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Biofilm assemblage and activity on plastic in urban streams at a continental scale: Site characteristics are more important than substrate type

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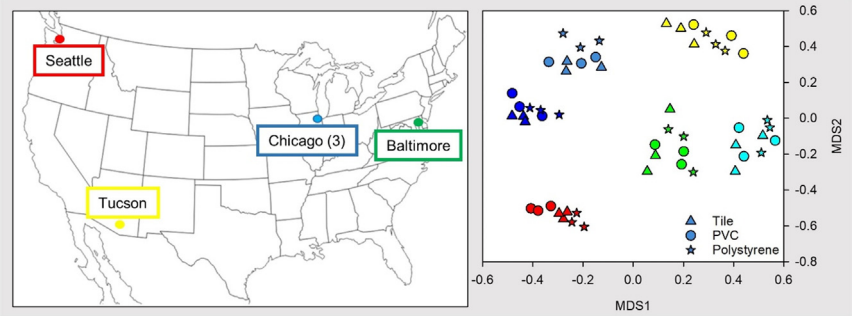
HIGHLIGHTS

- We incubated plastic and natural substrates in streams across the United States.
- Microbes on plastic were similar in type and activity to natural surfaces.
- Microbes on substrates were more influenced by incubation site than substrate type.
- Understanding microbial growth on plastic in streams is needed to predict its fate.

GRAPHICAL ABSTRACT



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ABSTRACT

The fate of plastics in rivers is a key component of the global plastic cycle. Plastics entering freshwater ecosystems are colonized by microbial biofilms, and microbe-plastic interactions can influence ecosystem processes and plastic fate. While literature examining the role of geographic region on plastic biofilms is quickly expanding, research which covers large (i.e., continental) spatial scales and includes freshwater ecosystems is warranted. In addition, most research focuses on bacterial communities, while biofilm eukaryotes are less commonly studied. We assessed biofilm metabolism and community structure on plastic (foamed polystyrene and polyvinyl chloride; PVC) and natural substrates (unglazed ceramic tile) in urban streams spanning a nested geographic gradient in the continental United States. We measured biofilm biomass, community respiration, and chlorophyll *a*, in addition to assessing marker gene-based community diversity of bacterial, fungal, and algal assemblages. Results demonstrated some substrate-specific trends in biofilm characteristics, including higher biofilm biomass on polystyrene across sites, and lower diversity of bacterial assemblages on both types of plastic litter versus tile. However, there were no differences among substrates for chlorophyll, respiration, and the abundance and diversity of algal and fungal assemblages. Thus, we concluded that the primary driver of biofilm metabolism and community composition were site characteristics, rather than substrate type. Additional studies are needed to quantify which site-specific characteristics drive biofilm dynamics on plastic litter in streams (e.g., water chemistry, light, seasonality, hydrology). These results add to the growing literature on the biofilm 'plastisphere' in aquatic ecosystems, demonstrating that the factors which control the assembly and activity of biofilm communities on plastic substrates (including bacteria, algal, and fungal assemblages together) in urban streams are similar to those driving biofilm dynamics on natural substrates.

1. Introduction

The accelerating rate of production and disposal of plastic since the 1950s has resulted in the accumulation of plastic litter in ecosystems worldwide (Geyer et al., 2017). Designed to be durable and resist degradation, plastic persists in the environment for long periods of time (Gewert et al.,

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2015). Negative interactions between plastic pollution and biota include ingestion, entanglement, and the dispersal of chemical additives (e.g., flame retardants, dyes) (Barnes et al., 2009; Gewert et al., 2015). Early research efforts emphasized the fate of plastic litter in oceans, with freshwaters note as a key source of the material (Jambeck et al., 2015; Rochman and Hoellein, 2020). Research on the input and distribution of plastic within freshwaters is increasing, but the relationship between plastic and freshwater biota is an understudied, yet critical, part of the global 'plastic cycle' (Hoellein and Rochman, 2021; Windsor et al., 2019).

Microbial biofilms, which consist of bacteria, algae, and fungi in a mucilaginous matrix, are ubiquitous in aquatic ecosystems (Battin et al., 2016). Biofilm communities on natural surfaces (e.g., leaf litter, wood, sand, rocks) are key sites for ecosystem metabolism and nutrient cycling in streams (Battin et al., 2016; Tank et al., 2010). Biofilms also form the base of aquatic food webs, serving as a food resource for higher trophic level organisms (Cross et al., 2007; Hall et al., 2000). Microbial communities in streams are sensitive to environmental heterogeneity, and changes in discharge, light, and nutrients can alter biofilm community structure and function (Battin et al., 2003; Cross et al., 2007). Biofilm communities are also distinct in composition and activity on different types of surfaces, such as leaf litter, rocks, and organic matter (Battin et al., 2016; Hoellein et al., 2009). Given the widespread interest in plastic pollution, studies of the activity and community dynamics of plastic-associated aquatic biofilms are quickly emerging (Amaral-Zettler et al., 2020; Harrison et al., 2018; Wright et al., 2021).

Biofilms in stream ecosystems are affected by urbanization. Human population density, infrastructure (e.g., roads), stormwater runoff, wastewater, and the input of a wide array of synthetic chemicals (e.g., plastic) can influence the growth, dispersal, and activity of biofilm constituents (Binh et al., 2016; Booth et al., 2016; Kolpin et al., 2004; Peterson et al., 2011; Schade et al., 2011). Urban landscapes are considered 'homogenized' when human-induced environmental change selects for similar taxa or species guilds at sites spanning large geographic distances (Groffman et al., 2014; McKinney, 2006). For example, relative to biofilms in forested systems, urban streams support a distinct microbial 'pool' including taxa from waste- and stormwater infrastructure, while rare, native taxa are inactivated or extirpated (Hosen et al., 2017). Therefore, urban streams in different regions show similarity of microbial communities, and thus are considered 'homogenized', even across large geographic distances.

In aquatic ecosystems, plastic particles are substrates for microbial biofilm growth that share some properties with natural surfaces such as rocks and wood (e.g., relatively recalcitrant, often hard surface), but with some unique chemical and physical properties (e.g., leaching and adsorbed chemicals, potential hydrophobicity) (Amaral-Zettler et al., 2020; Harrison et al., 2018). The role of plastic-mediated selection of biofilm constituents has been a subject of study in marine ecosystems. For example, an experimental incubation of plastic and natural substrates at 4 globally distributed marine locations showed that bacterial community composition was driven by site-specific characteristics rather than substrate type (Coons et al., 2021). Recent reviews have also suggested that site characteristics (e.g., water chemistry, temperature) may be more important than substrate type for determining biofilm growth and activity, although comparing among studies with varied endpoints, contexts (i.e., laboratory vs in situ), and experimental designs is challenging (Amaral-Zettler et al., 2020; Oberbeckmann and Labrenz, 2020). In freshwaters, distinct microbial assemblages and metabolic processes may occur on plastic relative to natural substrata, especially for organic particles such as leaves and wood (Hoellein et al., 2014, 2017); however, no studies have tested the role of site relative to substrate type on biofilm assemblage in streams for bacterial, algal, and fungal assemblages in parallel. Most studies have focused on bacteria, while the algal and fungal components of plastic-associated biofilms are less commonly considered (Amaral-Zettler et al., 2020). More analyses are needed that incorporate multiple taxa into biofilm studies, as all groups are important components of aquatic biofilms that contribute to community structure and function.

The aim of this study was to determine if plastic litter affects biofilm community structure, function, and composition, including bacterial, algal, and fungal assemblages, in urban streams spanning a nested geographic gradient. We hypothesized that the chemical and structural properties of plastic surfaces may select for similar communities of microbes in different streams. Thus, we predicted plastic would select for "homogenized" biofilms of reduced complexity and similar community composition when incubated in streams in different biomes, regardless of the distance among sites. In contrast, we expected biofilms on natural surfaces to host diverse and distinct assemblages with unique organisms for each biome.

2. Materials and methods

2.1. Study sites

The study sites were in densely populated areas with strong historical records of ecological research, including 3 sites local to Chicago and 3 others across the United States, representing 4 different biomes (Fig. 1, Table 1). The 3 streams in the Chicago-metropolitan region were the North Branch Chicago River (Chicago, IL), Higgins Creek (Des Plaines,

Fig. 1. A) Study sites included Gwynns Falls (Baltimore, Maryland), the Santa Cruz River (Tucson, Arizona), Thorton Creek (Seattle, Washington), and B) 3 sites in Chicago, Illinois, Springbrook Creek (SC), Higgins Creek (HC), and the North Branch Chicago River (NB).

Table 1
Summary of sampling dates, as well as the physical and chemical measurements for 6 study sites. N Br = North Branch, Cr. = Creek, R. = River, Temp. = Temperate, Temp = temperature, Cond = specific conductivity.

