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Plastic microbiome development in a freshwater ecosystem

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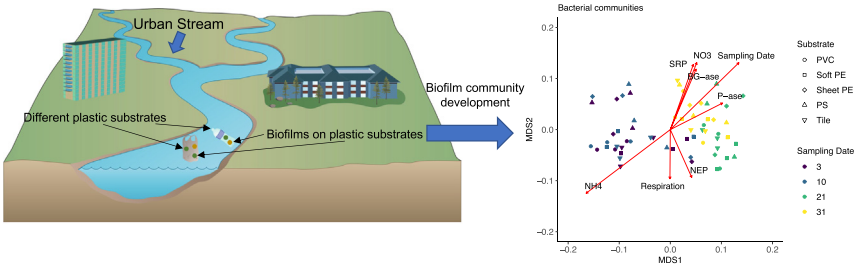
HIGHLIGHTS

- Microbial colonization of plastic litter impacts its fate in urban streams.
- We assessed succession in microbial activity and diversity on 4 plastic substrates.
- Time, not substrate type, was major factor in driving microbial community trends.
- Plastic biofilms active and dynamic, and can contribute to key ecosystem processes.

GRAPHICAL ABSTRACT



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ABSTRACT

To understand biological interactions of plastic litter in freshwater ecosystems, as well the potential effects of plastics on ecosystem processes, studies of the activity and composition of plastic-associated microbial communities are needed. The physical properties and chemical composition of plastic polymers are key components of plastic product design, and may also select for distinct microbial biofilms colonizing plastic litter. We monitored growth and succession of biofilm communities on plastic substrates of common morphotypes (i.e., hard, soft, foam, and film) and a natural surface (i.e., an unglazed ceramic tile) incubated in an urban stream. We measured biofilm biomass, metabolism, extracellular enzyme activity, and bacterial, fungal and algal community composition over four weeks during primary succession. Results demonstrated a general increase in biofilm biomass and enzymatic activity corresponding to carbon, nitrogen and phosphorus metabolism during biofilm development for all substrate types. We observed higher respiration rates and negative net ecosystem productivity on foam and tile surfaces in comparison to hard, soft and film plastic surfaces. Biofilm bacterial, fungal and algal assemblages showed few significant differences in composition among substrates. However, all microbial communities changed significantly in composition over time. While substrate type was not the major factor driving biofilm composition and activity, these data show plastic litter in streams is well colonized by an active and dynamic biofilm community. As plastic litter is increasing across all types of aquatic ecosystems, it should be considered a medium for biologically active organisms that contribute to key ecosystem processes.

1. Introduction

Since its industrialization in the mid-1900s, rates of plastic production and waste generation have accelerated to approximately 288 million tons per year (Geyer et al., 2017; Jambeck et al., 2015). Plastics that escape waste management can accumulate in terrestrial and riverine systems before transport to global oceans (Eerkes-Medrano et al., 2015; Reddy et al., 2006). Plastic pollution can release compounds to soil and water (e.g., phthalates) (Teuten et al., 2009) which may affect human and ecosystem health (Koch and Calafat, 2009; Meeker et al., 2009; Oehlmann et al., 2009). Degradation of plastic pollution is largely through physical process (i.e., ultraviolet light, abrasion), as biologically mediated breakdown of plastic polymers is slow and enzymatically challenging (Andrady et al., 2011). However, colonization of plastic litter surfaces by microorganisms is common, and the activity and assemblage of plastic-associated microbial

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communities is an emerging focus of research in ecosystems worldwide (Hoellein et al., 2017; Zettler et al., 2013).

Microbial biofilms consist of bacteria, archaea, algae, and fungi in an extracellular matrix, and form on all submerged surfaces in aquatic ecosystems (Battin et al., 2016). In streams, microbial biofilms are critical for nutrient cycling and ecosystem metabolism (Battin et al., 2016; Thompson and Sinsabaugh, 2000). Key physical drivers of biofilm growth include water velocity and hydrodynamics, nutrient and light availability, and the topography and chemistry of the substrate surface (reviewed in Battin et al., 2016; Bridier et al., 2017; Lyautey et al., 2005). Microbial biofilms on natural surfaces in streams (e.g., wood, fine sediments, and rocks) show distinct biofilm community composition, metabolism, and nutrient uptake rates (Hoellein et al., 2009).

Plastic surfaces are an abundant and novel substrate for microbial biofilm colonization in urban streams (Hoellein et al., 2017; McCormick and Hoellein, 2016). Plastics commonly found in streams include food containers, bags, textiles, and construction debris (Hoellein et al., 2014; McCormick and Hoellein, 2016; Rech et al., 2014). Plastic surfaces can select for distinct biofilm communities due to the physical and chemical novelty of plastic compared to organic (e.g., leaf litter and fine particulate organic matter; FPOM) or inorganic (e.g., rock and sand) surfaces (Oberbeckmann et al., 2018). However, the mechanisms whereby plastic can select for the growth and succession of novel biofilm communities have not been identified (McCormick et al., 2014).

The chemistry of plastic polymers determines the physical properties of plastic products, the durability of plastic pollution in the environment, and could be a mechanism for the selection of distinct microbial biofilms on plastic litter (Lee et al., 2008). Harder plastic compounds have polymers arranged in parallel lines with little inter-polymer pore space, while softer plastics have more frequent polymer branching which increases inter-polymer space and flexibility (Roff and Scott, 1971b). Plasticizers such as biphenyl-A (BPA) and phthalates are added during manufacturing to enhance rigidity, decrease brittleness, and provide resistance to UV degradation (Roff and Scott, 1971a). Hydrophobic plastic polymers may adsorb chemicals from the environment including poly-chlorinated biphenyls (PCBs), organo-chlorines (i.e., pesticides), poly-aromatic hydrocarbons (PAHs), and pharmaceuticals (Endo et al., 2005; Haritash and Kaushik, 2009; Teuten et al., 2009).

While the internal chemistry and chemical associations of plastic litter may affect microbial communities, plastic surfaces could also select for growth of heterotrophic microbes that produce enzymes that can potentially break down plastic polymers. The degradation of plastic debris by microbes can enhance the rate of decomposition or release toxic plasticizers (Khatoon et al., 2014). Recent work in marine coastal ecosystems has provided evidence for selection of potential hydrocarbon degrading bacteria during early stages of microbial colonization of weathered polyethylene (Erni-Cassola et al., 2020). However, selection of microbes with potential for plastic polymer breakdown in biofilms colonizing plastic litter compared to natural surfaces in streams has yet to be investigated in in situ conditions (Kelly et al., 2020).

In this study, we monitored growth and succession of biofilm communities on plastic substrates of common morphotypes (i.e., hard, soft, foam, and film) and a natural surface (i.e., an unglazed ceramic tile) incubated in an urban stream. We measured biofilm biomass, metabolism, extracellular enzyme activity, and community composition over four weeks during primary succession. In our recent work, we demonstrated the importance of site characteristics on biofilm community activity and composition on plastic litter in urban streams (Vincent et al., 2022). Here, we focus on investigating the temporal/successional patterns of biofilm community development on various plastic substrates. Measuring biofilm colonization and function on plastic surfaces in streams will illustrate the effect of substrate characteristics on biofilm succession and the role of plastic in stream ecosystem processes. We hypothesize that the differences in structural and chemical characteristics among substrates would generate differences in biofilm characteristics and community composition during biofilm development. We expected the role of substrate type to be most important during early successional period, as later in succession the biofilm growth will be less directly affected by substrate composition.

2. Methods

2.1. Site description

The North Branch of the Chicago River drains urban and suburban landscapes in the Chicago Metropolitan Region, Chicago, IL, USA. Watershed land use is predominantly urban (60.6 %) with 25.5 % of the landscape as impermeable surfaces (Cook and Hoellein, 2016). Water quality is also affected by wastewater effluent (Water Reclamation Facility of the Village of Deerfield, IL) and combined sewer overflows are situated along the length of the river. We conducted this study in a 50 m reach of the river within Harm's Woods Forest Preserve District of Cook County (latitude: 42.058330, longitude: −87.772088). This site has been used to study ecosystem processes in urban streams including leaf litter breakdown, sediment nitrogen cycling, and plastic litter movement (Cook and Hoellein, 2016; Hoellein et al., 2014; McCormick and Hoellein, 2016; Turek and Hoellein, 2015).

