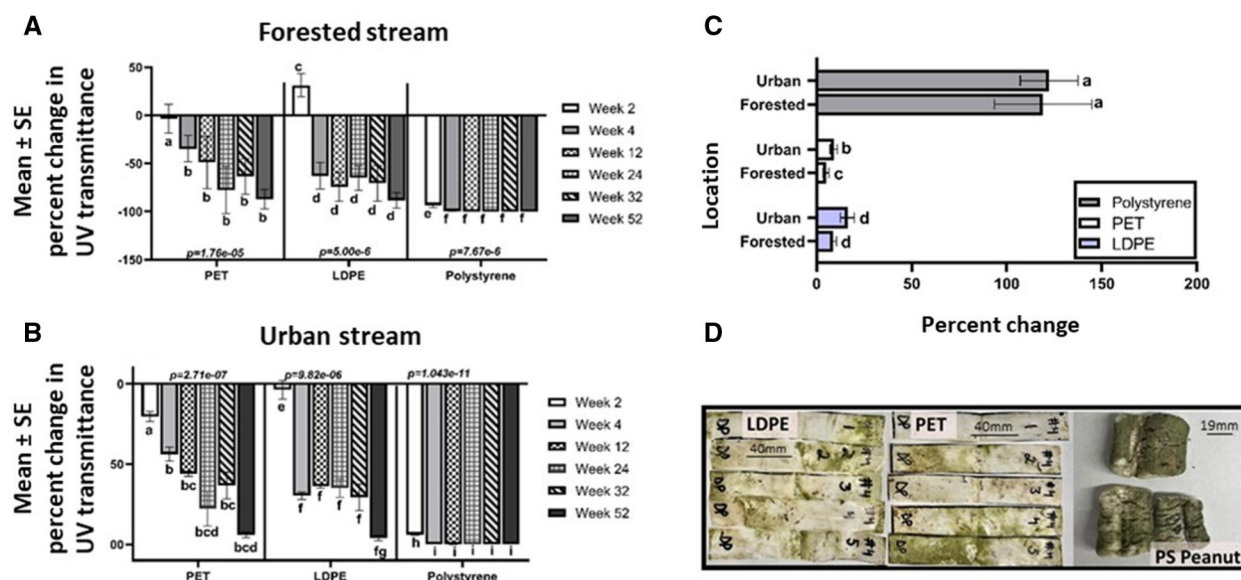


Figure 1. Mean \pm SE changes in ultraviolet (UV) and mass data. (A) Depicts changes in UV transmittance for items deployed in forested locations. (B) Depicts changes in UV transmittance for items deployed in urban locations. (C) Depicts changes in item mass in both locations. (D) Visualizes biofilm development on the surface of field-aged plastics from urban stream location (Week 24 samples). Graphs with letters depict significant differences for each specific polymer type. Letters that are the same are not significantly different. Statistical analysis was grouped by polymers; data do not depict analysis across polymer groups.

are specific to certain degradation mechanisms. Field-aged macropastics were compared to the initial reference spectra (see [online supplementary material, Table S2](#)). Across polymers, we noted differences in the fingerprint region of each spectrum ($500\text{--}1,500\text{ cm}^{-1}$; [Li et al., 2019](#); [Nandiyanto et al., 2023](#)), following aging in the field (i.e., 24 and 52-weeks; see [online supplementary material, Table S2](#)). In samples from our forested site, we saw that in the $900\text{--}1,200\text{ cm}^{-1}$ region, there were new functional groups formed after 24 weeks, which increased in absorbance for all polymers at 52 weeks (see [online supplementary material, Figure S7](#)). Because PET has a C–O backbone, the formation of groups at approximately $1,027\text{ cm}^{-1}$ highlights O–C–C stretching (see [online supplementary material, Figure S7](#)), which has been reported to be the result of mechanical stress from water or hydrolysis as well as physical stress, as demonstrated with polylactic acid, a thermoplastic polymer ([Carrasco et al., 2010](#); [Oliveira et al., 2016](#)). Low-density polyethylene and PS exhibited C–O stretches along this region. At 52 weeks, PET and PS exhibited O–H stretches at the $3,000\text{--}3,800\text{ cm}^{-1}$ wavelength; the increase in this region is indicative of photo-oxidation, where hydroxyl groups are formed as a byproduct more often than carbonyl groups (see [online supplementary material, Figure S7](#)). Photo-oxidation and hydrolysis can also be attributed to the formation of –OH groups in the PS spectra (see [online supplementary material, Figure S7](#); [De Monte et al., 2022](#)). For PET, we saw the ester bonds at $\sim 1,119\text{ cm}^{-1}$ decrease following aging in the field, revealing the cleavage of ester bonds (see [online supplementary material, Table S2](#); [Liu & Lei, 2019](#)). Low-density polyethylene samples in the urban stream also exhibited CH_3 and CH_2 bond degradation but no O–H stretching, which was not surprising given that it is the only non-aromatic hydrocarbon polymer, so it can absorb less UV light (see [online supplementary material, Figure S7](#)). In all our recovered samples, surface characterization of the field-aged polymers demonstrated noticeable differences in functional groups between forested and urban stream habitats. One commonality is that by 52 weeks, the macroplastic

surface functional profile of the samples varied compared to our initial FTIR analysis.

