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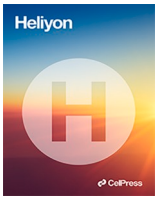

Freshwater algal biofilm assemblages are more effective than invertebrate assemblages at aggregating microplastics

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ABSTRACT

Microplastics, plastic particles less than 5 mm in length, are a ubiquitous pollutant in the environment, but research on freshwater microplastic contamination is lacking. A possible fate of microplastics in freshwater environments is to become entangled or aggregated in biofilms, which are matrices of algae, bacteria, and micro invertebrates that grow on underwater surfaces, following a progression of settling algae, periphyton, and finally invertebrate colonization. This *in-situ* study at the Oasis Marina at National Harbor in Oxon Hill, Maryland, examined how the taxonomic assemblages of freshwater biofilms in the Potomac River are associated with the number of microplastics aggregated within them. Aluminum discs, acting as artificial substrate for biofilm growth, were deployed at the water's surface and at 2 m depth to survey biofilm assemblage and were sampled monthly from October 2021–October 2022. Microplastic abundances in the water column were measured every 2 weeks over the same period. Spatial and temporal trends in trapped and suspended microplastics, water quality parameters (temperature, dissolved oxygen, pH, salinity, conductivity, turbidity, ammonia, nitrate, and phosphate), and biofilm assemblages were measured and compared to explore factors affecting the abundance of microplastics and their partitioning between the water column and biofilms. Water quality had no measurable impact on microplastic abundance in the water column at either depth, but temperature was negatively correlated to microplastic abundance in biofilms. As the weather warmed and biofilms progressed to invertebrate settling, they tended to contain fewer microplastics. This may have occurred because less biologically rich biofilms, primarily composed of unicellular algal colonies, provide a favorable surface for microplastic deposition. Understanding seasonal changes in biofilm assemblage and microplastic abundance may help track the fate of microplastics in freshwater systems, particularly in their interactions with lower trophic organisms.

1. Introduction

Microplastics, plastic particles less than 5 mm in length, are a ubiquitous contaminant found in nearly all terrestrial and aquatic

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environments and organisms studied to date [1–4]. Microplastics in freshwater ecosystems primarily originate from urban sources, including stormwater runoff across impervious surfaces, automobile tire and brake system degradation, litter, and even wastewater treatment plant effluent [5–9]. Some studies have looked into microplastic movement and deposition within lakes and rivers (i.e. [10]); however, a detailed understanding of microplastic abundance, transport, and seasonal spatial dynamics in freshwater river systems is still lacking.

As microplastics travel around waterways, they have opportunities to interact with the flora and fauna in the water column, including biofilm assemblages of algae, bacteria, and invertebrates growing on surfaces. These biofilms can offer shelter to the organisms that compose and inhabit them, helping them withstand environmental stressors like water currents, competition, and predation [11–14]. Biofilms can form at any water depth when bacteria and algae settle out of the water onto a surface, creating a suitable

Fig. 1. Research location (black asterisk) at National Harbor, with inset showing National Harbor's location relative to Washington, D.C. and the Potomac River. Figure made in ArcGIS.

environment for periphyton and invertebrates [12,15]. A biofilm's mass is affected by nutrient availability, water quality, and trophic status of the organisms comprising the biofilm. These variables, in turn, vary seasonally and with depth in the water column [12,16].

The growth and loss of biofilm biomass can influence the fate of microplastics in aquatic environments, but the physical and biological interactions with taxonomic assemblages remain unclear [3]. Varying organismal assemblages offer multiple mechanisms for biofilms to aggregate microplastics, one example being periphyton growth entangling microplastics, therefore increasing the bioavailability of microplastics for invertebrate grazers, filter feeders, or tube-dwellers [17–26].

This research aimed to explore seasonal variability in: (1) the taxonomic composition of freshwater biofilms, (2) the quantity of microplastics trapped in the biofilms per unit area, and (3) the quality of the surrounding water, including its microplastic concentration. We measured the numbers of microplastics aggregated in biofilms on upward- and downward-facing substrates at two depths (surface and 2 m), concurrently documenting water quality parameters, the concentration of microplastics in the water column, and the areal extent, biomass, and taxonomic composition of the biofilms. This study can help elucidate correlations between biofilm assemblages and the fate of microplastics in freshwater river and estuary systems.

2. Materials and methods

2.1. Field site and materials

The field component of this study took place at the Oasis Marina in National Harbor, Oxon Hill, Maryland from October 2021 to October 2022 (Fig. 1). National Harbor is an urbanized site with vehicular traffic, pedestrians, impervious surfaces, and stormwater runoff located on the Potomac River downstream of its union with the Anacostia River. Washington, D.C., and the Blue Plains Wastewater Treatment Plant are also located immediately upstream. Estuarine environments, like the Potomac River and nearby Chesapeake Bay, can serve as microplastic sinks [27], making National Harbor well-suited to a study of microplastic fate.