2.2. Experiment assembly, deployment, and collection

We assembled thirty 'litterbags' using mesh bags (3.3 mm pore size polypropylene) commonly used for litter decomposition experiments (Cady Bag, Pearson, Georgia) and nylon zipties. Each litterbag contained 20 individual substrate pieces ($N = 4$ each) of five substrate types (4 × 4 cm^2). We used unglazed ceramic tile as a surrogate for rock surfaces that support periphyton (thickness = 5 mm) (Hoellein et al., 2014). Four common plastic types with distinct combinations of hardness and flexibility were used. PVC represented a hard and rigid plastic surface (thickness = 3.5 mm; Model WPVC-0125-F, Small Parts Inc., Logansport, IN, USA). Low density polyethylene (thickness = 2 mm; Part LDPS-0060-F, Small Parts Inc., Logansport, IN, USA) was used for intermediate hardness and rigidity, while low density polyethylene in 'film' form (i.e., a plastic drop cloth purchased from a hardware store; thickness = 0.15 mm) represented a soft and flexible surface. Finally, foamed polystyrene provided a plastic surface that is relatively soft and fragile (thickness = 2 mm; Model 80HT1R foam hinged lid food containers, Dart, Inc., Mason, MI, USA).

Pieces were cut into 4 × 4 cm^2 pieces and arranged to form a "quilt" separated by zipties and arranged so that no two pieces of the same substrate were adjacent to one another. Prior to deployment in the river, we cleaned the bags by first blowing surfaces with a compressed air source and then sterilizing with 95 % ethanol. Litterbags were placed in the river in blocks of three at random points throughout the 50 m reach. Rebar was inserted into the stream bed and the block of three bags was secured ~3 cm above the stream bottom downstream of the rebar. At no point after deployment were any bags exposed to the air. The litterbags representing day 0 were brought to the field site, not deployed, and processed upon returning to the lab.

We collected litterbags every 3–4 days from August 15th to September 15th 2017. One block of three bags ($N = 60$ substrates, 12 replicates for each of 5 substrates) was collected per date and stored in a plastic zip-top bag until processing in the lab (approximately 1 h). We also collected 40 L of stream water, which was refrigerated until use. In the lab, litterbags were placed in separate trays that were sterilized with alcohol. We opened the litterbags and gently rinsed individual pieces of substrate with deionized water to remove all non-attached material, including invertebrates. We placed each individual substrate into trays or 160 ml specimen containers for laboratory assays using sterilized forceps. Care was taken to avoid sloughing or scraping off attached biofilm while handling or moving materials.

2.3. Physiochemical measurements

At each sample collection we measured water temperature, dissolved oxygen (DO) (HQ40d portable meter, Hach, Loveland Colorado), and

specific conductivity (Model 30, Yellow Springs Instruments, Yellow Springs, OH, USA). We measured discharge across a stream width transect by recording depth and velocity at 0.5 m intervals (Marsh-McBirny Flow-Mate 2000 Flow Meter, Hach). We collected triplicate 20 mL water samples and filtered them into new 20 mL scintillation vials in the field using glass microfiber filters (0.2 μm, Sigma-Aldrich, St. Louis MO). Water samples were frozen within 2 h and stored for later analysis (within 6 months). We quantified dissolved soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate (NO_3^-) on a Seal Analytical Autoanalyzer (Seal Analytical, Mequon WI) using the antimonyl tartarate, phenol hypochlorite, and Cd reduction methods, respectively (APHA, 1998a).

2.4. Biofilm metabolism

We measured net ecosystem production (NEP) and community respiration (CR) using chamber incubations in the light and dark (Hoellein et al., 2014). Collected river water was removed from the fridge, poured into a clean 20 L bucket and left to stand for 1 h to equilibrate to lab temperature. We measured dissolved oxygen (DO) concentration in the bucket using a portable DO meter (HQ40d portable meter, Hach). We placed a set of the freshly collected substrates ($N = 18$, triplicates of each of 5 substrates and 3 water-only controls) in individual 160 mL specimen containers and slowly submerged and capped the containers under water in the bucket (i.e., free of bubbles). A second set of specimen containers were filled in the same manner ($N = 18$), but were wrapped in foil and placed under an inverted tray to eliminate light. Both the covered and uncovered containers were placed in an environmental chamber set at 20 °C (similar to the ecosystem temperature) with constant illumination for a 3 h incubation. Afterwards, containers were individually uncapped, the substrate carefully removed using forceps, and DO measured. We noted the start time and end time for each individual incubation. Net change in DO concentration was used to calculate community respiration (dark) and net ecosystem production NEP (illuminated), and GPP was calculated by difference (Bott, 2007).

2.5. Biofilm biomass

Crystal violet (hexamethyl pararosaniline chloride) binds to the extracellular matrix produced by biofilms (Burton et al., 2007). It can be detected in very small quantities which makes it a useful tool for quantifying biofilm development and as a proxy for biomass (Burton et al., 2007). Here, we note that the use of crystal violet for quantification purposes included a measure of both the exopolysaccharides and biofilm biomass. We stained one set of freshly collected substrates from each replicate litterbag ($N = 12$) with 2 mL of a 1 % aqueous crystal violet solution for 45 min. After the initial exposure period, excess unbound stain was washed off the samples using deionized water and they were left to dry in a warm oven (~30 °C) overnight. The bound stain was eluted using 15 mL of 95 % ethanol that was repeatedly washed over the sample for 15 min. We collected ethanol with the stain in solution into glass test tubes for dilution and quantification at 595 nm using a spectrophotometer (Shimadzu Pharmaspec 1700 UV–Vis Spectrophotometer, Shimadzu North America, Columbia MD, USA). Optical density at 595 nm was recorded for all samples, and samples were diluted if needed. Effective optical density per unit area of substrate was reported as absorbance cm^{-2}.

2.6. Extracellular enzyme activity potential

The activity potential of three common enzymes involved in heterotrophic C, N, and P cycling (β-glucosidase (BG), *N*-acetyl-β-D-glucosaminidase (NAG), and phosphatase, respectively) were quantified following (Jackson et al., 2013). After the metabolism assays, we scraped biofilms from one side of the substrates using a sterile razor blade ($N = 15$) and collected the scrapings into 15 mL centrifuge tube (Corning, Corning NY) using 8 mL of deionized water. Samples were vortexed for 15 s and then stored in a refrigerator overnight. The next day, samples were vortexed for ~15 s to homogenize and suspend the biofilm. We loaded sample, buffer, reagents, and standards into a black 96-well plate and fluorescence was quantified immediately at 460 nm following excitation at 360 nm (FLUOstar Omega Fluorometric plate reader, BMG Labtech, Ortenberg, Germany) ($N = 3$ analytical reps for each sample). Sample plates were re-quantified after 3 h of incubation at room temperature in the dark. The increase in fluorescence over the course of the incubation is due to consumption of reagent by the microbes in the scraped biofilm and is proportional to their activity potential. Pilot experiments showed enzyme activities peaked within 3 h and then remained stable for all enzymes and samples, so we reported the maximum rates at 3 h of incubation.

2.7. Chlorophyll

The substrates incubated for NEP were destructively sampled for chlorophyll-a using the hot ethanol method. We did not measure chlorophyll-a content directly after sampling from the field. We acknowledge this may have affected the overall value for chlorophyll, but any impact on chlorophyll would be uniform across substrates and limit the effect on the comparison across materials. We wiped each substrate using a sterile cotton swab (Puritan Products, Guilford Maine). We placed the cotton tip of the swab in a 15 mL centrifuge tube (Corning, Corning NY), and immediately froze tubes until analysis within two months. Samples were thawed in a refrigerator (4 °C) the night before extraction. Samples were extracted in 5 mL of 95 % ethanol in a hot water bath at 75 °C for 15 min, and then at room temperature in the dark for 2 h. We recorded absorbance at 750 nm and 665 nm for all samples using the multi-lambda function of a spectrophotometer (Shimadzu Pharmaspec 1700 UV–vis, Shimadzu Corp, Columbia MD). Next, samples were acidified with 200 μL of 0.25 M hydrochloric acid (HCl) and absorbance was re-measured at the same wavelengths. The difference between acidified and non-acidified samples represents the absorbance due to chlorophyll-a and not to phaeophytin (APHA, 1998b).