To evaluate oxidation processes as a means of polymer degradation, the carboxyl index (CI) was calculated (mean \pm SE) across samples and locations (see [online supplementary material, Table S2](#); [Ramanujam, 2013](#)). We found that every polymer exhibited an increase in mean CI values, except for PET, at 52 weeks from the urban stream location. Relative to the control, the polymer with the greatest change in CI was PET at 24 weeks. The CI values declined at 52 weeks, as previously shown in marine systems by [Cheung and Not \(2024\)](#) and [Fernández-González et al. \(2021\)](#). Notably, PS from the forested stream declined in CI between Week 24 and Week 52 (see [online supplementary material, Table S2](#)). [Cheung and Not \(2024\)](#) reported that the oscillation of CI values over time can be attributed to removing oxidation processes like fragmentation and microbial consumption, which aligns with our fragmentation data (see below) and biofouling due to biofilm colonization ([Bedia et al., 2003](#); [Sudhakar et al., 2007](#)).

For each polymer across both stream types, hydroxyl index (HI) and vinyl index (VI) values (mean \pm SE) were higher than the 0-month or control sample value in [online supplementary material, Table S2](#). The HI was higher for all polymers in the urban stream habitat versus the forested habitat at 52 weeks. Low-density polyethylene was the only polymer whose HI decreased from 24 weeks to 52 weeks, suggesting LDPE began mineralizing into smaller molecules. Mean VI values for all polymers across both sites increased by 52 weeks relative to the control measurements (see [online supplementary material, Table S2](#)). In a few instances, there was a decrease from 24 weeks to 52 weeks, indicative of a decrease due to the biodegradation of double bonds by microbes ([Martínez et al., 2021](#)). Our observed increases in VI are indicative of degradation from photochemical reactions from UV light exposure ([Heacock et al., 1968](#); [Jabarin & Lofgren, 1994](#); [La Mantia, 1984](#)). The HI values observed can be attributed to photo-degradation processes that can form radicals ([Fairbrother et al., 2019](#)). Despite reductions in UV transmittance, our findings suggest that oxidative, biodegradation, and photodegradation each

contributed to the degradation of plastic items in both stream locations.

SEM and biofilm visualization

The control items imaged with SEM were clean with smooth surfaces (Figures 2A1–C1 and 3A1–C1). Our 32-week samples demonstrated a mature biofilm across all polymers (Figures 2A2–C2 and 3A2–C2). Scanning electron microscopy imaging showed that diatoms were well established on the surface of the biofilm (Figure 4A and B). Because diatoms have silica shells, their presence was confirmed by SEM-EDS analysis when filtering for silica-based compounds (Figure 4A and B; Weinstein et al., 2016). Across polymers, there were noticeable cracks on the surface of the macroplastics that resulted from the bonds breaking (Figures 2A2–C2 and 3A2–C2; Yakimets et al., 2004). Cracks in the surface of MPs have been demonstrated in laboratory scenarios with UV light (Eich et al., 2015; Sun et al., 2022). Despite reduced UV transmittance at 12 weeks (Figure 1A and B), cracking could have occurred before biofilm development impeded UV rays or from surface tensions during drying once items were returned to the lab before cleaning (Lebreton et al., 2018). Ultimately, the cracks can compromise the polymer's integrity, allowing for fragmentation into MPs (Awaja et al., 2016). When the macroplastic surfaces were cleaned of biofilm through H₂O₂ digestion, we observed scratches, punctures or pits, and rigid surfaces on all polymers (Figures 2A3–C3 and 3A3–C3). Aside from UV

radiation contributing to surface cracking and degradation of polymers (Webb et al., 2009), the altered surface could also result from diatom colonization on the surface via biofilm formation (Figure 4A and B; Smith et al., 2021). Similar to previous work by Weinstein et al. (2016), diatom shells made of silica (see online supplementary material, Figure S8) and trapped sediment particles could have created an abrasive surface, resulting in physical or mechanical stress to the items, resulting in fragmentation and altered surface characterization.