	Location	Latitude, longitude	Biome	Start date	End date	Incubation (days)	Water temp. (°C)	Cond. (μS cm^{-1})
Higgins Cr.	Des Plaines, IL	42.01909, −87.93675	Temp. grassland	3-Jul-18	24-Jul-18	21	22.5	1048
N Br Chicago R.	Chicago, IL	41.97774, −87.73949	Temp. grassland	10-Jul-18	28-Jul-19	18	22.2	1119
Springbrook Cr.	Wheaton, IL	41.84074, −88.14643	Temp. grassland	3-Jul-18	23-Jul-18	20	21.9	966
Santa Cruz R.	Tucson, AZ	32.35096, −111.09601	Desert	1-Jun-18	22-Jun-18	21	31.7	1198
Gwynns Falls	Baltimore, MD	39.35173, −76.73990	Temp. deciduous forest	7-Jun-18	27-Jun-18	20	20.6	554
Thornton Cr.	Seattle, WA	47.70111, −122.30733	Temp. rain forest	25-Jun-18	16-Jul-18	21	16.3	261

IL), and Springbrook Creek (Wheaton, IL). The remaining sites were the Santa Cruz River (desert; Tuscon, AZ), Gwynns Falls (temperate deciduous forest; Baltimore, MD), and Thornton Creek (temperate rain forest; Seattle, WA; Fig. 1).

2.2. Substrate preparation and incubation

Substrates with different physical and chemical properties were deployed at each site. Foamed polystyrene (i.e. Styrofoam), polyvinyl chloride (PVC), and unglazed ceramic tile were arranged randomly in mesh bags commonly used for leaf breakdown studies (3.3 mm pore size; Cady Bag, Pearson, Georgia) (Hoellein et al., 2014). Ceramic tile is a commonly used substrate in stream ecology as a surrogate for rock surface. Mesh bags contained 12 individual pieces ($N = 4$ each of polystyrene, PVC, and unglazed tile) secured with nylon zipties (Supplemental Fig. 1). Polystyrene containers (thickness = 2 mm) and sheets of PVC (thickness = 3.5 mm) were cut into 16 cm^2 squares. Tile (thickness = 5 mm) was 23 cm^2. Four bags were attached to iron rebar (Tucson, AZ) or concrete cinderblocks (other sites) and submerged for 18–21 days (Hoellein et al., 2014). Incubations occurred from June 1 to July 28 in summer 2018.

2.3. Biofilm respiration and substrate collection

Biofilm respiration was measured in situ during each collection event. Triplicate samples of each substrate were carefully rinsed with stream water to remove settled debris and macroinvertebrates, and placed in 160 mL specimen containers. We also prepared 3 controls with water only to account for abiotic and water column effects on dissolved oxygen. All sample containers ($N = 3$ for each substrate) were filled with stream water to eliminate air bubbles, enclosed in a black plastic bag to prevent light exposure, and submerged (Hoellein et al., 2009). We measured dissolved oxygen (DO; HQ40d portable meter, Hach, Loveland, CO, USA) at the beginning of a 3-hour incubation, and in each specimen container following the incubation. Afterwards, all substrates were wrapped in aluminum foil and stored on ice for transport to the laboratory and frozen (−20 °C). At each site, we recorded water temperature and specific conductivity (μS; Yellow Springs Instruments, Yellow Springs, Ohio) at the time of collection. We recorded stream depth to the top of the substrate (cm). Future studies would benefit from collection of additional site-specific characteristics (e.g., light, water chemistry, and turbidity) that were not recorded here.

2.4. Biofilm biomass and chlorophyll-a

We measured biofilm biomass using aqueous crystal violet (hexamethyl pararosaniline chloride) dye and ethanol elution. Triplicates of each colonized substrate and un-colonized 'control' substrates ($N = 3$ each of polystyrene, PVC, and unglazed tile) were rinsed with DI water and placed in 150 mL aluminum weigh pans to dry. We used 2 mL of 1% aqueous crystal violet to stain the biofilm extracellular matrix on the surface of each substrate (Burton et al., 2007). We waited 45 min to allow for adherence to the biofilm. Substrates were rinsed with DI water to remove excess stain and transferred to weigh dishes for ethanol (EtOH) elution. We added 15 mL of 95% EtOH to each weigh dish and agitated for 30 s to ensure complete coverage. After 10 min, we decanted the EtOH and unbound stain into 5 mL disposable glass test tubes (Kimble, DWK Life Sciences, Millville, NJ), and we completed a 1:5 dilution prior to measuring absorbance. Optical density was measured at 595 nm using a spectrophotometer (Shimadzu Pharmaspec 1700 UV–Vis Spectrophotometer, Shimadzu North America, Columbia MD). We corrected for background absorbance using uncolonized controls.

We measured chlorophyll-a using a hot ethanol extraction. Triplicate samples of each substrate were swabbed with sterile cotton swabs (Puritan Products, Guilford, ME) and the swabs were placed in 15 mL centrifuge tubes (VWR International, Radnor, PA) and frozen until 24 h before processing. Clean cotton swabs ($N = 3$) were placed in 15 mL centrifuge tubes to serve as laboratory controls. We added 5 mL of 95% EtOH to each tube, inverted 4–5 times to mix, and placed in a 75 °C water bath for 15 min. Once removed from the bath, centrifuge tubes were left at room temperature for 2 h in the dark. We decanted the extracted solution into 5 mL glass Kimble tubes (Kimble, DWK Life Sciences, Millville, NJ) and allowed 15 min for suspended particles to settle in the tubes. Then, we measured the absorbance of chlorophyll and phaeophytin at 750 and 650 nm using a spectrophotometer (Thermo SPECTRONIC® 20 Genesys®, Spectrum Chemical, New Brunswick, NJ). Following the initial readings, all samples were acidified with 0.2 mL of 0.25 M hydrochloric acid (HCl). Absorbance by the acidified samples was re-measured at the same wavelengths to assess phaeophytin absorbance. All measurements were corrected by subtracting the average absorbance of the 3 swab controls. The difference between pre- and post-acidification measurements allows for calculation of chlorophyll according to standard methods (APHA, 1998).

2.5. Biofilm community assemblages

We swabbed triplicates of each substrate from each study site with sterile cotton swabs (Puritan Products) and completed DNA extraction following manufacturer instructions using Qiagen Power Soil DNA extraction kits (Qiagen, Hilden, Germany). One blank swab per extraction was included to control for contamination. We also extracted 2 'kit' controls (i.e., no swab) to test for contamination. For bacteria, we conducted polymerase chain reaction (PCR) with the 515F and 806R primers to amplify the V4 hypervariable region of 16S rRNA gene (Caporaso et al., 2012). For fungi we used the ITS1f and ITS2 primers for the ITS regions (Gardes and Bruns, 1993; White et al., 1990). For algae we used the p23SrV_f1 and p23SrV_r1 primers for the plastid 23S rRNA gene in eukaryotic algae and cyanobacteria (Sherwood and Presting, 2007). Successful DNA extraction and PCR amplification was confirmed by agarose gel electrophoresis. Amplified samples were sequenced by the University of Illinois at Chicago Sequencing Core using the Illumina MiSeq platform in 2 × 250 paired-end format.

Community assemblage data were processed in MOTHUR (version 1.40.0; (Schloss et al., 2009)) following the MiSeq standard operating procedure (Kozich et al., 2013). Sequences were assembled and demultiplexed, and sequences with ambiguities or homopolymers >8 bases were removed from the dataset. We aligned bacterial sequences against the SILVA ssu database (updated 13Dec2017), algal sequences against the SILVA lsu database (updated 17Dec2017), and the fungal sequences against the UNITE ITS reference (updated 1Dec2017). We used Uchime to remove chimeric sequences (Edgar et al., 2011), and sequences were clustered according to 97% similarity in operational taxonomic units (OTUs). Data sets were randomly subsampled to 26,945 sequences per sample for bacterial assemblages,

12,265 sequences per sample for the algal assemblages, and 5056 sequences per sample for the fungal assemblages. Procedures used for microbial community analyses are consistent with previous analyses from urban streams (Hoellein et al., 2014; McCormick et al., 2016). All sequence data are available from the National Center for Biotechnology Information Sequence Read Archive, accession number PRJNA823476.