2.8. Biofilm community composition

Attached biofilm samples used in the respiration assays were destructively sampled for community composition using the 16S, ITS, and 23S markers. We used sterile cotton swabs (Puritan Products) to remove all biofilm from the surface of each substrate, then cut the cotton tip, placed it into a sterile 2 mL screwcap tubes (VWR, New York City, NY), and stored it at −20 °C until extraction, purification, and amplification (within 4 months). We used the Qiagen Power Soil DNA extraction kit (Qiagen, Hilden, Germany) following manufacturer instructions to extract DNA. We selected four time points that cover the range of biomass-equivalent dynamics we observed (3, 10, 21, and 31 days) for biofilm community measurements. Extracted DNA was amplified using the 515F and 806R primers for the bacterial and archaeal 16S rRNA gene (Caporaso et al., 2012), CS1_ITS1f and CS2_ITS2 primers for the fungal ITS regions (Gardes and Bruns, 1993; White et al., 1990), and CS1_p23SrV_f1 and CS2_p23SrV_r1 for the plastid 23S rRNA gene in eukaryotic algae and cyanobacteria (Sherwood and Presting, 2007). We confirmed DNA isolation and amplification via agarose gel electrophoresis for all PCR reactions. Amplicons samples were sent to the University of Illinois at Chicago Sequencing Core for sequencing using the Illumina MiSeq platform. Sequence data were cleaned and analyzed using MOTHUR (version 1.40.0) (Schloss et al., 2009) according to the MiSeq SOP (Kozich et al., 2013). Paired reads were assembled, demultiplexed, and screened for homopolymers and ambiguities. All sequences were trimmed to appropriate lengths and chimeric sequences were removed using Uchime (Edgar et al., 2011). We clustered sequences into OTUs (operational taxonomic units, 97 % identity level) prior to statistical analyses and classification. We aligned our bacterial 16S rRNA gene sequences against the SILVA ssu database (updated 2017-12-13), our plastid 23S rRNA gene sequences against the SILVA lsu database (updated 2017-12-17), and our fungal ITS sequences against the UNITE ITS reference (updated 2017-12-01). All sets of aligned sequences were randomly subsampled to appropriate levels to allow for downstream analysis. Bacterial

assemblages were subsampled to 27,166 sequences per sample, algal assemblages were subsampled to 6006 sequences per sample, and fungal assemblages were subsampled to 8545 sequences per sample. All sequence data are available from the National Center for Biotechnology Information Sequence Read Archive, accession number PRJNA851479.

2.9. Statistical analyses

We tested all environmental and metabolic data for effects of substrate type ($n = 5$) and sampling date ($n = 10$) during succession using linear mixed effect models (LME) with blocking for repeated measurements (Pinheiro et al., 2018). Where there was a significant effect of substrate we ran a Tukey's post hoc test for pairwise comparisons between different substrates. All analyses were conducted and figures produced using R unless noted otherwise.

While annotation of most of the sequences at broad taxonomic levels was successful for each of the assemblages, annotations at more resolved levels for the algal and fungal sequences were successful for only a small fraction of the total sequences. Thus, to incorporate most of the sequences into the analyses and to keep comparisons consistent across the assemblages, we performed most of community analyses at broad taxonomic levels. To compare biofilm community composition across different substrates and across time we calculated Bray-Curtis distance matrices for each assemblage at the class level (i.e., bacterial, algal, fungal) in the R package *vegan* (Oksanen et al., 2018) and then used PERMANOVA, the *adonis* function, to compare communities between substrates and sampling dates. Bray-Curtis distance matrices for each set of primers were plotted using non-metric multidimensional scaling (NMDS). We then used the *envfit* function from the package *vegan* to find correlations between environmental variables (laboratory assays) and our assemblage distance matrices. When significant, these variables were plotted as vectors in the NMDS space with vector length being scaled to strength of correlation. We calculated Shannon diversity at the genus level for all assemblage and selected unique taxa at the order level for each substrate and timepoint to generate Venn diagrams using the *venn.diagram* function (Chen, 2018). Heatmaps showing relative abundance of bacterial, algal, and fungal taxa were generated at the phylum level in R (for bacterial assemblages, Proteobacteria were subdivided into classes). In addition to the above analyses, testing whether specific bacterial groups change in abundance during biofilm development was an important study question. For this, we aimed to identify differentially abundant microbial genera during the initial stages of biofilm succession (between day 3 and day 10) for each of the substrates, using DESeq 2.0 package in R (Love et al., 2014). The usage of DESeq 2.0 first involved a normalization of taxonomic count data as per the default method, followed by differential abundance testing using the Wald test with *p*-value adjusted for multiple hypothesis-testing across the taxa.

3. Results

Our study period from Aug 15–Sept 15, 2017 was late summer for the region and some variation in physiochemical parameters occurred. Discharge was stable and low relative to annual variation, with only two brief periods of increased discharge (Supplemental Fig. 1). However, there was a significant increase in dissolved NO_3^- and SRP over the incubation, (linear regression, $p < 0.001$, $r^2 = 0.42$ and 0.49, respectively) and no significant change in NH_4^+ (Supplemental Fig. 2).

All substrates exhibited biofilm growth over the course of the 31 day incubation (Fig. 1a). Biofilm biomass was not significantly different among substrate types (LME, $p = 0.932$), but changed significantly over sampling dates (LME, $p = 0.026$), with no significant interaction between substrate and time (LME, $p = 0.994$; Table 1). Biofilm biomass increased after day 10 for all substrate types, followed by a period of high variability between days 15 and 25, and then a decline on days 28 and 31 (Fig. 1a). We quantified chlorophyll-a concentrations as an indicator of primary producers (Fig. 1b). As for biomass, chlorophyll was not significantly different among substrate types (LME, $p = 0.249$), was significantly different among sampling dates (LME, $p < 0.001$), and there was no interaction between substrate and time (LME, $p = 0.918$; Table 1). Chlorophyll concentration significantly increased after day 20, remained elevated, and then declined on the final sampling date (Fig. 1b).

For biofilm metabolism, we observed that the substrates fell into two groups: a) tile and polystyrene (PS) which had negative NEP and higher rates of respiration, and b) PVC, sheet PE, and soft PE which had positive or neutral NEP and lower respiration (Fig. 2). The patterns over time and across substrates were different for GPP, respiration and NEP. GPP showed significant difference over time (LME, $p = 0.005$), with no effect of substrate type (LME, $p = 0.098$) or interaction between date and substrate (LME, $p = 0.330$; Table 1; Fig. 2a). Respiration was significantly different among substrate types (LME, $p < 0.001$) and over time (LME, $p < 0.001$), with no significant interaction ($p = 0.055$; Table 1; Fig. 2b). Respiration rates were significantly lower on PVC, soft PE, and sheet PE, relative to PS and tile. The 3 substrates with lower respiration rates were not significantly different from each other, and PS and tile were not significantly different from one another (Tukey's post hoc test, $p < 0.001$). Finally, NEP was different only by substrate type (LME, $p < 0.001$), with no effect of date or interaction between date and time ($p = 0.586$; Table 1; Fig. 2c). For NEP, the same pattern among the different substrates were observed as for respiration (Tukey's post hoc test, $p < 0.001$).

Extracellular enzyme activity assays revealed the biofilms' potential for general C (BG), N (NAG), and P (phosphatase) activity (Fig. 3). For all the three enzymes, there was a significant difference among dates (LME, $p < 0.001$), with no effect of substrate or date and substrate interaction (Table 1). Patterns for phosphatase showed lower rates on the first 3 dates and higher values on the last 5 (Fig. 3a). NAG showed a similar temporal trend as phosphatase, however, rates declined on the last sampling date (Fig. 3b). BG showed the overall same temporal and substrate patterns as NAG and P (Fig. 3c).