Diatom-dominated biofilm has been reported in laboratory and field assessments of macroplastics aged in water (Nava et al., 2022; Pinto et al., 2019; Smith et al., 2021). In our study, the diatom community was mainly composed of *Cocconeis placentula* and *Planothidium lanceolatum*, which have been commonly detected in freshwater (Figure 4A and B). *Cocconeis placentula* is primarily an epiphyte found in high abundance growing on a variety of macrophytes (Eminson & Moss, 1980); it has also been found growing on stones and synthetic surfaces (Cesarini et al., 2023). It is considered a pioneer species that can be found in monospecific assemblages, sometimes blooming and reaching high abundances in recently disturbed streams (Biggs & Lowe, 1994). *Planothidium lanceolatum* is primarily epipsammic (i.e., grows on sand grains) or epilithic (i.e., grows on rock surfaces; Wetzel et al., 2014, 2019). The species is cosmopolitan and found in stagnant and flowing water bodies (Reynolds, 1984). Thus,

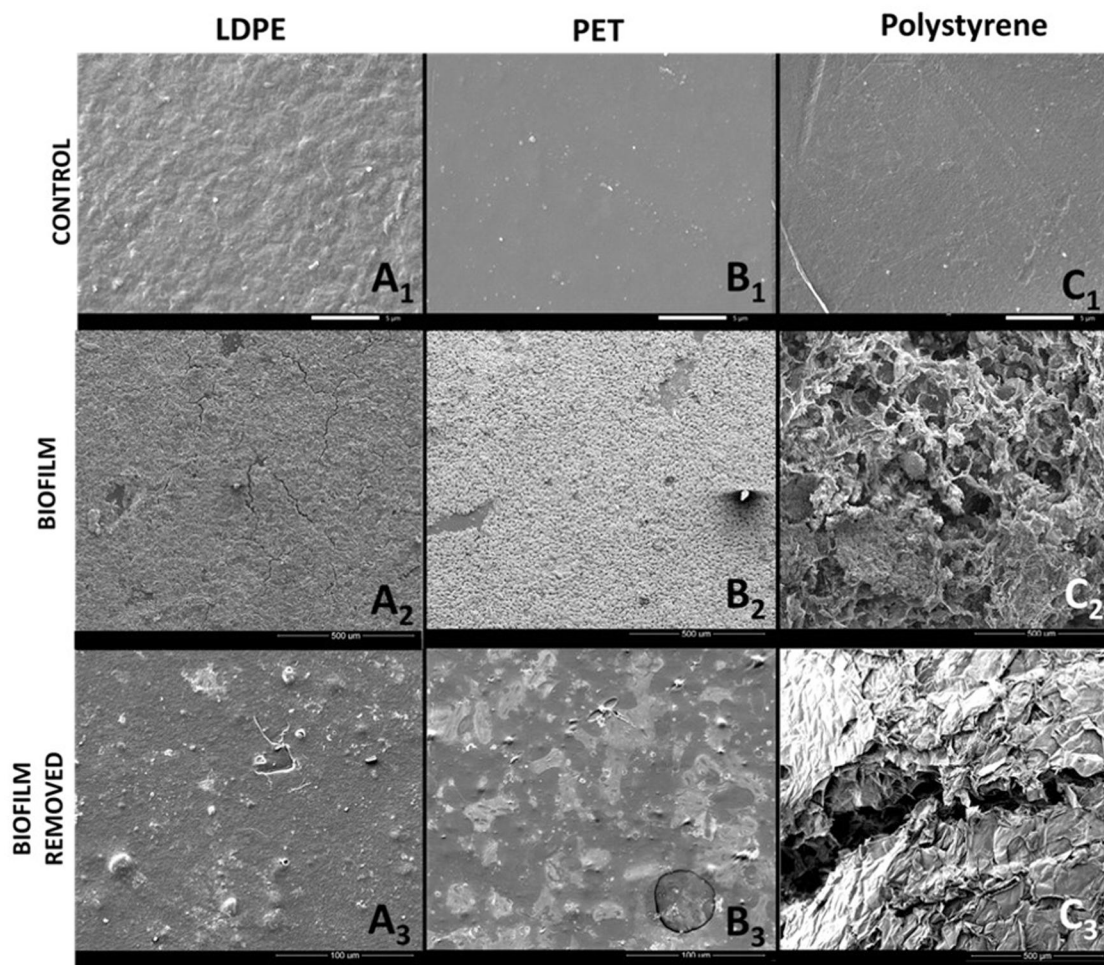


Figure 2. Scanning electron microscopic images of the surface topography of macroplastics at 0 week and 24 weeks post-deployment in the Forested stream. Surface topography for the Week 0 strips was nearly featureless for low-density polyethylene (LDPE) (A₁), polyethylene terephthalate (PET) (B₁), and polystyrene (PS) (C₁). Samples with biofilm demonstrate the presence of diatoms and surface cracking (A₂–C₂). The surface topography of plastic strips with biofilm removed exhibited a degraded surface with pits and holes in PET, LDPE, and microcracks in PS (A₃–C₃).