2.6. Data analysis

We used 2-way analysis of variance (ANOVA) to compare heterotrophic respiration rates (mg O_2 cm^{-2} h^{-1}) among polystyrene, PVC, and tile substrates at our six study streams. Following a significant interaction between sites and substrates, we used 1-way ANOVA to compare respiration among substrates at each site individually. We used a Bonferroni correction to account for multiple comparisons ($\alpha = 0.05/6 = 0.008$). Biofilm biomass (optical density cm^{-2}) and autotroph abundance (chlorophyll cm^{-2}) were compared among sites and substrates using 2-way ANOVA. We used Tukey's multiple comparison test to identify differences among seasons or habitats following a significant *P*-value (≤ 0.05).

We used MOTHUR to assess bacterial diversity based on observed OTUs and the Shannon-Weiner (H′) index. Differences in OTU richness and Shannon diversity among sites and substrates were identified using 2-way ANOVA and Tukey's post-hoc test. We generated a Bray-Curtis dissimilarity index and subsequent distance matrix in MOTHUR to assess differences in community assemblage among sites and substrates. Distance matrices were visualized using nonmetric multi-dimensional scaling (nMDS). Differences in bacterial assemblage among sites and substrates were identified using analysis of similarity (ANOSIM). All data were assessed to meet the statistical assumptions. Analyses were completed using MOTHUR and SYSTAT 13 (SYSTAT Software, Crane Software International, Chicago, IL, USA). For algal and fungal assemblages, all the corresponding statistical analyses as for the bacterial assemblage were performed in R v4.0.4.

3. Results

3.1. Biomass, respiration, and chlorophyll

Biofilm biomass, measured as optical density (cm^{-2}) was different among substrates (2-way ANOVA $F_2 = 8.16$, $P = 0.001$) and sites (2-way ANOVA $F_5 = 10.21$, $P \leq 0.001$), with no significant interaction ($F_{10} = 1.03$, $P = 0.441$; Fig. 2A). Optical density was highest on polystyrene, with no difference between tile and PVC. Among sites, biomass was highest at the North Branch Chicago River, while streams in Arizona, Maryland, and Washington were intermediate, and Higgins Creek and Springbrook Creek were lowest (Fig. 2A).

Biofilm respiration was variable among sites and substrates (Fig. 2B). Respiration was greater at Higgins Creek and the Santa Cruz River relative to the other sites. Due to a significant interaction between site and substrate (2-way ANOVA $F_{10} = 8.60$, $P \leq 0.001$), we analyzed differences among substrates for each site individually, using a Bonferonni-corrected *P*-value of $0.05/6 = 0.008$. At Higgins Creek, respiration rates were highest for biofilms on polystyrene, tile was intermediate, and PVC was lowest (1-way ANOVA $P = 0.006$; Fig. 2B). Springbrook Creek showed a different pattern, where respiration on tile was highest, followed by PVC and polystyrene. There were no differences among substrates at the other 4 sites (Fig. 2B).

Chlorophyll concentration differed among sites (2-way $F_5 = 10.25$, ANOVA $P = 0.001$), but there were no significant differences among substrates (2-way ANOVA $F_2 = 2.59$, $P = 0.089$) and no significant interaction (2-way ANOVA $F_{10} = 2.09$, $P = 0.052$; Fig. 2C). Chicago-area sites and Thornton Creek (Washington) showed the highest chlorophyll, Gwynns Falls (Maryland) was intermediate, and the Santa Cruz River (Arizona) was lowest (Fig. 2C).

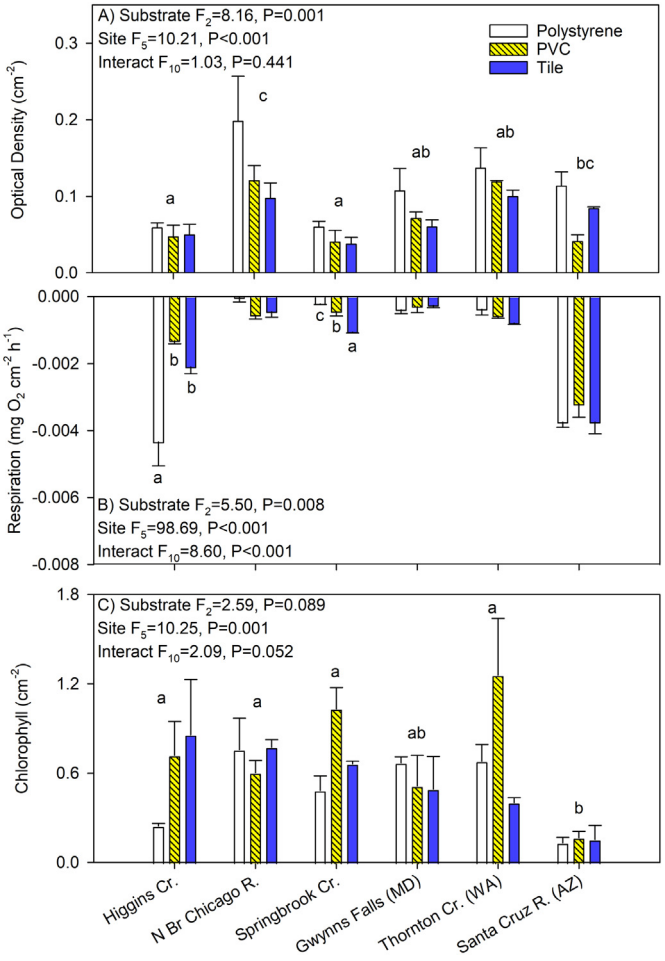

Fig. 2. Mean (±SE) (A) optical density (cm^{-2}) of crystal violet (biomass), (B) respiration rate, and (C) chlorophyll on polystyrene, PVC, and tile incubated at 6 sites. Letters on (A) and (C) represent Tukey's Test results by site. On panel (B), small letters are from 1-way ANOVA analyses among substrata for each site individually, completed after a significant site x substrate interaction. Abbreviations: N Br = North Branch, Cr. = Creek, R. = River.

3.2. Biofilm assemblages: richness and diversity

Bacterial assemblage patterns varied across substrate types and sites. The mean number of observed OTUs was significantly higher on tile, relative to polystyrene and PVC (2-way ANOVA $F_2 = 6.47$, $P = 0.004$; Fig. 3A). Among sites, OTU richness was highest at Higgins Creek (4255 ± 148), while Gwynns Falls, Santa Cruz River, North Branch Chicago River, and Springbrook Creek were intermediate, and Thornton Creek was lowest (1281 ± 47, 2-way ANOVA, $F_5 = 152.64$, $P \leq 0.001$; Fig. 3A). For Shannon diversity of bacterial assemblages, the interaction between substrate and site was significant (2-way ANOVA $F_{10} = 2.39$, $P = 0.027$, Fig. 4A). Thus, we used a 1-way ANOVA among substrates, for each site individually (with Bonferonni-correction of $P = 0.008$ for significant differences) and found that biofilm diversity among substrates was different only at Higgins Creek (1-way ANOVA $F_2 = 18.69$, $P = 0.003$) and Gwynns Falls (1-way ANOVA $F_2 = 13.48$, $P = 0.006$). In both streams, polystyrene had the least diverse community relative to PVC and tile (Fig. 4A).

Algal community metrics showed differences among sites but not among substrates. Algal OTU richness was highest in Gwynns Falls (Maryland) and lowest in Thornton Creek (Washington) (2-way ANOVA $F_5 = 33.02$, $P < 0.001$), with no difference among substrates (2-way ANOVA, $F_2 = 0.36$, $P = 0.509$; Fig. 4B). Algal Shannon diversity was highest in Gwynns Falls and the Santa Cruz River (Arizona) compared to other sites

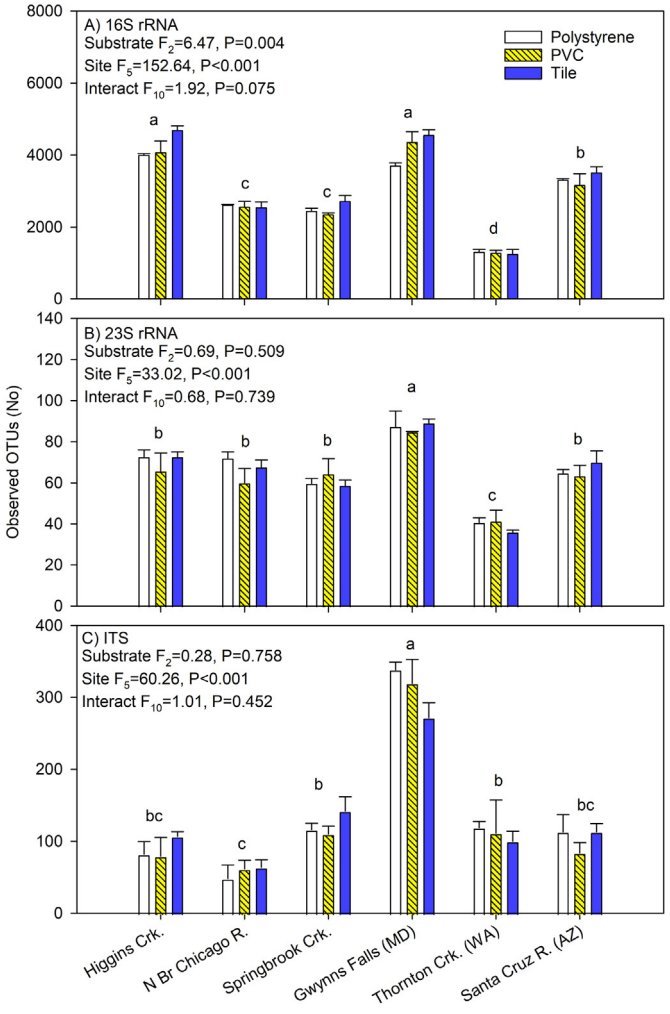

Fig. 3. Mean (±SE) number of observed operational taxonomic units (OTUs) for assemblages of (A) bacteria (16S rRNA gene), (B) algae (23S rRNA gene), and (C) fungi (ITS region) on polystyrene, PVC, and tile incubated at 6 different sites. Letters represent Tukey's Test results by site. Abbreviations: N Br = North Branch, Crk. = Creek, R. = River.