3.1. Bacterial, algal, and fungal assemblages

We obtained a total of 1,684,292 sequence reads of the 16S rRNA gene across our four sampling events (Days 3, 10, 21, 31) after bioinformatics processing. Using PERMANOVA and Bray-Curtis distances we observed significant differences in bacterial assemblages by date and among substrates (PERMANOVA $p = 0.001$ and $p = 0.002$ for date and substrates, respectively), with no significant interaction (PERMANOVA), $p = 0.605$; Table 2. Pairwise-PERMANOVA post hoc tests identified significant differences in the bacterial assemblages between soft PE and tile ($p = 0.027$), and sheet PE and tile ($p = 0.030$). Based on the phylum level community composition (Supplemental Fig. 3), this difference in bacterial community assemblages between the soft and sheet PE and tile substrates was likely driven by the higher relative abundance of Actinobacteria on the PE substrates. Other than this difference, the bacterial assemblages across all the substrates were dominated by Alpha-, Beta- and Gamma-Proteobacteria and Bacteroidetes (Supplemental Fig. 3).

We visualized bacterial community differences between dates and substrates using NMDS calculated using Bray-Curtis dissimilarity distances (Fig. 4a). Overall, respiration, NEP, extracellular enzyme activity (BG- and P-ase), chlorophyll-a, day of incubation, and water chemistry were significantly correlated with ordinated data (Table 3). Grouping of assemblages by date occurred according to the x-axis and showed the strongest correlations with NH_4^+ concentration and day of incubation (Fig. 4a; Table 3). In addition, using DESeq 2.0 we evaluated potential changes in the relative abundance of bacterial genera during early stages of biofilm succession (between days 3 and 10), the time period which we predicted plastic substrate may have the strongest impact on community composition. Results demonstrated a significant increase in the relative abundance (DESeq 2.0, Wald Test, adjusted $p < 0.05$) of *Aeribacillus* and an unclassified genus within *Enterobacteriaceae* on day 10 as compared to day 3 for all the substrates. In addition, the substrate types PS, soft PE and tile also demonstrated a significant increase in the relative abundance of *Halomonas* on day 10 as compared to day 3.

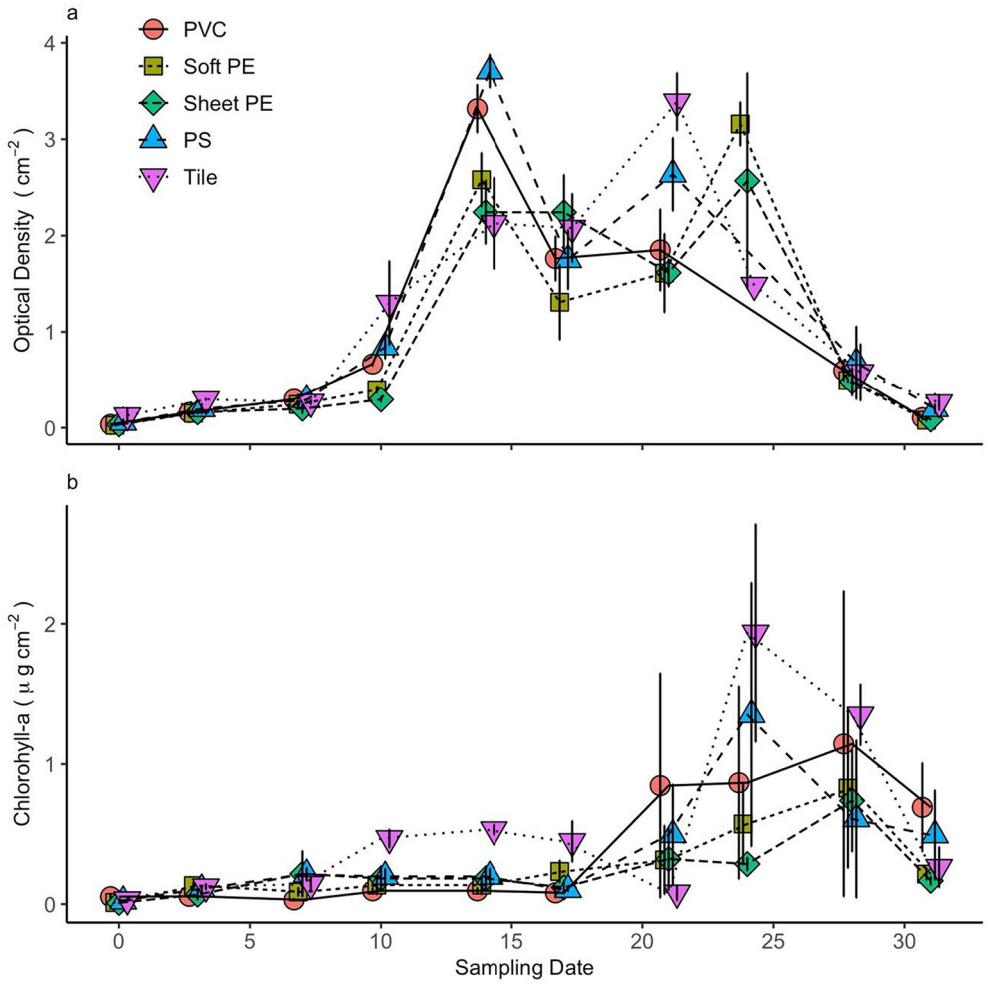

Fig. 1. Mean (±SE) (a) optical density of crystal violet stain (biofilm size) and (b) chlorophyll *a* during biofilm incubation on polyvinyl chloride (PVC), soft and sheet polyethylene (PE), polystyrene (PS, Styrofoam), and tile.

We obtained a total of 414,414 plastid sequences across dates and substrates after bioinformatics processing. Bray-Curtis distances showed a significant difference among dates (PERMANOVA, $p = 0.033$), but not among substrate types (PERMANOVA, $p = 0.132$) and no interaction between date and substrate (PERMANOVA, $p = 0.424$; Table 3). Phylum Stramenopiles and unclassified algae were the dominant groups for the algal assemblages across all the substrates (Supplemental Fig. 4). Using NMDS we plotted the Bray-Curtis distances for algal assemblages (Fig. 4b). Overall, sampling date and water chemistry (NH_4^+) were significantly correlated with ordinated data (Table 3). Grouping of assemblages by date occurred according to the y-axis and showed the strongest correlations with NH_4^+ concentration and sampling date (Fig. 4b). From the DESeq-based differential abundance analysis, we did not observe any significant changes in the relative abundance of algal genera between day 3 and day 10 for any of the substrates.

We obtained a total of 91,978 fungal ITS sequences across dates and substrates after bioinformatics processing. We compared community composition between substrates and across sampling dates using PERMANOVA and Bray-Curtis distances. We observed significant differences among dates (PERMANOVA, $p = 0.001$) but not among substrates (PERMANOVA, $p = 0.193$) and no significant interaction (PERMANOVA, $p = 0.106$; Table 2). The fungal assemblages across the different substrates were predominantly composed of phylum Ascomycota and unclassified Fungi (Supplemental Fig. 5). Using NMDS we plotted the Bray-Curtis distances for sequenced fungal communities (Fig. 4c). We observed significant correlations between ordinated data and extracellular enzyme activity (BG-, NAG- and P-ases), GPP, sampling date, and water chemistry (NO_3^-, NH_4^+) (Table 3). Grouping of assemblages by date occurred according to the x-axis and showed the strongest correlations with NH_4^+ concentration, sampling date, and phosphatase activity (Fig. 4c). From the DESeq-based differential abundance analysis, we observed large changes in relative abundance of a few fungal genera from day 3 to day 10 for all the substrates (Table S2). These included *Aureobasidium*, *Taphrina*, and unclassified genera within *Pseudeurotiaceae* and Basidiomycota that dropped in relative abundance on day 10; and unclassified genera within Fungi and *Rhizophydiales* that increased in relative abundance on day 10. However, only the increases in relative abundance of unclassified Fungi and *Rhizophydiales* in tile-associated assemblages were statistically significant (DESeq 2.0, Wald Test, adjusted $p < 0.05$, Table S2).