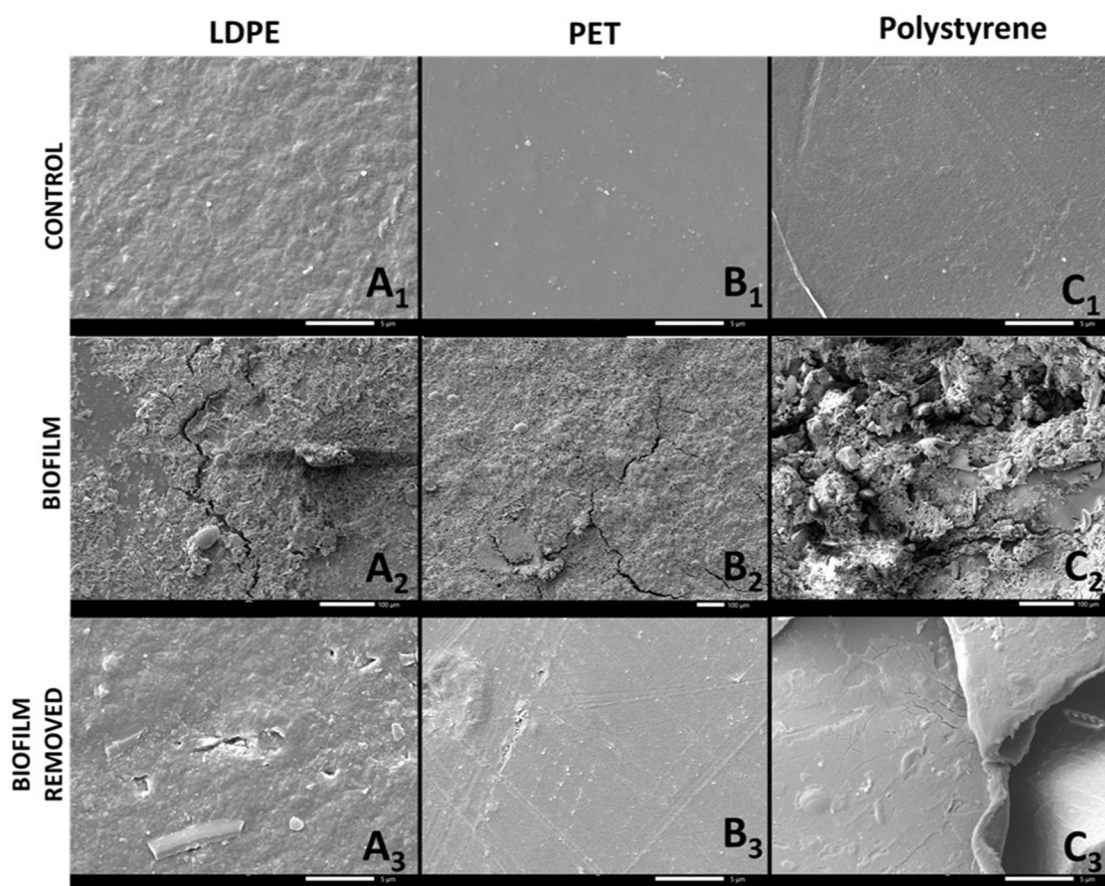


Figure 3. Scanning electron microscopic images of the surface topography of macroplastics at 0 week and 24 weeks post-deployment in the Urban stream. Surface topography for the Week 0 strips was nearly featureless for low-density polyethylene (LDPE) (A₁), polyethylene terephthalate (PET) (B₁), and polystyrene (PS) (C₁). Samples with biofilm demonstrate the presence of diatoms and surface cracking (A₂–C₂). The surface topography of plastic strips with biofilm removed exhibited a degraded surface with pits and holes in PET, LDPE, and microcracks in PS (A₃–C₃).

future investigation into biofilm assemblage and diatom composition on macroplastic surfaces is warranted to understand their role in macroplastic degradation in streams. Additionally, further investigation is needed into why these specific diatoms colonize our field-aged macroplastics dominantly and whether their abundance and community composition differ between synthetic and natural substrates. Diatom physiology and vertical distributions can be changed by environmental variables such as the presence of plastics, such as sinking or burying, which may decrease their density and influence the macroplastics in-situ location (Andrady, 2011). The colonization of biofilm can alter the buoyancy of MPs, resulting in them being captured in sediments or aphotic zones of freshwater (Hao et al., 2022). Plastic–diatom interactions are complex and affect carbon cycling in freshwater systems (Hayer et al., 2022).

Laboratory macroplastic fragmentation test

Results from our laboratory fragmentation test revealed that macroplastics deployed in streams produced MPs in as little as 2 weeks for each polymer, including our natural comparison (i.e., cellulose strips; Figure 5). Mean \pm SE of our Pearson's R from polymer analysis using Open Specy can be found in [online supplementary material, Table S3](#). Of the three polymers deployed, PS particles were mainly present as fragments (long axis length of $486 \pm 53.4 \mu\text{m}$ and an aspect ratio of 6.1 ± 0.92 ; see [online supplementary material, Figure S10](#)). Low-density polyethylene and PET particles were found as both fragments and fibers (see [online supplementary material,](#)