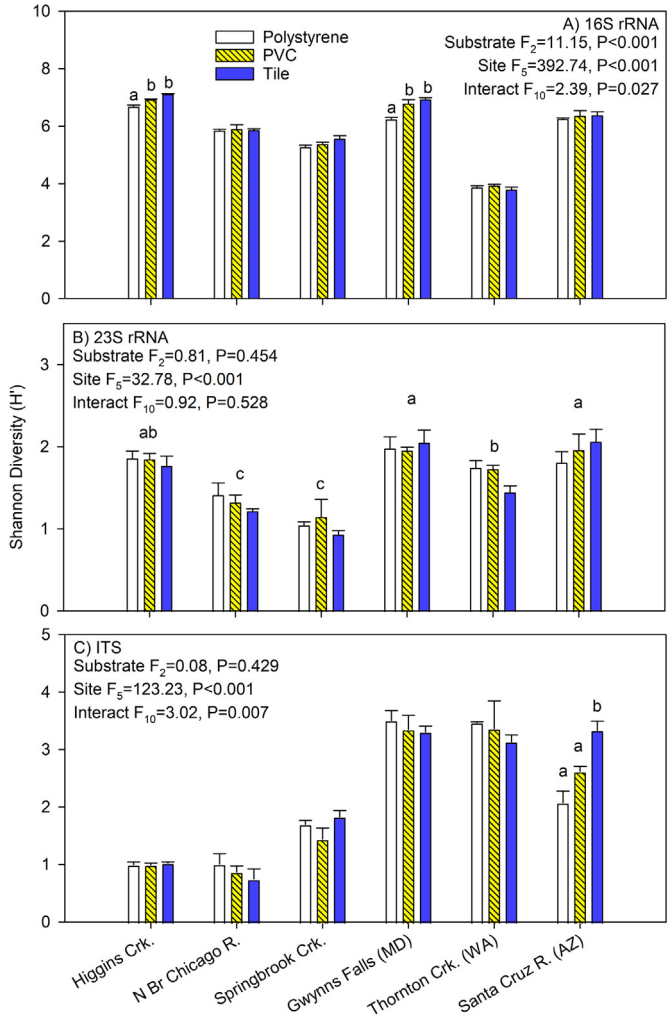

Fig. 4. Mean (±SE) Shannon-Weiner diversity index (H′) for assemblages of (A) bacteria (16S rRNA gene), (B) algae (23S rRNA gene), and (C) fungi (ITS region) on polystyrene, PVC, and tile at 6 different sites. Letters on (B) represent Tukey's Test results by site. On panel (A) and (C), small letters are from 1-way ANOVA analyses among substrata for each site individually, after a significant site x substrate interaction. Abbreviations: N Br = North Branch, Crk. = Creek, R. = River.

(2-way ANOVA $F_5 = 32.78$, $P < 0.001$) with no differences by substrate (2-way ANOVA, $F_2 = 0.81$, $P = 0.454$; Fig. 4B).

Fungal OTU richness and diversity across substrates and streams showed different patterns. Fungal OTU richness was significantly higher at Gwynns Falls compared to other sites (2-way ANOVA $F_5 = 60.26$, $P < 0.001$), and there was no difference among substrates (2-way ANOVA $F_2 = 0.28$, $P = 0.758$; Fig. 3C). However, fungal Shannon diversity showed a significant interaction of substrate and site (2-way ANOVA $F_{10} = 3.02$, $P = 0.007$), so we compared across substrates for each stream individually (using a Bonferonni-corrected *P*-value). The Santa Cruz River was the only site with a significant difference among substrates (1-way ANOVA $F_2 = 16.01$, $P = 0.004$), where fungal taxa diversity was higher on tile than PVC and polystyrene (Fig. 4C). With respect to site, fungal diversity was highest at Gwynns Falls, Thornton Creek, and Santa Cruz River, and lowest at the three Chicago sites.

3.3. Biofilm assemblages: community composition

We analyzed the 16S rRNA sequence results using NMDS and ANOSIM to assess differences by geographic scale and substrate type. Analysis of similarity showed the bacterial community composition across sites was significantly different, where each of the 6 sites was representative of a distinct assemblage of OTUs (ANOSIM $P \leq 0.001$ for all pairwise comparisons; Table 2). In contrast, there were no differences in community composition when comparing among tile, polystyrene, and PVC substrata at each site. The 6 study sites formed distinct clusters on the nMDS, showing the differences among streams (Fig. 5A). The three Chicago-area sites were spread across the x-axis of the NMDS, so variation in biofilm community composition among the 3 streams in close proximity was in equal proportion to the variation among streams from different biomes in the United States. In contrast, differences in community composition among substrates were much smaller than among sites, and there is little clustering of substrate-specific biofilm communities within each site (Fig. 5A).

Similar to the bacterial assemblages, the OTU-based composition for the algal assemblages significantly differed across each site (ANOSIM, $P \leq 0.002$; Table 2). Although there were significant site-specific differences in algal composition, the three Chicago-area sites were closely clustered on the NMDS and there was little clustering for the other sites (Fig. 5B). There was no substrate-specific clustering of algal assemblages within each site.

Fungal assemblages showed similar trends as algae. The composition of the fungal assemblages at each site was significantly different from the other sites (ANOSIM, $P \leq 0.002$; Table 2), but the fungal assemblages for the Chicago-area sites clustered closer to each other on the NMDS plot

Table 2
Results from pairwise ANOSIM analysis of differences among sites for assemblage of bacteria, algae, and fungi. Top and bottom values are the *P*-value and R value, respectively. Abbreviations: Crk = creek, R = River, N = North.

Site	Higgins Crk.	N. Branch Chicago R.	Springbrook Crk.	Santa Cruz R.	Gwynns Falls
Bacteria					
N. Branch Chicago R.	≤0.001 1.000				
Springbrook Crk.	≤0.001 1.000	≤0.001 1.000			
Santa Cruz R.	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000		
Gwynn's Falls	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000	
Thornton Crk.	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000	≤0.001 1.000
Algae					
N. Branch Chicago R.	≤0.001 0.956				
Springbrook Crk.	≤0.001 0.728	≤0.001 0.719			
Santa Cruz R.	≤0.0010.980	≤0.001 0.808	≤0.001 0.913		
Gwynns Falls	≤0.001 0.859	0.002 0.985	≤0.001 0.798	≤0.001 0.976	
Thornton Crk.	≤0.001 0.801	≤0.001 0.784	0.002 0.603	≤0.001 0.951	≤0.001 0.885
Fungi					
N. Branch Chicago R.	≤0.001 0.768				
Springbrook Crk.	0.002 0.778	≤0.001 0.579			
Santa Cruz R.	≤0.001 0.963	≤0.001 0.664	≤0.001 0.730		
Gwynns Falls	≤0.001 1.000	≤0.001 0.801	≤0.001 0.786	≤0.001 0.918	
Thornton Crk.	≤0.001 0.982	≤0.001 0.686	≤0.001 0.691	≤0.001 0.800	≤0.001 0.806

relative to the other three sites (Fig. 5C). There was no clustering of substrate-specific fungal assemblages within each site.

3.4. Common taxa among and within sites

Comparison of the taxonomic groups which make up the biofilm communities from each of the study sites showed distinct and diverse assemblages of bacteria. The 25 most abundant families in the entire dataset represented >74% of the sequences across each of the sites. Taxa that were common across sites included unclassified Bacteria, Comamonadaceae, Rhodobacteraceae, Sphingomonadaceae, Rhizobiales (unclassified) and Burkholderiales (unclassified) (Supplemental Fig. 2). Some taxa showed site-specific variations in abundance, for example, Comamonadaceae was more abundant in Thornton Creek than elsewhere.