We calculated Shannon diversity for bacterial, algal, and fungal assemblages on each substrate and sampling date (Supplemental Fig. 6). Two-way ANOVA showed no significant differences in diversity across the substrates for bacteria ($p = 0.286$), algae ($p = 0.176$), or fungi ($p = 0.153$; Supplemental Table 1). However, the fungal and algal communities showed a difference by date (Two-way ANOVA, $p < 0.001$ for each), while the bacterial communities did not (Two-way ANOVA), $p = 0.286$; (Supplemental Table 1). The major change in Shannon diversity for fungal and algal communities happened between day 3 and day 10 where the diversity decreased for most of the substrates, after which the changes in the Shannon diversity remained similar thereafter (Supplemental Fig. 6). The Shannon diversity metrics showed a similar pattern to analysis of taxa grouping illustrated with Venn diagrams (Supplemental Fig. 7). For bacteria, algae, and fungi, most taxa were present across all substrates, with relatively few taxa unique to each substrate.

Table 1
Results from 2-way ANOVA using linear mixed effects (LME) models comparing effects of time and substrate on biofilm structure and function (p-value < 0.05 for significant factor effects, represented in the table in bold). Abbreviations = chl = chlorophyll, GPP = gross primary production NEP = net ecosystem production, BG-ase = β-glucosidase, NAG-ase = *N*-acetyl-β–D-glucosaminidase, P-ase, phosphatase, df = degrees of freedom.

Assay	df	Factor	F-ratio	p-Value
Biomass	1	Date	75.08	**0.026**
	4	Substrate	2.92	0.932
	4	Interaction	1.55	0.994
Chl a	9	Date	5.28	**<0.001**
	4	Substrate	1.06	0.249
	36	Interaction	0.7	0.918
Respiration	1	Date	16.33	**<0.001**
	4	Substrate	35.45	**<0.001**
	4	Interaction	4.86	0.055
GPP	1	Date	12.31	**0.005**
	4	Substrate	3.22	0.098
	4	Interaction	2.16	0.330
NEP	1	Date	12.49	0.503
	4	Substrate	64.03	**<0.001**
	4	Interaction	3.82	0.586
BG-ase	1	Date	173.31	**<0.001**
	4	Substrate	3.57	0.652
	4	Interaction	2.83	0.142
NAG-ase	1	Date	56.2	**<0.001**
	4	Substrate	2.56	0.227
	4	Interaction	0.33	0.927
P-ase	1	Date	76.37	**<0.001**
	4	Substrate	0.85	0.878
	4	Interaction	0.87	0.921

3.2. NEP model

We developed three models to explain the disparities in NEP observed between negative NEP (PS and tile) and positive NEP substrates (sheet PE, soft PE, and PVC) based on measurements of all the water chemistry and biofilm metabolism parameters in this study. The overall model was created using generalized linear model selection without an a priori formula for the explanatory variables, and found that NEP was best predicted by SRP, NH_4^+ and the relative abundance of Flavobacteriia and unclassified Cyanobacteria (Table 4). However, the model predicting positive NEP values (sheet PE, soft PE, and PVC substrates) was the most supported model with an R^2 of 0.89. In this model NEP is best predicted by SRP, date of sampling and the relative abundance of Sphingobacteria (class). The model predicting negative NEP values (PS and Tile substrates) had an R^2 of 0.12, and in this model NEP is best predicted by time (date), NO_3^- and unclassified Eukaryotic algae (Table 4).

4. Discussion

4.1. Biofilm attributes more strongly driven by incubation time than substrate type

Based on the enzymatic assays, biofilm biomass, and chlorophyll-a measurements, we observed a similar overall pattern of biofilm development and succession among the plastic and natural surfaces (i.e., tile) in an urban stream. As biofilms matured on all substrates, there was an increase in biofilm biomass, chlorophyll-a, and C, N and P-related enzymatic activity, followed by either a decrease or stabilization of these parameters near the end of the incubation. We conclude that the different plastic substrates, just like tile, support the development of mature freshwater biofilms that are metabolically active, as has been documented previously (Chen et al., 2019; Hoellein et al., 2014). Thus, through the growth of biofilms that were similar in composition and activity to those on a natural surface, plastic substrates in our study supported biofilm communities that were biologically and chemically reactive to the same degree as natural surfaces. Therefore, plastic litter can be a viable habitat from the perspective of biofilm organisms in streams, and plastic-based biofilms represent a previously overlooked habitat that contributes to biogeochemical dynamics in polluted ecosystems.

One important exception to the overall pattern in biofilm characteristics across substrates was a distinction in respiration and NEP. Tile and polystyrene showed negative NEP while both types of polyethylene and PVC showed positive NEP. The pattern was driven by differences in respiration rather than GPP. This shows that while substrates had similar metrics for primary production (i.e., GPP and chlorophyll), tile and polystyrene sustained more heterotrophic activity than PVC or polyethylene. In addition, the NEP patterns were consistent throughout the month-long incubation period, suggesting that some physical attributes of the substrates could explain the discrepancy. The polyethylene and PVC used for this study were newly manufactured and had not experienced any weathering or use prior to the start of the experiment. These surfaces may not have provided 3-dimensional complexity in micro-topography and are non-porous, and may thereby not retain organic matter which supports enhanced heterotrophic activity. In contrast, the unglazed ceramic tile and the foamed polystyrene surfaces could offer higher surface area and microsite heterogeneity for heterotrophic respiration and retention of fine particulate organic matter. We speculate this could occur in the rough surface of the unglazed ceramic tile and in the pore spaces of the foamed polystyrene, although we did not measure microtopography or pore-space organic matter. Substrate-specific development of adsorbed organic matter layer on different plastic substrates in stream water was recently observed elsewhere (Rummel et al., 2021). Our work on biofilm metabolism on plastic substrates in streams across continental United States showed similar findings (Vincent et al., 2022). Over longer time scales, the heterotrophic nature of biofilms on foamed polystyrene compared to polyethene and PVC might contribute to more rapid degradation. The 'spongy' texture of foamed polystyrene also would facilitate fragmentation via abiotic and biotic mechanisms.

We note there was a distinction in NEP among substrates without a corresponding difference in community composition or biofilm biomass, so the enhanced respiration on tile and polystyrene was most likely due to changes in activity of the same community across surfaces, rather than a selection for biofilm constituents with distinct metabolic capabilities. However, our analyses suggested disparities in NEP could potentially be explained by site data and a number of key taxa, in particular the class Sphingobacteria (which were higher on PS and tile substrate). This bacterial group consists of taxa that are known to degrade complex organic matter in diverse ecosystems including freshwater (Newton et al., 2011), and this metabolic versatility could be associated with its importance in explaining the biofilm metabolism trends. More work which compares degradation pathways across polymer types in urban rivers is needed, including assessments of the relationships between biofilm metabolism, community composition, and breakdown. In particular, we did not characterize the physicochemical properties of plastic surfaces in this study, but suggest future analyses will benefit from combining metrics of particle topography and chemical associations with biofilm community composition and activity.

4.2. Biofilm community composition: succession of bacterial, fungal and algal taxa across substrates

We expected that differences in biofilm community composition across substrates would be most evident early in succession (day 3 of incubation), while biofilm microorganisms were colonizing bare surfaces, relative to later in succession, when biofilms were growing on a surface already colonized by microbes. The mechanisms we expected to drive differences in succession among substrates were the distinct physical structure of plastic surfaces as well as its possible chemical associations that might affect selection of a unique microbial consortium. We assessed this hypothesis using DESeq analysis between days 3 and 10. Contrary to our predictions, the successional patterns from these results showed no difference in successional