[Figures S9 and S11](#)). The long axis length for PET fibers was $1,215 \pm 121 \mu\text{m}$, and the aspect ratio was 74.7 ± 15.3 . The long axis length of fragments was $252 \pm 86.9 \mu\text{m}$, and the aspect ratio was 3.0 ± 0.13 . Low-density polyethylene particles were found to be primarily fragments rather than fibers. The long axis length of fragments was $231.1 \pm 25.6 \mu\text{m}$ with an aspect ratio of 8.12 ± 1.3 . Low-density polyethylene fibers had a mean long axis length of $1,431 \pm 74.3 \mu\text{m}$ and a mean aspect ratio of 56.2 ± 6.2 . Our analysis showed that for each polymer, there was a significant interaction effect of time (sampling time) and site (urban vs. forest stream location) on MP counts for PS, PET, and LDPE ($F_{5,24} = 4.46, p = 0.005$; $F_{5,47} = 7.49, p = 3.07 \times 10^{-5}$; $F_{5,41} = 8.35, p = 1.63 \times 10^{-5}$, respectively). The subset of MP images and polymer matches can be found in [online supplementary material, Figures S9–S11](#).

Except for cellulose, macroplastics deployed in urban stream locations had significantly higher particle counts than items from the forested location (Figure 5). The formation of MPs in as little as 2 weeks proved to be faster than a previously reported in-situ fragmentation study by Weinstein et al. (2016), which found macroplastic polymers in salt marshes produce MPs in as little as 4–8 weeks. Low-density polyethylene had the lowest particle counts out of the three polymers, ranging between 0–34 particles/item at the forested stream location and 0–57 particles/item at the urban stream location across the study period (Figure 5A). Polystyrene yielded the highest particle counts (urban stream only), ranging between 0 and 25 particles/item at the forested stream location and 0–304 particles/item at the urban