Algal communities in biofilms were different among sites, although Bacillariophyta, unclassified Bacillariophyceae, and unclassified Eukaryota combined made up an average of 73.1% of sequences (Supplemental Fig. 3). Other taxa showed site-specific patterns. For example, unclassified Cyanobacteria made up 5.9–10% of sequences in Gwynns Falls, Higgins Creek, and the Santa Cruz River, but were <0.7% of sequences at the other 3 sites. Tetradesmus was 11.5% of sequences from the Santa Cruz River, but was <0.4% of sequences at other sites. Finally, unclassified Ulvophyceae was 8% of sequences at Thorton Creek, but <0.3% of sequence at the other sites (Supplemental Fig. 3).

Fungal community composition across sites revealed unique patterns for each location. 'Unclassified fungi' was the most common taxon noted, representing an average of 52% of sequences across the 6 sites. The most abundant taxon identified was Chytridiomycota, which ranged from a high of 30.3% of sequences in Higgins Creek to a minimum of 0.4% of

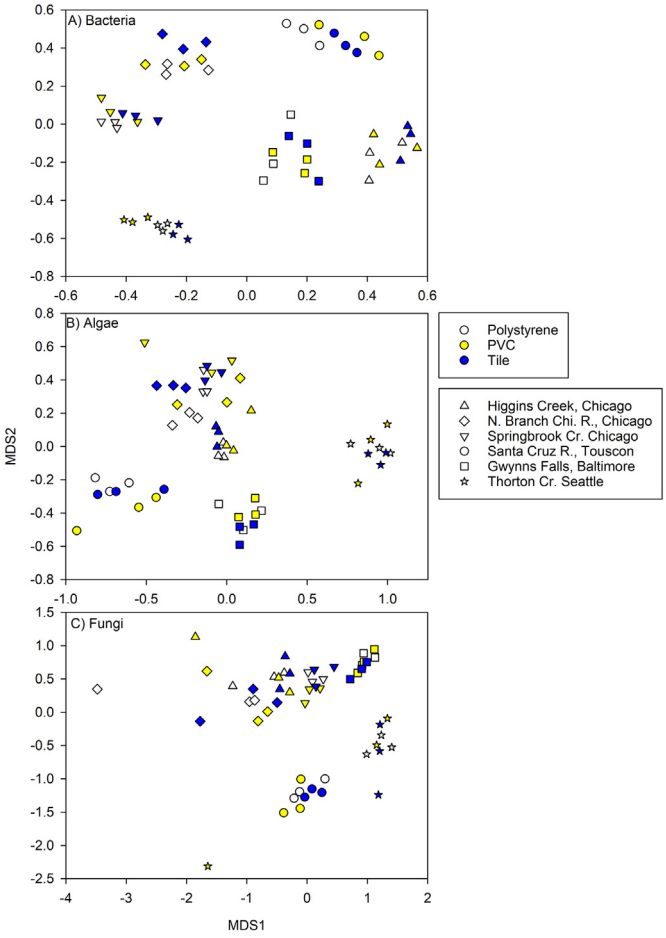

Fig. 5. Nonmetric multidimensional scaling (nMDS) ordination of assemblages of (A) bacteria (16S rRNA gene), (B) algae (23S rRNA gene), and (C) fungi (ITS region) (from 3 different substrates incubated at 6 different sites). Stress = 0.26, 0.12, 0.16 for bacteria, algae, and fungi, respectively. Ordinations were created using 100 iterations.

sequences in the Santa Cruz River. Other taxa abundant at individual sites include Rhizophydiales (20.6% of sequences at Springbrook Creek), Pleosporales (an average of 6.9% of sequences across Santa Cruz River, Thorton Creek, and Gwynns Falls), and Cladosporium (5.4% of sequences at Thorton Creek) (Supplemental Fig. 4).

4. Discussion

Understanding microbial colonization and activity on plastic pollution is critical to quantify its transport, fate, and biological impacts (Amaral-Zettler et al., 2020; Wright et al., 2021). Microbial biofilms serve a crucial role in nutrient cycling and are the base of aquatic food webs, and they can affect plastic transport and contribute to plastic breakdown in aquatic habitats (Battin et al., 2016; Gewert et al., 2015; Hoellein et al., 2019). We found robust microbial growth on natural and plastic substrates incubated in streams across the continental United States. Some aspects of biofilm structure and composition were affected by substrate type, including enhanced biomass and reduced bacterial OTU richness for biofilms on polystyrene relative to PVC and tile. However, we found mixed evidence that plastic substrate supported distinct bacterial assemblages compared to a natural substrate, and no evidence that plastic influenced algal or fungal biofilm constituents in this study. Overall, we conclude that plastic did not select for 'homogenous' biofilm communities at different sites, and that biofilm assemblage was driven more strongly by site-specific environmental conditions than by the plastic itself.

4.1. Biofilm biomass, chlorophyll, and respiration across substrates

Organic and inorganic compounds immediately adsorb to the surface of a submerged substrate, forming a layer capable of supporting microbial growth (Rummel et al., 2017). The physical properties of a substrate (e.g., roughness, hydrophobicity) and the chemistry of adsorbed compounds can affect biofilm colonization (Lorite et al., 2011; Ogonowski et al., 2018). Because plastic has unique chemical structure and adsorbates, we expected plastic would select for similar and simplified biofilm community structure and function, which we quantified as biomass, respiration, and chlorophyll among sites.

Biomass, respiration, and chlorophyll were all significantly different among sites, but only biomass showed a difference among substrate types (i.e., higher on polystyrene compared to PVC and tile). We conjecture that the texture and surface area of polystyrene accounted for this pattern. Despite sharing hydrophobic properties with PVC, the porous, sponge-like nature of polystyrene provides additional surface area for the initial matrix of organic and inorganic compounds to develop and could retain fine particulate organic matter, thereby supporting microbial growth. Thus, we suggest that the high biofilm biomass on foamed polystyrene was due to the surface architecture rather than the chemistry of the material. Because this pattern was the same across sites, we conclude it is one factor affecting biofilm properties on polystyrene.

Enhanced biofilm biomass on polystyrene could be important at larger spatial scales in urban streams, given the abundance of polystyrene litter. Polystyrene represented 3–8% of all macro-scale litter (i.e., visible to the naked eye) in the benthic zones and 3–15% of macolitter in riparian zones of streams in the Chicago area (McCormick and Hoellein, 2016). In the Great Lakes, foamed polystyrene was most common form of plastic pollution observed in surface waters and shorelines (Eriksen et al., 2013; Zbyszewski et al., 2014). Because polystyrene is a major constituent of the 'community' of plastic litter, factors which affect biofilm growth differently than other types of plastic are important to predict its fate. We expect the high microbial biomass on polystyrene relative to other plastic types could ultimately contribute to faster degradation or enhanced retention (Gewert et al., 2015), and should be considered for polymer-specific models of plastic budgets and transport.

Substrate was not the key driver of biofilm respiration rates, which varied more strongly by site. Biofilm respiration rates showed no differences among substrata at 4 sites, and contrasting patterns among substrates at the remaining 2 sites. At Higgins Creek, polystyrene had significantly higher biofilm respiration rates than PVC and tile, while at Springbrook Creek, polystyrene had the lowest rates, PVC was intermediate, and tile was highest. Across sites, the high respiration at Higgins Creek and the Santa Cruz River were likely due to the treated wastewater (Eppehimer et al., 2021; Hamdhani et al., 2020; McCormick et al., 2016).

The patterns for chlorophyll also do not suggest a role for substrate as a strong driver in total autotroph abundance. Sites in the Chicago-area and in Washington supported higher chlorophyll abundance, while chlorophyll at Gwynns Falls and the Santa Cruz River were intermediate and low, respectively. We cannot attribute the differences to canopy cover or light among sites. For example, the Santa Cruz River had little riparian shading and low chlorophyll levels, Higgins Creek had no riparian shading (i.e., the riparian zone was concrete) and high chlorophyll, while Gwynns Falls had a dense riparian canopy and high chlorophyll. Other factors which could affect site-specific patterns in chlorophyll include scouring, turbidity, and water quality, but were not assessed here.