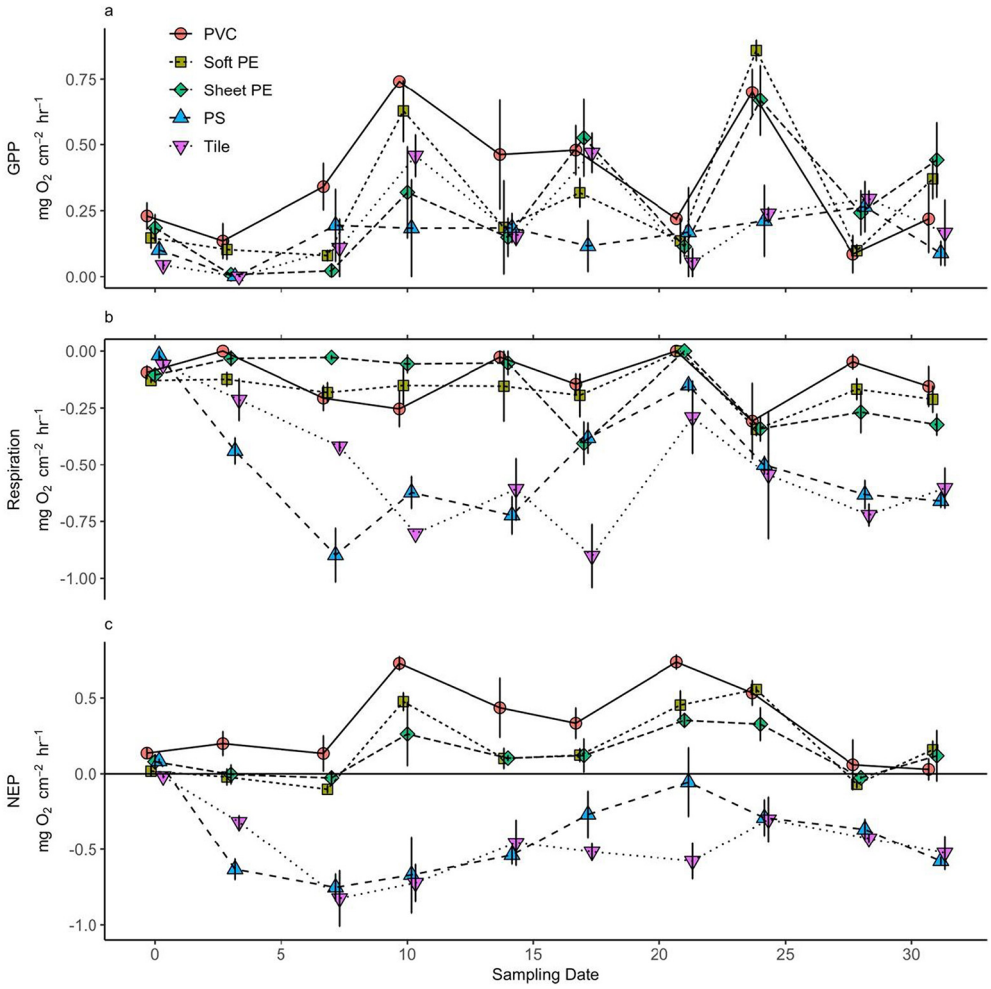

Fig. 2. Mean (±SE) of (a) gross primary production (GPP), (b) community respiration, and (c) net ecosystem production (NEP) on biofilms growing on polyvinyl chloride (PVC), soft and sheet polyethylene (PE), polystyrene (PS, Styrofoam), and tile.

patterns in the microbial assemblages across the different substrates for bacteria, algae, or fungi. We conclude from this comparison that there is no effect of substrate on early successional patterns, or that the impact of substrate on biofilm composition occurred on days 0–2, and the biofilms were already mature on the substrates by our first sample collection on day 3. There is some evidence from research elsewhere that 3 days may have been too late to capture a substrate effect. Recent work by Rummel et al. on conditioning film of dissolved organic matter on different plastic and glass substrates and development of early microbial community provided some evidence of early substrate-specific microbial colonizers within 24 h, but convergence of the biofilm community composition by 72 h (Rummel et al., 2021). In addition, assessment of biofilm community development patterns on weathered plastics in marine systems highlighted significant enrichment of plastic-degrading bacterial taxa in the first two days of biofilm development (Erni-Cassola et al., 2020). On day 9, these taxa were rare members of a microbial community predominantly comprised of taxa common to marine biofilms.

Although the overarching pattern in the data was similarity in successional patterns for the microbial communities across the substrates, we noted a significant change in alpha diversity over time for the fungal community across all substrate types, particularly between days 3 and 10. This is in contrast to the lack of change in bacteria and algae during this time. The reduction in alpha diversity for the fungal assemblages on day 10 was accompanied by an associated drop in the relative abundance of various fungal taxa based on the DESeq results (although not statistically significant). These included *Aureobasidium*, *Taphrina*, and unclassified genera within *Pseudeurotiaceae* and Basidiomycota. Together, these changes suggest more colonizer turnover between days 3 and 10 for fungi.

Despite the overall lack of difference in the microbial assemblages among the substrates during biofilm succession, bacterial community composition was different between the polyethylene substrates and tile, driven by the higher relative abundance of Actinobacteria on the polyethene. Actinobacteria are ubiquitous members of freshwater microbial communities both in the pelagic and sediment zones. However, the pelagic and sediment Actinobacteria have distinct genomic and metabolic characteristics. The highly abundant pelagic Actinobacteria are small sized with streamlined genomes and slow growth rates (Ghai et al., 2014; Newton et al., 2011). On the other hand, Actinobacteria inhabiting the sediments have large genomes with a diverse set of metabolic capabilities (Benimeli et al., 2007; Margesin et al., 2013). While Actinobacteria have previously been found as members of freshwater biofilms on artificial substrate (i.e., stainless steel; (Parfenova et al., 2013)), they were not as abundant as in the surrounding water and little is known about their distinct metabolic capabilities. Interestingly, Actinobacteria was also found to be higher in abundance in biofilms associated with PE sheets and microplastics as compared to polypropylene sheets in freshwater (Wang et al., 2021). We speculate that the enriched Actinobacteria on PE substrates might represent members of the phylum that are metabolically versatile and can utilize complex organic substrates, as noted for Actinobacteria in sediments and soil ecosystems (Alvarez et al., 2017; Shao et al., 2019). Genomics/Metagenomics based investigation of plastic-associated biofilms can potentially provide clues about the metabolic capabilities of these Actinobacteria

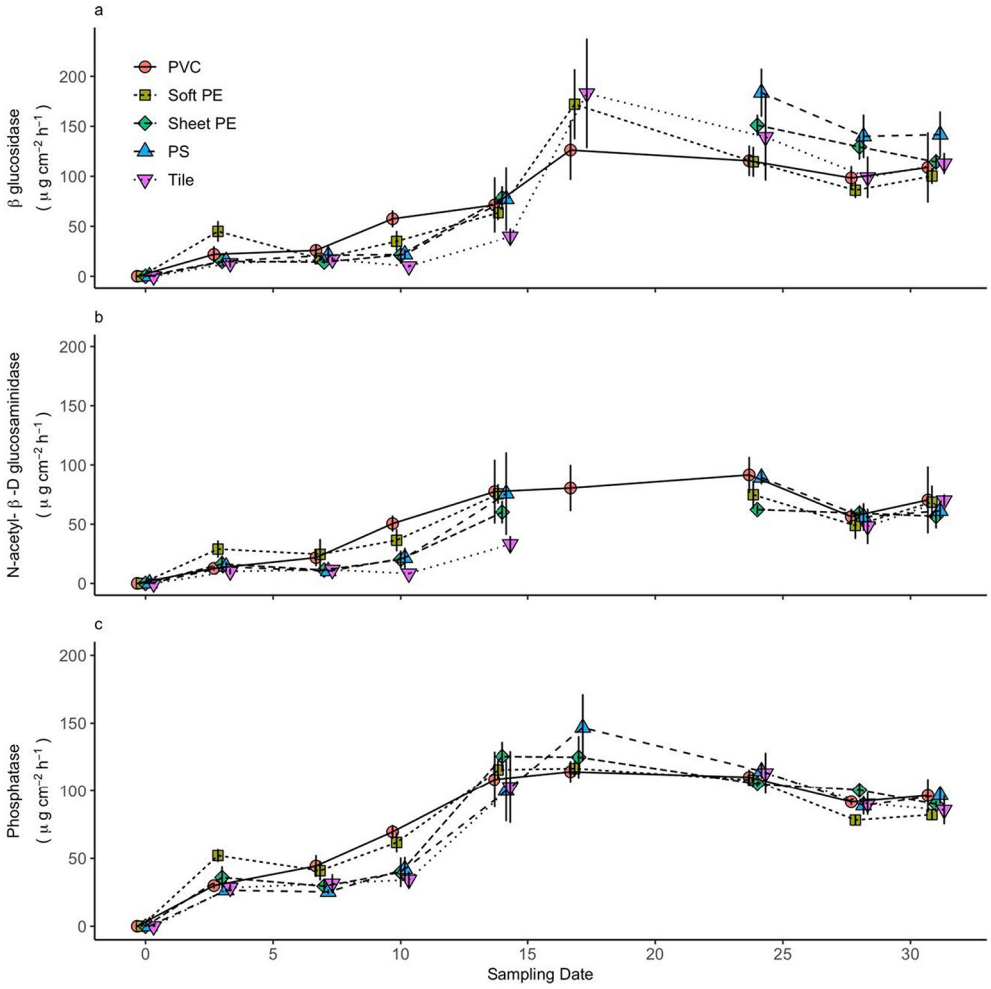

Fig. 3. Mean (±SE) for enzymatic activity potential of (a) β-glucosidase, (b) *N*-acetyl-β–D-glucosaminidase, and (c) phosphatase during biofilm incubation on polyvinyl chloride (PVC), soft and sheet polyethylene (PE), polystyrene (PS, Styrofoam), and tile.

that may be associated with their selective enrichment on polyethylene substrates.