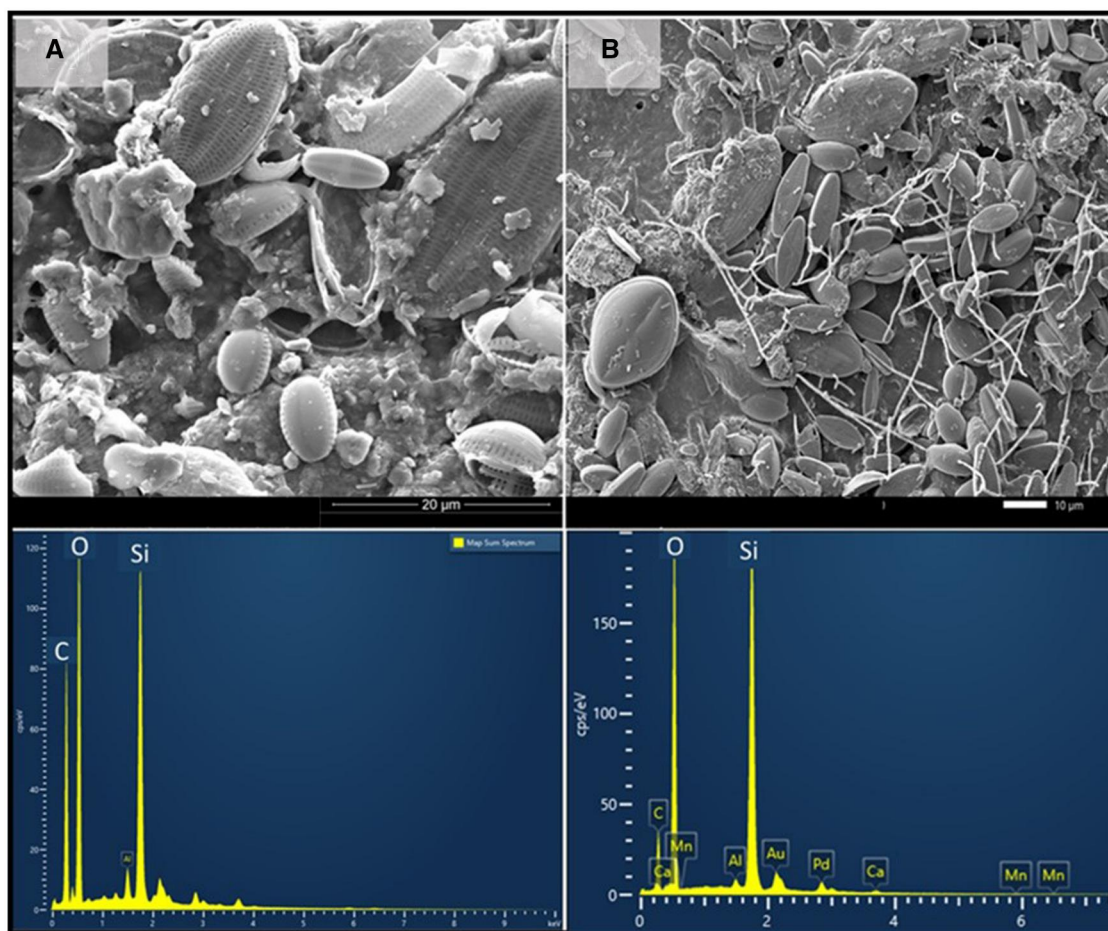


Figure 4. Surface scanning electron microscopy (SEM) imaging of biofilm at 1,000× to 5,000× magnification. (A) SEM of the forested site biofilm from low-density polyethylene (LDPE) samples, along with energy-dispersive spectroscopy (EDS) peaks, will be used to confirm the diatom structure as a result of the Si signature. (B) SEM of diatom community from LDPE samples in the urban site, along with EDS peaks to confirm diatom structure as a result of Si signature. Dominant communities of diatoms are *Cocconeis placentula* and *Planothidium lanceolatum*. Images are from LDPE but are representative of biofilm present across all plastic polymers. Note. PET = polyethylene terephthalate; PS = polystyrene.

stream location (Figure 5B), where at the time of collection, it became more brittle and prone to breakage. Polyethylene terephthalate was our intermediate, with particle counts ranging between 0 and 32 particles/item at the forested stream location and 0–114 particles/item at the urban stream location (Figure 5C).

Fragmentation rate

Regarding fragmentation rate, we found that PS and LDPE had significantly higher rates of fragmentation at the urban stream location versus forested ($F_{1,4} = 13.5$, $p=0.02$; $F_{1,7} = 10.83$, $p=0.013$, respectively); PET fragmentation rate did not differ between locations ($F_{1,8} = 2.06$, $p=0.19$). The fragmentation rate ranged between 0.83 and 0.91 particles/week for macroplastics in the forested location and ranged between 1.06 and 7.98 particles/week at the urban stream location (Figure 5). The urban location had greater visible light and UV-A penetration and coupled with flashier flows characteristic of urban streams draining human-altered landscapes, likely influenced the higher fragmentation rates observed (Walsh et al., 2005). Water chemistry over the 52-week period did not vary greatly, where pH can influence polymer degradation; our study system's pH was neutral with a mean (SE) value of 7.76 ± 0.002 (see online supplementary material, Text S2). The sediment profile of our sites shows that each site was mainly composed of sediment between 500 and 2,000 µm

in size (see online supplementary material, Figure S4). Thus, these particles could have caused abrasion to the surfaces. However, the similarity between sites suggests other mechanisms played a more influential role (see online supplementary material, Figures S3 and S4). Compared to synthetic polymers, we did observe higher fragmentation of our biodegradable strips made of cellulose. The higher fragmentation of the cellulose corroborates previously reported findings by Weinstein et al. (2020) that cellulose polymers degraded faster than synthetic polymers (e.g., LDPE, PET, PS) when placed in the environment. While Weinstein et al. (2016) demonstrated this in salt marsh habitats that underwent diurnal changes of tidal cycles, the fragmentation rate of cellulose observed between urban and forested locations may also be attributed to its complete submergence and decomposition by microbes because cellulose is among the most readily available sources of carbon in plant leaf litter (Hayer et al., 2022; Tiegs et al., 2013).

Gerritse et al. (2020) conducted work on macroplastic fragmentation in a laboratory setting using seawater, reporting fragmentation as a percent of mass loss compared to initial mass. Consistent with our findings, cellulose items fragmented at a higher rate than plastics. Interestingly, PET had a higher fragmentation rate than PS and LDPE. In our study, PS exhibited a higher fragmentation rate, followed by LDPE, and then by PET (see online supplementary material, Table S4). These differences