4.2. Bacterial assemblage: mixed impacts of substrate and site

While many metrics for biofilm characteristics did not differ on tile relative to PVC and polystyrene (i.e. respiration, chlorophyll, and communities of fungi and algae), the number of bacterial taxa on plastic was lower than tile across sites (Fig. 2A), suggesting some impact of plastic litter on bacterial constituents of biofilm. However, the *magnitude* of the impact of substrate type was much lower than the impact of study site, as illustrated by 2-way ANOVA (substrate F-value = 6.47 and site F-value = 152.64; Fig. 3A). In addition, given the relatively high number of OTUs in the biofilms overall, it is not clear what the ecological effects of reduced bacterial OTU richness will be, if any. The role of plastic in reducing bacteria OTU richness merits further exploration of plastic biofilms, however, including studies that span gradients of biofilm succession, different environmental conditions, and metagenomics approaches, to offer more insight into the effects of plastic on bacterial assemblages and activity (Wright et al., 2021).

The patterns for diversity of bacterial assemblages were mixed among sites. Four sites showed no differences in diversity among substrates, while the two sites which showed significant differences in bacterial diversity among substrata (Higgins Creek and Gwynns Falls) had the same pattern, where tile (i.e., the natural substrate) and PVC had more diverse communities than polystyrene. These sites also had the most OTUs and had similar community composition to one another. We do not have an ecological explanation for the low diversity on polystyrene only at Higgins Creek and Gwynns Falls or their similarity in taxonomic composition. We conclude from these patterns that the effects of plastic litter on bacterial diversity in freshwaters are context specific, and more analyses at sites of varying environmental conditions are needed (Amaral-Zettler et al., 2020; Harrison et al., 2018; Wright et al., 2021).

Assessing community composition using nMDS clearly demonstrated that site, and not substrate type, most strongly determined the assemblage of bacteria. Surprisingly, geographic region was not a strong factor either. In our partially 'nested' study design, we expected the 3 sites in close proximity (Higgins Creek, Springbrook Creek, and North Branch Chicago River) to show more similarities in bacterial assemblages compared to the 3 sites from other biomes. However, the nMDS groupings for the bacterial assemblages show the 3 Chicago-area sites were variable and distributed across the ordination space to the same degree as assemblages from the 3 sites in disparate biomes. Previous work on freshwater biofilms colonizing anthropogenic and natural substrates also found that location (e.g., river, pond, artificial stream, water column, sediment), and not substrate (e.g., tile, glass, plastic), was a key driver of biofilm assemblage (Hoellein et al., 2015, 2017). Therefore, the periphyton-like biofilm that develops on hard surfaces in streams has a similar bacterial assemblage as adjacent hard substrates, regardless of that surface material, and may conduct similar biofilm-mediated ecosystem processes.

4.3. Algal and fungal assemblages: no impact of substrate and role of geography

The algal and fungal assemblages provided similar evidence as the bacteria with respect to the lack of biofilm community homogenization on plastic across different sites. However, on the NMDS illustration of assemblages across sites, the bacterial results show the 3 Chicago-area sites were separate and spaced across the ordination, while the fungal and algal assemblages showed the 3 Chicago-area sites were grouped together. We acknowledge that ANOSIM pairwise comparisons showed distinctions among the 3 Chicago area fungal and algal communities. However, the relative similarity among those sites on the NMDS, compared to the 3 sites elsewhere in the country, merits some interpretation. This pattern may be due to the differing role of environmental factors in driving the assembly of the biofilm communities. For instance, the predominantly heterotrophic and highly diverse bacterial assemblages may be more influenced by local variation in water chemistry and organic matter composition, and thus reflect greater differences in assemblages across sites. In contrast, algal and fungal assemblages may be driven by abiotic factors that show regional patterns (e.g., sunlight and temperature), thus sites in close geographic proximity could exhibit greater similarity in algal and fungal assemblages. Our understanding of the biogeography of non-bacterial constituents of microbial biofilms on plastic and natural surfaces would benefit from additional study (Amaral-Zettler et al., 2020; Lacerda et al., 2020).

The literature which examines the role of geographic region on plastic biofilms is developing rapidly, and offers mixed conclusions and a role for biofilm successional stage as a key driver of patterns. Amaral-Zettler et al.

(2020) and Oberbeckmann and Labrenz (2020) reviewed studies on biofilm colonization of plastic and non-plastic controls (i.e., water, organic matter, or non-organic surfaces), under experimental and in situ conditions (Amaral-Zettler et al., 2020; Lacerda et al., 2020). While comparing among publications is challenging and there is much remaining to learn, both reviews concluded there was less evidence for plastic-based microbial selection relative to other factors such as climate, season, and water chemistry. Coons et al. (2021) conducted incubations of different plastic polymers and a control (i.e., glass slides) for 5 weeks at 4 widely dispersed locations in coastal areas globally, and showed location, not substrate type, was the driving factor for bacterial assemblages.

4.4. Taxonomic groups in stream biofilms

Across biomes, plastic and tile substrata were colonized by similar bacterial taxa in varying abundances. Communities of bacteria were dominated by Proteobacteria, Bacteroidetes, and Actinobacteria, three phyla that are well-documented in freshwater biofilms (Besemer et al., 2007; Hoellein et al., 2015; McNamara and Leff, 2004). Together, these three taxa represented 70–95% of all sequences across sites. Some sites showed unique community constituents, which while present at lower abundance may have ecologically significant roles. The stream which showed the most distinct grouping of bacterial taxa was Thornton Creek in Washington, which also had the lowest number of observed OTUs. For example, Thornton Creek biofilms included high levels of Comamonadaceae, a documented denitrifier and colonizer of artificial substrata, perhaps due to historically high nitrate concentrations at that site (Embrey and Frans, 2003). In addition, Thornton Creek had a higher abundance of Rhodobacteraceae and Sphingomonadaceae, which are well documented across rural-urban land-use gradients and can contain antibiotic and metal resistance genes (Roberto et al., 2019). Rhizobiales is a well-documented family in freshwater biofilms in drinking water systems (Liu et al., 2012) and was more common in the Santa Cruz River relative to the other sites. Though low in abundance overall, unclassified Actinomycetales was found at all sites and was also in higher abundances in the Santa Cruz River. Finally, Gwynns Falls had a higher abundance of Methylophilaceae, a taxa linking methanol uptake and denitrification in freshwaters (Kalyuhznaya et al., 2009).

We can supplement our understanding of the taxa found on biofilms in Chicago-area streams by comparing to previous research at the same sites. Bacterial assemblages from the North Branch Chicago River had several taxa found in a previous analysis of plastic biofilms (Hoellein et al., 2015), such as Burkholderiales, unclassified Betaproteobacteria, unclassified Gammaproteobacteria, and unclassified Bacteria. In Higgins Creek and Springbrook Creek, McCormick et al. (2016) measured differences in the microbial assemblages on microplastic (i.e., plastic <5 mm) and seston, where unclassified Gammaproteobacteria, Pseudomonadaceae, and Campylobacteraceae (three taxa common in wastewater effluent and colonizers of novel substrata) (Adav and Lee, 2008) were more abundant on microplastic (McCormick et al., 2016). Bacterial assemblages in biofilms from Higgins and Springbrook Creeks in this study contained those taxa as well, although in similar abundance on plastic and tile biofilms. Previous work suggested the family Pseudomonadaceae, which contains some plastic-degrading bacterial strains (Arkatkar et al., 2010; Balasubramanian et al., 2010; Tribedi and Sil, 2013), may be selected for growth on plastic. McCormick et al. (2016) noted Pseudomonadaceae was 0.8–2.5% of sequences for bacteria in the water column and seston, and 12.2% of total sequences on microplastic (McCormick et al., 2016). However, in our study, Pseudomonadaceae represented 0.1–0.4% of sequences across all sites. The comparisons suggest microplastic in the water column may offer a distinct environment for biofilms compared to benthic macroplastic. Further research that examines the community composition of all sizes of plastic litter, in all stream habitats, will elucidate patterns for community selection among plastic types.

Biofilm algal assemblages across the sites were dominated by the diatoms (Bacillariophyta/Bacillariophyceae) and unclassified Eukaryotic organisms (Supplemental Fig. 3), except for Santa Cruz River where unclassified Eukaryotes and *Tetradesmus* were dominant. The abundance of the diatoms on biofilms across most sites is similar to patterns recorded on plastic substrates in marine ecosystems (Oberbeckmann et al., 2016; Zettler et al., 2013), indicating a major role of diatoms in primary production on natural and plastic substrates in aquatic ecosystems. The site-specific enrichment of specific algal taxa such as *Tetradesmus*, Cyanobacteria, and *Ulvophyceae* is interesting, and there is some evidence for their enrichment on plastic biofilms in other aquatic ecosystems. For instance, Chlorophyta (green algae that include *Tetradesmus* and *Ulvophyceae*) were one of the most abundant algal groups on the PET-attached biofilms in the North Sea (Oberbeckmann et al., 2016). Cyanobacterial groups were also found to be abundant in plastic-associated biofilms in marine and freshwater ecosystems (Wang et al., 2021; Zettler et al., 2013). The site-specific enrichment of these groups may reflect geographical and/or local differences in abiotic conditions and possibly different successional trends within the biofilms, and these results warrant further work to link their occurrence and abundance to specific biotic and abiotic factors.