The question of plastic-mediated selection of microbial communities is a topic of interest and has been subject to recent meta-analysis. A recent review of 400 studies documenting plastic degradation by isolated environmental microbes reported degradation of 72 different plastic types by 436 species of fungi and bacteria (Gambarini et al., 2021; Lear et al., 2021). The bacterial species were affiliated with primarily three phyla: Proteobacteria (n = 133), Actinobacteria (n = 88) and Firmicutes (n = 60). Fungal species were affiliated with phyla Ascomycota (n = 118), Basidiomycota (n = 19) and Mucoromycota (n = 13). Since most of these isolates were either from soil, waste dumping sites, or microbial culture collections, our results which suggested selection of Actinobacteria on PE substrates are consistent with the meta-analyses as well as other recent evidence (Wang et al., 2021) and suggest that further investigation into the enzymatic capacity of the phylum is needed.

Table 2
Results from PERMANOVA to compare Bray-Curtis distances by substrate and sampling date for assemblages of bacteria, algae, and fungi (p-value < 0.05 for significant factor effects, represented in the table in bold).

	Degrees of freedom	F-ratio	p-Value
Bacterial community			
Substrate	4	3.03	**0.002**
Date	3	15.72	**0.001**
Interaction	12	0.93	0.605
Algal community			
Substrate	4	1.56	0.132
Date	3	3.01	**0.033**
Interaction	12	1.04	0.424
Fungal community			
Substrate	4	1.45	0.193
Date	3	26.26	**0.001**
Interaction	12	1.44	0.106

A potential caveat in our experimental design could be the use of tile as a surrogate for natural benthic substrates such as rocks. We recognize that the use of mesh bags to place the substrates could affect the light availability and cause shear/stress to the tile substrates, potentially leading them to have different dynamics than actual benthic rocks. However, the use of tile as surrogates for natural substrate resembles work in other stream ecology studies (Peckarsky et al., 2013). In addition, our study aims also made it relevant to set up the tile substrate in the same way as the plastic substrates. Another potential limitation in our study design includes lack of microbial community analysis of the surrounding stream water. We recognize that this sampling effort could provide insights into the relationship between the biofilm communities and the microorganisms in the surrounding water at various stages of the incubation. Future work can potentially benefit from sampling both the plastic substrates and the water-column microbial communities, as done in other studies (Oberbeckmann et al., 2016). Finally, our study was conducted in an urban stream as that is the type of stream with the most plastic litter (Cowger et al., 2019; Kiessling et al., 2021). Environmental conditions in urban streams drive biofilm characteristics (e.g., elevated levels of nutrients and chemical contaminants) which may affect our results. Follow-up studies on the effect of plastic-mediated

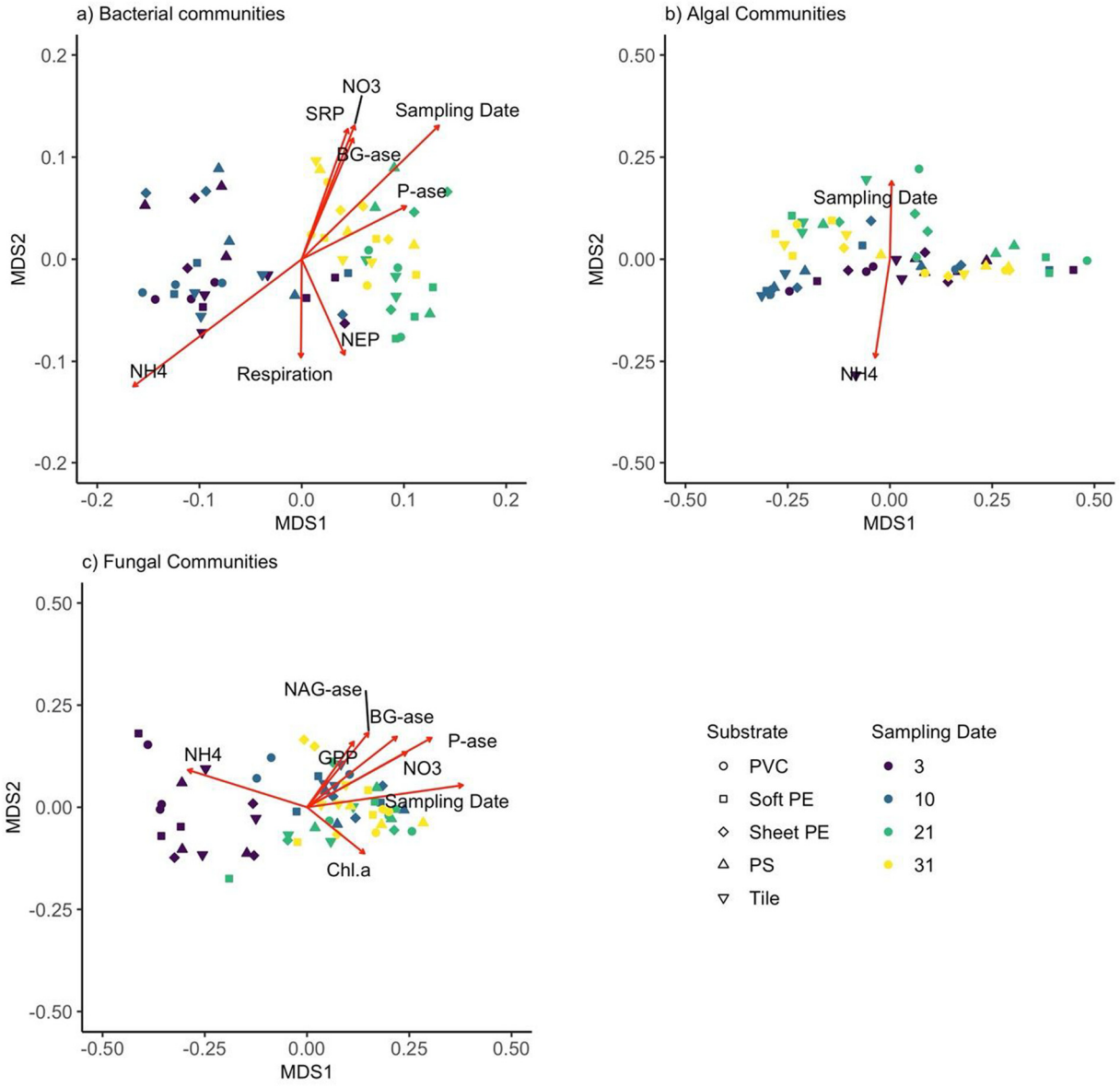

Fig. 4. Nonmetric multidimensional scaling (nMDS) ordination of sequencing data (Bray–Curtis dissimilarity) for (a) bacterial assemblages, (b) algal assemblages, and (c) fungal assemblages at class-level of 4 dates during biofilm incubation on polyvinyl chloride (PVC), soft and sheet polyethylene (PE), polystyrene (PS, Styrofoam), and tile. nMDS stress values for bacterial, algal, and fungal assemblages are 0.13, 0.04, and 0.08, respectively. Lines show significant correlations between biofilm and water column attributes relative to NMDS axes (Table 3). Abbreviations: NEP = net ecosystem production, GPP = gross primary production, BG-ase = β-glucosidase, NAG-ase = *N*-acetyl-β–D-glucosaminidase, P-ase, phosphatase, SRP = soluble reactive phosphorus, NO_3^- nitrate, NH_4^+ = ammonium, and chl = chlorophyll.

selection for biofilms should consider conducting studies at sites that span a gradient of land-use types and physico-chemical conditions of streamwater.