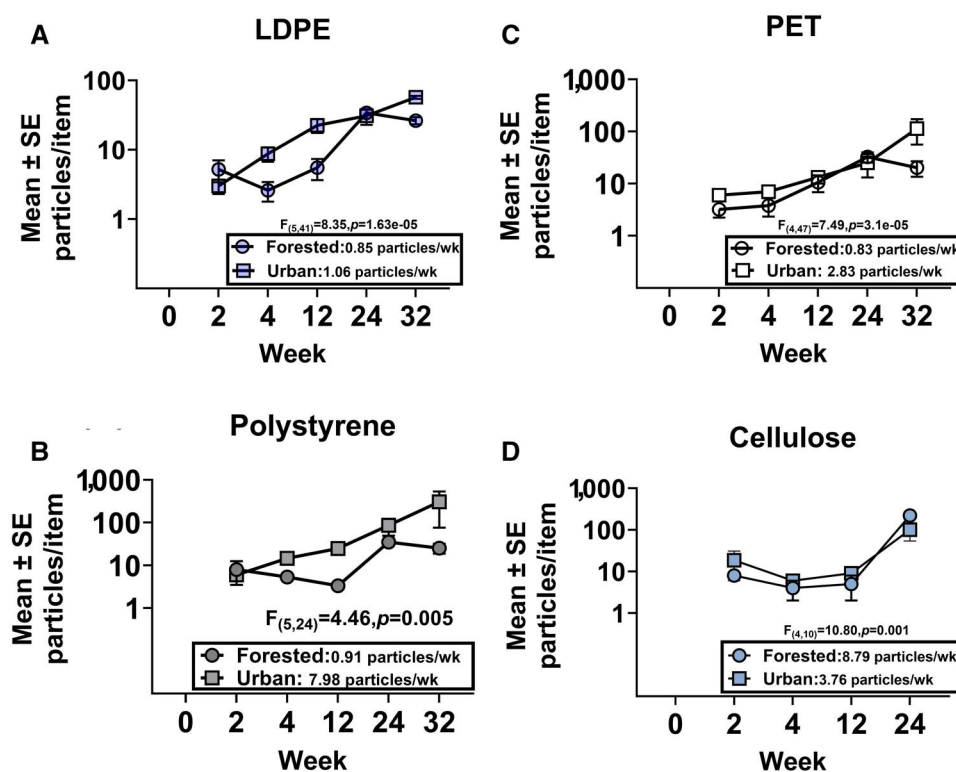


Figure 5. The abundance of microplastic particles produced in the laboratory fragmentation test. No microplastic particles were produced by the 0 week (control). (A) Low-density polyethylene comparison (LDPE). (B) Polystyrene comparison. (C) Polyethylene terephthalate (PET) comparison. (D) Cellulose comparison. Statistics and p values are reflective of the interaction effect of time and site.

are likely influenced by the matrix, where [Gerritse et al. \(2020\)](#) used seawater, and our study used freshwater. Additionally, it is worth noting that our work was conducted in situ, as opposed to a controlled laboratory setting.

Plastic-derived organic carbon leaching experiment

Polymers taken from the field at 24 weeks following biofilm removal exhibited measurable levels of DOC leaching when introduced to different aquatic matrices (see [online supplementary material, Table S5](#); see [online supplementary material, Figure S12](#)). Using Ultrapure water, we found that LDPE leached between 34 and 151 $\mu\text{mol C L}^{-1}$, PET between 41 and 81 $\mu\text{mol C L}^{-1}$, and PS between 35 and 48 $\mu\text{mol C L}^{-1}$ (see [online supplementary material, Table S5](#); see [online supplementary material, Figure S12](#)). When samples were added to filtered stream water, LDPE ranged between 113 and 269 $\mu\text{mol C L}^{-1}$, PET ranged between 103 and 378 $\mu\text{mol C L}^{-1}$, and PS ranged between 77 and 79 $\mu\text{mol C L}^{-1}$ (see [online supplementary material, Table S5](#); see [online supplementary material, Figure S12](#)). These results suggest that as polymers age, they can leach DOC, and future research into these processes is necessary to understand potential assimilation by microbes; the DOC released can have inhibitory or stimulatory effects on microbes due to the chemicals associated with plastics that may alter growth or function ([Romera-Castillo et al., 2018](#)).

Challenges and limitations

Plastics in riverine or fluvial systems can remain for years to centuries due to capture in sediment, riparian zones, and vegetation. However, there is limited data available on the process of macroplastic fragmentation ([Delorme et al., 2021](#); [Liro et al., 2023, 2024](#); [Williams & Simmons, 1996](#)). Compared to the other literature regarding plastic fragmentation in river systems, our results are

comparable. However, we did not deploy items like [Liro et al. \(2024\)](#) did, using bottles to track macroplastic mass loss during item transport. As detailed, riverine plastics can persist for long periods ([Liro et al., 2023](#)). Our approach differed to reflect those that are captured. While we demonstrated and confirmed secondary MP production in as little as 2 weeks, we took detailed steps to ensure we did not count particles from other sources that could be adhered to the surface of our items by targeting polymers not reported in our study area ([Gray et al., 2024](#)) but also acknowledge that our estimates are conservative at best as we did not count particles below 63 μm . We reported higher fragmentation rates in our urban stream for each plastic polymer. However, we did not assess hydrodynamics and how these processes may influence degradation and fragmentation. As previously detailed, our urban stream site is characterized by faster flows ([Plont et al., 2022](#)) and more pronounced flashiness due to its urban setting, with the imperviousness of the entire watershed being 32% ([Ketabchy et al., 2023](#)). It warrants more research that details how hydrodynamics impact fragmentation along sites within the same network. There may be differences, as [Liro et al. \(2024\)](#) explained, mountain streams with more intense flows (average flow rate of 11 m^3/s) and a streambed mainly composed of gravel and cobbles (2–4 mm or greater), allowing for more contact with physical obstacles that can damage plastic items resulting in higher fragmentation rates. Our stream sites were predominantly composed of particles ranging from 0.5 to 2 mm (see [online supplementary material, Figure S4](#)) and had varying flow rates, with 2,419 m^3/d (0.028 m^3/s) at our forested site and 10,187 m^3/d (0.1179 m^3/s) at our urban site ([Plont et al., 2022](#)). Compared to [Liro et al. \(2024\)](#) extrapolations (PET bottle; mass loss of 0.26 g per year), our data show fragmentation being lower in our system (1 mg to 23 mg over 3 months or 4 mg to 92 mg per year for each of our polymers; see [online](#)

supplementary material, Table S4) versus the mountain stream Liro et al. (2024) investigated. Thus, corroborating their statement regarding differences between mountain and lowland systems. We have a caveat of importance to note, which is that in the present study, we used smaller pieces of macroplastics rather than bulk macroplastics (e.g., bottles and bags).