Unclassified fungi were the dominant group among the fungal assemblages across sites, in addition to some site-specific abundant fungal taxa. Unclassified Chytridiomycota were significantly more abundant in the Chicago region sites in comparison to the other sites. Chytridiomycota are saprotrophs that likely play a key role in decomposing organic matter in biofilms. *Rhizophydiales*, a family of Chytridiomycota, were abundant in Gwynns Falls and Thornton Creek, suggesting that while a different group of Chytridiomycota was dominant at these sites they could likely perform a similar function. Evidence for high abundance of Chytridiomycota on plastic biofilms has been documented in oceans (Kettner et al., 2017; Oberbeckmann et al., 2016), but similar trends in freshwaters have not yet been studied. In addition, we note the Ascomycota group *Pleosporales* was more abundant in all the non-Chicago region sites. Many freshwater *Pleosporales* decompose decaying plant material (Zhang et al., 2012). Ascomycota were also abundant in plastic-associated biofilms elsewhere (Oberbeckmann et al., 2016; Wang et al., 2021). A recent review of studies documenting microbial plastic degradation showed the largest number of fungal isolates were Ascomycota ($n = 118$) (Gambarini et al., 2021). It is unclear what factors contribute to the geographic patterns of Ascomycota seen here, but if certain taxa in this group degrade plastic, the rates of plastic degradation could vary by geographic region.

5. Conclusion

Despite recent strides in documenting the abundance, distribution, and movement of plastic litter in freshwater ecosystems (McCormick and Hoellein, 2016; Windsor et al., 2019), the role of plastic in shaping the community composition and activity of freshwater biofilms, including bacteria, algae, and fungi, is not well understood (Harrison et al., 2018). This study showed plastic is readily colonized by stream biofilms. Plastic showed a modest role for affecting biofilm biomass and OTU richness for bacteria, especially on polystyrene, which could influence its breakdown rates, and thereby its form during transport downstream. However, the strongest patterns indicated assemblages of bacteria, algae, and fungi among substrate types are more driven by incubation site. More research is needed to understand the patterns of microbial succession on plastic and natural surfaces over varying time scales, the differences in biofilms on floating and benthic plastic litter, and the effect of plastic size, degradation, and polymer type on microbial community composition and activity. Documenting how urban stream biofilms interact with plastic pollution will be critical to understand its ecological effects and global fate.

CRediT authorship contribution statement

Conceptualization AV, TH, JK; Data curation; AV, AC, JK, TH Formal analysis; AV, AC, TH, Funding acquisition; TH Investigation; AV, TH, JK Methodology; AV, TH, JK, Project administration; TH Resources; TH, JK Software; TH, JK, Supervision; TH, JK, Validation; AV, AC, TH Visualization; Roles/

Writing - original draft; AV, TH, JK, AC, Writing - review & editing. AV, TH, JK, AC.

Declaration of competing 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2022.155398.

References

Adav, S.S., Lee, D.J., 2008. Physiological characterization and interactions of isolates in phenol-degrading aerobic granules. Appl. Microbiol. Biotechnol. 78, 899–905. https://doi.org/10.1007/s00253-008-1370-0.

Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151.

APHA, 1998. Standard Methods for the Examination of Water And Wastewater. 20th edition. United Book Press, Inc., Baltimore, MD.

Arkatkar, A., Juwarkar, A.A., Bhaduri, S., Uppara, P.V., Doble, M., 2010. Growth of Pseudomonas and Bacillus biofilms on pretreated polypropylene surface. Int. Biodeterior. Biodegrad. 64, 530–536. https://doi.org/10.1016/j.ibiod.2010.06.002.

Balasubramanian, V., Natarajan, K., Hemambika, B., Ramesh, N., Sumathi, C.S., Kottaimuthu, R., Rajesh Kannan, V., 2010. High-density polyethylene (HDPE)-degrading potential bacteria from marine ecosystem of Gulf of Mannar,India. Lett. Appl. Microbiol. 51, 205–211. https://doi.org/10.1111/j.1472-765X.2010.02883.x.

Barnes, D.K., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond.B Biol. Sci. 364. https://doi.org/10.1098/rstb.2008.0205.

Battin, T.J., Kaplan, L.A., Newbold, J.D., Hansen, C.M.E., 2003. Contributions of microbial biofilms to ecosystem processes in stream mesocosms. Nature 426, 439–442. https://doi.org/10.1038/nature02152.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M., Packmann, A.I., 2016. The ecology and biogeochemistry of stream biofilms. Nat. Rev. Micro 14, 251–263. https://doi.org/10.1038/nrmicro.2016.15.

Besemer, K., Singer, G., Limberger, R., Chlup, A.K., Hochedlinger, G., Hodl, I., Baranyi, C., Battin, T.J., 2007. Biophysical controls on community succession in stream biofilms. Appl. Environ. Microbiol. 73, 4966–4974. https://doi.org/10.1128/aem.00588-07.

Binh, C.T.T., Adams, E., Vigen, E., Tong, T., Alsina, M.A., Gaillard, J.-F., Gray, K.A., Peterson, C.G., Kelly, J.J., 2016. Chronic addition of a common engineered nanomaterial alters biomass, activity and composition of stream biofilm communities. Environ. Sci. Nano 3, 619–630.

Booth, D., Roy, A.H., Smith, B., Capps, K.A., 2016. Global perspectives on the urban stream syndrome. Freshw. Sci. 35 (1), 412–420.

Burton, E., Yakandawala, N., LoVetri, K., Madhyastha, M.S., 2007. A microplate spectrofluorometric assay for bacterial biofilms. J. Ind. Microbiol. Biotechnol. 34, 1–4.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S.M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J.A., Smith, G., Knight, R., 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J. 6, 1621–1624.

Coons, A.K., Busch, K., Lenz, M., Hentschel, U., Borchert, E., 2021. Biogeography rather than substrate type determines bacterial colonization dynamics of marine plastics. PeerJ 9, e12135.

Cross, W.F., Wallace, J.B., Rosemond, A.D., 2007. Nutrient enrichment reduces constraints on material flows in a detritus-based food web. Ecology 88, 2563–2575.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Embrey, S.S., Frans, L.M., 2003. Surface-water Quality of the Skokomish, Nooksack, And Green-Duwamish Rivers And Thornton Creek, Puget Sound Basin, Washington, 1995-98. U.S. Geological Survey, Tacoma, Washington.

Eppehimer, D.E., Hamdhani, H., Hollien, K.D., Nemec, Z.C., Lee, L.N., Quanrud, D.M., Bogan, M.T., 2021. Impacts of baseflow and flooding on microplastic pollution in an effluent-dependent arid land river in the USA. Environ. Sci. Pollut. Res. 1–15.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77, 177–182. https://doi.org/10.1016/j.marpolbul.2013.10.007.

Gambarini, V., Pantos, O., Kingsbury, J.M., Weaver, L., Handley, K.M., Lear, G., 2021. Phylogenetic distribution of plastic-degrading microorganisms. mSystems 6. https://doi.org/10.1128/msystems.01112-20.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts.pdf. Mol. Ecol. 2, 113–118.

Gewert, B., Plassmann, M.M., MacLeod, M., 2015. Pathways for degradation of plastic polymers floating in the marine environment. Environ. Sci. Process. Impacts 17, 1513–1521.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782.

Groffman, P.M., Cavender-Bares, J., Bettez, N.D., Grove, J.M., Hall, S.J., Heffernan, J.B., Hobbie, S.E., Larson, K.L., Morse, J.L., Neill, C., 2014. Ecological homogenization of urban USA. Front. Ecol. Environ. 12, 74–81.

Hall, R.O., Wallace, J.B., Eggert, S.L., 2000. Organic mater flow in stream food webs with reduced detrital resource base. Ecology 81, 3458–3463.

Hamdhani, H., Eppehimer, D.E., Bogan, M.T., 2020. Release of treated effluent into streams: a global review of ecological impacts with a consideration of its potential use for environmental flows. Freshw. Biol. 65, 1657–1670.

Harrison, J.P., Hoellein, T.J., Sapp, M., Tagg, A.S., Ju-Nam, Y., Ojeda, J.J., 2018. Microplastic-associated biofilms: a comparison of freshwater and marine environments. Freshwater Microplastics. Springer, Cham, pp. 181–201.