4.3. Differences among bacteria, algae, and fungal components of biofilms

Few studies have used molecular techniques to simultaneously examine the composition of bacterial, algal, and fungal assemblages within stream biofilms. Comparison of alpha diversity for all the three microbial assemblages in our study suggest the highest diversity for bacteria. This is not surprising, as bacteria are known to harbor higher diversity in many ecosystems in comparison to their algal and fungal counterparts (Wagg et al., 2019). In addition, as the database for inferring taxonomic information for bacteria from 16S rRNA gene sequences is much more developed relative to databases available for the other two groups, our results for the alpha diversity observed here could also be influenced by this methodological artifact.

Our analyses of biofilm community composition for algal and fungal taxa represent a relatively early assessment of community composition across taxonomic groups using next generation sequencing techniques. While studies in freshwater ecosystems have documented the bacterial component of plastic-associated biofilms (Hoellein et al., 2014; McCormick et al., 2014; McCormick et al., 2016), work characterizing the fungal and algal constituents is much more limited (González-Pleiter et al., 2021).

Stramenopiles and unclassified Eukaryotes were the dominant algal taxa across all the substrates, with Cyanobacteria present in low abundance, which is similar to previous assessments. Using scanning electron microscopy and next-generation sequencing, Zettler et al. (2013) found a dominant presence of diatoms on microplastics in the North Atlantic waters. Similarly, Oberbeckmann et al. (2016) found a significant presence of diatom groups in PET-associated biofilms in the North Sea, along-with brown-algae Phaeophyceae, ciliate group Conthreep and the green algae Chlorophyta. Diatoms are a major group of algae classified within the

Table 3
Correlations between biofilm and incubation characteristics relative to axes for non-metric multidimensional scaling (NMDS) of bacterial, algal, and fungal assemblages. Abbreviations: NEP = net ecosystem production, GPP = gross primary production, BG-ase = β-glucosidase, NAG-ase = *N*-acetyl-β–D-glucosaminidase, P-ase, phosphatase, date = length of incubation, SRP = soluble reactive phosphorus, NO_3^- nitrate, NH_4^+ = ammonium, and chl = chlorophyll.

Assay	NMDS 1	NMDS 2	p-Value
Bacterial assemblage			
NEP	0.147	−0.387	0.011
Respiration	−0.012	−0.393	0.025
BG-ase	0.211	0.484	0.002
P-ase	0.407	0.191	0.006
Date	0.555	0.513	0.001
SRP	0.196	0.517	0.002
NO_3^-	0.222	0.533	0.002
NH_4^+	−0.679	−0.475	0.001
Algal assemblage			
Date	0.021	0.999	0.026
NH_4^+	−0.147	−0.989	0.003
Fungal assemblage			
GPP	0.226	0.327	0.010
BG-ase	0.435	0.357	0.001
NAG-ase	0.301	0.366	0.002
P-ase	0.609	0.341	0.001
Chl. a	0.267	−0.244	0.038
Date	0.760	0.128	0.001
NO_3^-	0.481	0.289	0.002
NH_4^+	−0.587	0.162	0.001

Stramenopiles, and could likely be associated with the plastic substrates in this study as well. These results show their ability to colonize diverse plastic substrates in aquatic ecosystems and contribute to primary production, and show more work is needed to examine relationships between diatoms and plastic pollution in freshwater ecosystems.

For the fungal components of the biofilms, Ascomycota and unclassified Fungi were the dominant groups across the substrates. Ascomycota and Chytridiomycota were the most abundant identified fungal phyla on biofilms sampled from microplastics incubated in an Arctic freshwater lake for 11 days (González-Pleiter et al., 2021). However, the fungal community on microplastic biofilms was not statistically different from the fungal biofilms on the lake microbial mats. Here, Ascomycota were the most dominant across all the substrates on day 3 of the incubation, and dropped in abundance afterwards. This result and the associated drop in alpha diversity after day 3 for fungal assemblages potentially highlight an important role for this fungal group in early stages of biofilm formation. Notably, 14 Ascomycota-affiliated OTUs were significantly enriched on PET and glass biofilms as compared to free-living and particle-attached seawater communities in the North Sea, the highest for any fungal phyla (Oberbeckmann et al., 2016). In addition, phylum Ascomycota comprises the largest number of fungal species (n = 118) reported to degrade plastic across all the fungal phyla (Gambarini et al., 2021). Overall, these results reflect the need for more investigation of the metabolic capabilities of early fungal colonizers of plastic biofilms in aquatic ecosystems.

Table 4
Results for generalized linear model selection for explanatory variables that best predict the observed trends in NEP measurements. Model 'PE and PVC' corresponds to data for only PE and PVC substrates (model R^2 = 0.89), model 'PS and tile' corresponds to data for only PS and tile substrates (model R^2 = 0.12), whereas model 'All data' selects the explanatory variables to predict NEP values for all the substrates (model R^2 = 0.11). Abbreviations: SRP = soluble reactive phosphorus, NO_3^- = nitrate, NH_4^+ = ammonium, uncl = unclassified.

Term	Estimate	Std. error	Statistic	p-value	Model
(Intercept)	0.681	0.024	28.16	<0.001	PE and PVC
Date	0.016	0.001	13.60	<0.001	PE and PVC
SRP	−0.001	>0.001	−25.59	<0.001	PE and PVC
Sphingobacteria	6.752	4.185	1.61	0.111	PE and PVC
(Intercept)	−0.251	0.055	−4.58	<0.001	PS and tile
Date	0.009	0.002	3.48	0.001	PS and tile
NO_3^-	−<0.001	<0.001	−4.72	<0.001	PS and tile
Eukaryota uncl.	3.071	1.793	1.71	0.089	PS and tile
(Intercept)	0.722	0.244	2.96	0.003	All data
SRP	−0.001	<0.001	−5.35	<0.001	All data
NH_4^+	−0.006	0.002	−2.91	0.004	All data
Cyanobacteria uncl.	−47.970	26.540	−1.81	0.072	All data
Flavobacteria	−32.980	21.370	−1.54	0.124	All data

In conclusion, the results from this study highlight the similarity in successional trends for biofilm metabolism and microbial community structure in an urban stream across diverse plastic substrates as well as in comparison to a natural surface (tile). The metabolic activity of diverse bacterial, fungal and algal assemblages on plastic substrates occur at similar rates to natural surfaces, even though plastic litter is a relatively new substrate for streams and is not traditionally considered as a microbial habitat that contributes to ecosystem processes in streams. In addition to the overall similarity in these trends across substrata, there were patterns for certain microbial taxa and specific metabolic parameters that warrant further investigation for understanding their role in microbial community dynamics in plastic biofilms. These include the higher respiration and NEP rates on polystyrene and tile biofilms, the higher prevalence of Actinobacteria in polyethylene biofilms as compared to tile, and the higher abundance of fungal group Ascomycota during early stages of biofilm succession across all substrates. The study also provides a relatively early characterization of fungal and algal components of plastic biofilms in freshwater ecosystems, and highlights the potential analytical limitations in characterizing these important biofilm components. Future work aiming to understand the specific mechanisms and pathways active in substrate-specific microbial members of plastic biofilms can utilize metagenomics/metatranscriptomics-based approaches (Wright et al., 2021). This can potentially shed further light on their contributions to ecosystem processes and fate of plastic substrates in freshwater ecosystems.

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CRediT authorship contribution statement

Conceptualization SD, TH, JK; Data curation AC, SD, JK, TH; Formal analysis AC, SD, TH.

Funding acquisition TH; Investigation AC, SD, TH, JK; Methodology SD, TH, JK.

Project administration TH; Resources TH, JK; Software TH, JK; Supervision TH, JK.

Validation AC, SD, TH; Visualization, Roles/Writing - original draft AC, SD, JK, TH.

Writing - review & editing AC, SD, TH, JK.

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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