Although outside our scope, certain microbes and enzymes, such as PETases, can degrade plastic polymers through biofilm colonization and warrant further exploration (Howard & McCarthy, 2023; Nava & Leoni, 2021). For example, Oberbeckmann et al. (2016) found that filamentous cyanobacteria (*Phormidium*) can degrade hydrocarbons of macroplastics. While we did not constrain this directly, the presence of diatoms in our biofilms suggests this may be an important mechanism, as Dudek et al. (2020) indicated that diatoms provide an important habitat for these hydrocarbon-degrading bacteria. We did not perform SEM imaging on cellulose materials, because it has been demonstrated in freshwater that biofilm composition can differ between plastics and cellulose (Sabatino et al., 2024). In future work, we recommend that when looking at a more diverse group of polymers, it would be ideal to consider the following when investigating their degradation and fragmentation: observe biofilm development more closely to determine how the community changes over time, evaluate copollutants aside from MPs and synergies they may pose on degradation, measure flow more closely or place bags in areas with long term gauges, use site-specific data loggers for each bag to track how microclimates may influence degradation behavior, and isolate and assess enzymes from biofilms to determine their role in macroplastic degradation.

Environmental implications

Limited studies have investigated macroplastic degradation and fragmentation in freshwater, with even fewer that have evaluated these factors in situ to provide data on respective rates, a major knowledge gap in plastic research, according to Thompson et al. (2024). Low-order streams contribute greatly to the transport of water across landscapes and receive considerable amounts of plastic litter. Liro et al. (2023) highlighted the relatively unexplored nature of rivers as environments for plastic degradation and fragmentation. Riverine networks are valuable because of the connection between plastic pollution in rivers and stream networks that ultimately end up in oceans. During their journey, plastic debris traveling along rivers can release macroplastics, which can be a significant source of pollution (Liro et al., 2024). The present work contributes to the sparse literature from Liro et al. (2023), Liro et al. (2024), and Gerritse et al. (2020) regarding quantification methods for assessing macroplastic fragmentation in freshwater systems (Ford et al., 2022). Our results indicate that low-order riverine systems can be areas where oxidative processes lead to the degradation and fragmentation of macroplastics into MPs, with noticeable differences between urban and forested locations. The higher fragmentation in urban locations highlights the need for more attention, as urban streams are influenced by human activity and are subjected to high amounts of litter (Hoellein et al., 2024). Urban streams are traditionally flashier and experience faster flows compared to other stream types (Mogollón et al., 2016), which may influence plastic degradation and subsequent fragmentation. As Gray et al. (2024) found, non-target freshwater organisms can be exposed to MPs and accumulate MPs, where the impact on these important ecosystems is not well known compared to marine (Bexeitova et al., 2024). Also, we provide further insight into DOC leaching from aged macroplastics, where microbes that can utilize plastic-derived DOC may have inhibited or stimulated activity

due to additives and chemicals associated with plastic leachates. This warrants further investigation due to the implications regarding carbon cycling in freshwater and other biogeochemical processes facilitated by microbial communities that have not been fully elucidated under the umbrella of plastic pollution.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

Raw data can be provided upon request. Data used for statistical analysis and water quality can be found at <https://github.com/Golfballs13/Plastic-Degradation-Gray/blob/main/R%20output%20data.pdf> and <https://docs.google.com/spreadsheets/d/1EpyMwFerKjDkOjDTAJ3WzL4xwioYpggZ/edit?usp=sharing&ouid=109024147001144048768&rtpof=true&sd=true>

Author contributions

Austin Douglas Gray (Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing), Beija Gore (Data curation, Investigation, Writing—original draft, Writing—review & editing), Megan Gaesser (Data curation, Investigation, Writing—original draft, Writing—review & editing), Tessa Thibodeau (Data curation, Investigation, Writing—review & editing), Sam Purvus (Data curation, Investigation, Writing—review & editing), Allison Montgomery (Data curation, Investigation, Writing—review & editing), Kathryn Ouimet (Data curation, Formal analysis, Investigation, Writing—review & editing), Luisana Rodriguez Sequeira (Data curation, Investigation, Writing—original draft, Writing—review & editing), Tina Dura (Investigation, Methodology, Validation, Writing—original draft, Writing—review & editing), and Kathleen Mayer (Conceptualization, Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing—original draft, Writing—review & editing)

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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