Hoellein, T.J., Rochman, C.M., 2021. The plastic cycle: pools and fluxes of plastic litter at the watershed scale. Front. Ecol. Environ. 19, 176–183. https://doi.org/10.1002/fee.2294.

Hoellein, T.J., Tank, J.L., Rosi-Marshall, E.J., Entrekin, S.A., 2009. Temporal variation in substratum-specific rates of N uptake and metabolism and their contribution at the stream-reach scale. J. North Am. Benthol. Soc. 28, 305–318.

Hoellein, T.J., Rojas, M., Pink, A., Gasior, J., Kelly, J.J., 2014. Anthropogenic litter in urban freshwater ecosystems: distribution and microbial interactions. PLoS One 9, e98485.

Hoellein, T.J., Westhoven, M., Lyandres, O., Cross, J., 2015. Abundance and environmental drivers of anthropogenic litter on 5 Lake Michigan beaches: a study facilitated by citizen science data collection. J. Great Lakes Res. https://doi.org/10.1016/j.jglr.2014.12.015.

Hoellein, T.J., McCormick, A.R., Hittie, J., London, M.G., Scott, J.W., Kelly, J.J., 2017. Longitudinal patterns of microplastic concentration and bacterial assemblages in surface and benthic habitats of an urban river. Freshw. Sci. 36, 491–507. https://doi.org/10.1086/693012.

Hoellein, T.J., Shogren, A.J., Tank, J.L., Risteca, P., Kelly, J.J., 2019. Microplastic deposition velocity in streams follows patterns for naturally occurring allochthonous particles. Sci. Rep. 9, 3740. https://doi.org/10.1038/s41598-019-40126-3.

Hosen, J.D., Febria, C.M., Crump, B.C., Palmer, M.A., 2017. Watershed urbanization linked to differences in stream bacterial community composition. Front. Microbiol. 8, 1452.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science(80-.) 347, 768–771. https://doi.org/10.1126/science.1260352.

Kalyuhznaya, M.G., Martens-Habbena, W., Wang, T., Hackett, M., Stolyar, S.M., Stahl, D.A., Lidstrom, M.E., Chistoserdova, L., 2009. Methylophilaceae link methanol oxidation to denitrification in freshwater lake sediment as suggested by stable isotope probing and pure culture analysis. Environ. Microbiol. Rep. 1, 385–392. https://doi.org/10.1111/j.1758-2229.2009.00046.x.

Kettner, M.T., Rojas-Jimenez, K., Oberbeckmann, S., Labrenz, M., Grossart, H.P., 2017. Microplastics alter composition of fungal communities in aquatic ecosystems. Environ. Microbiol. 19, 4447–4459. https://doi.org/10.1111/1462-2920.13891.

Kolpin, D.W., Skopec, M., Meyer, M.T., Furlong, E.T., Zaugg, S.D., 2004. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci. Total Environ. 328, 119–130.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microbiol. 79, 5112–5120.

Lacerda, A.L.d F., Proietti, M.C., Secchi, E.R., Taylor, J.D., 2020. Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. Mol. Ecol. 29, 1903–1918.

Liu, R., Yu, Z., Zhang, H., Yang, M., Shi, B., Liu, X., 2012. Diversity of bacteria and mycobacteria in biofilms of two urban drinking water distribution systems. Can. J. Microbiol. 58, 261–270. https://doi.org/10.1139/W11-129.

Lorite, G.S., Rodrigues, C.M., De Souza, A.A., Kranz, C., Mizaikoff, B., Cotta, M.A., 2011. The role of conditioning film formation and surface chemical changes on Xylella fastidiosa adhesion and biofilm evolution. J. Colloid Interface Sci. 359, 289–295.

McCormick, A.R., Hoellein, T.J., 2016. Anthropogenic litter is abundant, diverse, and mobile in urban rivers: insights from cross-ecosystem analyses using ecosystem and community ecology tools. Limnol. Oceanogr. 61, 1718–1734.

McCormick, A.R., Hoellein, T.J., London, M.G., Hittie, J., Scott, J.W., Kelly, J.J., 2016. Microplastic in surface waters of urban rivers: concentration, sources, and associated bacterial assemblages. Ecosphere 7, e01556.

McKinney, M.L., 2006. Urbanization as a major cause of biotic homogenization. Biol. Conserv. 127, 247–260.

McNamara, C.J., Leff, L.G., 2004. Bacterial community composition in biofilms on leaves in a northeastern Ohio stream. J. North Am. Benthol. Soc. 23, 677–685. https://doi.org/10.1899/0887-3593(2004)023<0677:bccibo>2.0.co;2.

Oberbeckmann, S., Labrenz, M., 2020. Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation. Annu. Rev. Mar. Sci. 12, 209–232.

Oberbeckmann, S., Osborn, A.M., Duhaime, M.B., 2016. Microbes on a bottle : substrate, season and geography influence community composition of microbes colonizing marine plastic debris. PLoS One 11, e0159289. https://doi.org/10.1371/journal.pone.0159289.

Ogonowski, M., Motiei, A., Ininbergs, K., Hell, E., Gerdes, Z., Udekwu, K.I., Bacsik, Z., Gorokhova, E., 2018. Evidence for selective bacterial community structuring on microplastics. Environ. Microbiol. 20, 2796–2808.

Peterson, C.G., Daley, A.D., Pechauer, S.M., Kalscheur, K.N., Sullivan, M.J., Kufta, S.L., Rojas, M., Gray, K.A., Kelly, J.J., 2011. Development of associations between microalgae and denitrifying bacteria in streams of contrasting anthropogenic influence. FEMS Microbiol. Ecol. 77, 477–492. https://doi.org/10.1111/j.1574-6941.2011.01131.x.

Roberto, A.A., Van Gray, J.B., Engohang-Ndong, J., Leff, L.G., 2019. Distribution and co-occurrence of antibiotic and metal resistance genes in biofilms of an anthropogenically impacted stream. Sci. Total Environ. 688, 437–449. https://doi.org/10.1016/j.scitotenv.2019.06.053.

Rochman, C.M., Hoellein, T., 2020. The global odyssey of plastic pollution. Science (80-.) 368, 1184–1185.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., Schmitt-Jansen, M., 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. 4, 258–267.

Schade, J.D., MacNeill, K., Thomas, S.A., McNeely, F.C., Welter, J.R., Hood, J., Goodrich, M., Power, M.E., Finlay, J.C., 2011. The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. Freshw. Biol. 56, 424–436. https://doi.org/10.1111/j.1365-2427.2010.02509.x.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., Sahl, J.W., Stres, B., Thallinger, G.G., Van Horn, D.J., Weber, C.F., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541. https://doi.org/10.1128/aem.01541-09.

Sherwood, A.R., Presting, G.G., 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. J. Phycol. 43, 605–608. https://doi.org/10.1111/j.1529-8817.2007.00341.x.

Tank, J.L., Rosi-Marshall, E.J., Griffiths, N.A., Entrekin, S.A., Stephen, M.L., 2010. A review of allochthonous organic matter dynamics and metabolism in streams. J. North Am. Benthol. Soc. 29, 118–146. https://doi.org/10.1899/08-170.1.

Tribedi, P., Sil, A.K., 2013. Low-density polyethylene degradation by pseudomonas sp. AKS2 biofilm. Environ. Sci. Pollut. Res. 20, 4146–4153. https://doi.org/10.1007/s11356-012-1378-y.

Wang, L., Tong, J., Li, Y., Zhu, J., Zhang, W., Niu, L., 2021. Bacterial and fungal assemblages and functions associated with biofilms differ between diverse types of plastic debris in a freshwater system. Environ. Res. 196, 110371. https://doi.org/10.1016/j.envres.2020.110371.

White, T.J., Bruns, T.D., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA egens for phylogenetics. In: Innis, M., Gelfand, D., Sninsky, J., White, T. (Eds.), PCR Protocols: A Guide to Methods And Applications. Academic Press, Orlando, FL, pp. 315–322.

Windsor, F.M., Durance, I., Horton, A.A., Thompson, R.C., Tyler, C.R., Ormerod, S.J., 2019. A catchment-scale perspective of plastic pollution. Glob. Chang. Biol. 25, 1207–1221.

Wright, R.J., Langille, M.G.I., Walker, T.R., 2021. Food or just a free ride? A meta-analysis reveals the global diversity of the plastisphere. ISME J. 15, 789–806.

Zbyszewski, M., Corcoran, P.L., Hockin, A., 2014. Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. J. Great Lakes Res. 40, 288–299.

Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/es401288x.

Zhang, Y., Crous, P.W., Schoch, C.L., Hyde, K.D., 2012. Pleosporales. Fungal Divers. 53, pp. 1–221. https://doi.org/10.1007/s13225-011-0117-x.