Sampling racks were deployed at the surface and at 2 m depth, each consisting of 2 pairs of 10-cm diameter aluminum discs that served as artificial substrate for biofilm growth [28]. Sampling racks were installed and left deployed at the site from October 2021 to October 2022. The paired discs were placed against each other so that the exposed sides faced either towards the water's surface or towards the riverbed, creating opportunities for up- and down-facing biofilm growth and surfaces for microplastics to settle out of the water column. Ten sampling racks were deployed: five at the water's surface and five at 2 m depth, totaling forty discs (Fig. 2). All of these sampling racks were needed to accommodate a year's worth of field sampling. Six sampling racks were deployed for biofilm collection, with each disc assigned a month for quarter-disc biofilm collections. Two racks were used for biodiversity analyses, and two served as backups (Fig. 3). For recording and analyzing data on taxonomic assemblages and microplastic abundance, two discs per rack were classified as "Surface-up", "Surface-down," "2m-up," and "2m-down" and data from duplicate discs were averaged together. Each disc on the sampling racks remained in the water for the duration of the year-long study. This study design allowed us to observe variability associated with seasonal changes in water temperature and biodiversity but did not permit us to distinguish season from time since the disc was installed.

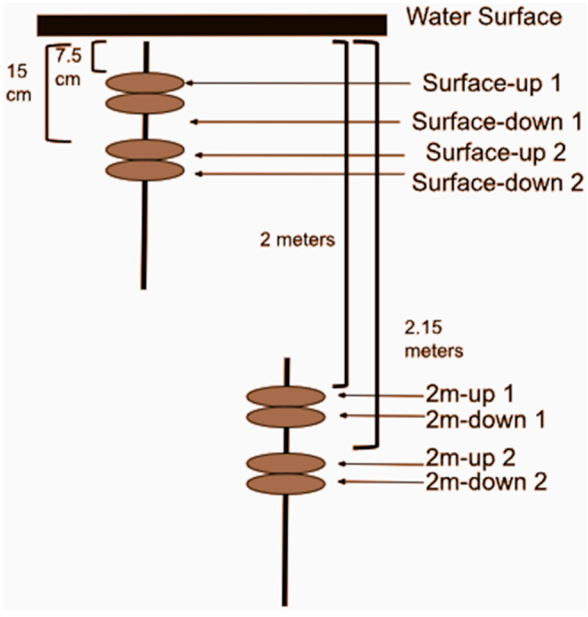

Fig. 2. Biofilm sampling rack field design.

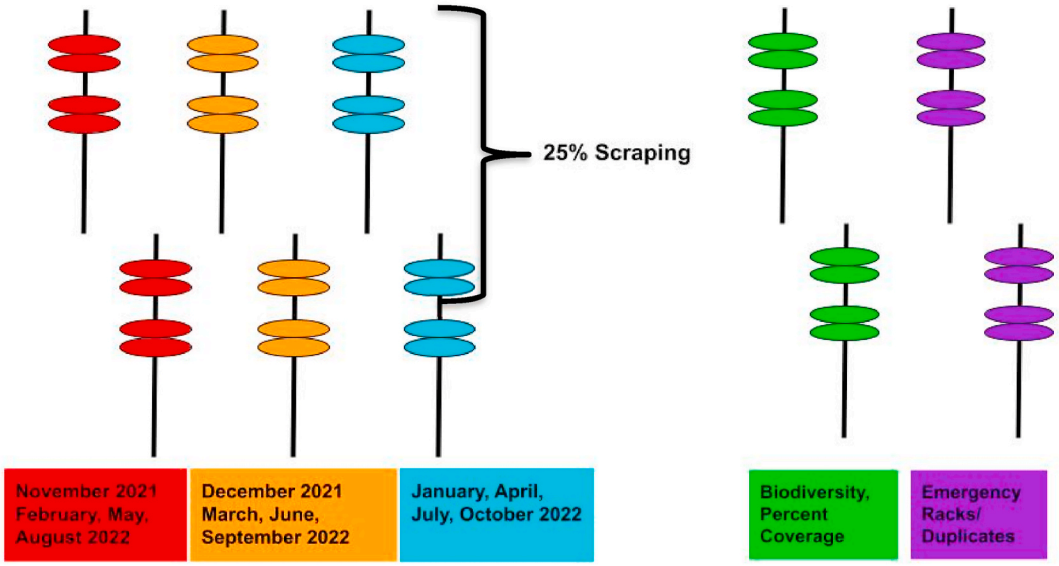

Fig. 3. Field design color coded by purpose of each sampling rack and sampling date.

2.2. Sample collection and Preparation

Biofilms were sampled monthly by slowly extracting the discs from the water so as not to disturb the biofilms, placing entire discs individually in glass dishes, covering them with ambient river water, and placing them under a dissecting microscope with an attached digital video camera. Four samples per biofilm were recorded by positioning the field of view scope 2.5 cm from the edge of the disc and rotating the disc clockwise to record a video of each quadrant regardless of organisms located within the field of view. The area of the field of view was 69 square millimeters (11 mm × 6.23 mm). Overall biofilm growth was measured on the total disc area in square centimeters (10 cm diameter disc = 12.174 cm^2), but the taxonomic assemblages, viewed from a camera with a smaller scope, were measured in square millimeters (mm^2). Videos were recorded to the camera, downloaded, and analyzed on a computer to measure taxonomic richness via unique taxa clustering, rather than species count [29–31]. Freshwater classification guidebooks were used to identify each taxonomic group (see Appendix A) [12,32,33]. The number of individual taxonomic groups were counted for richness, and the disc surface occupied by each taxon was measured in area (mm^2) using Image J software.

Microplastic abundance in biofilms was assessed monthly by scraping one quarter of each disc with a straight-edge razor blade into a pre-weighed aluminum container and immediately covering it with aluminum foil to prevent contamination, resulting in a total of 96 biofilm samples. Containers were placed under a fume hood for up to 48 h until all standing water evaporated.

One liter water samples were collected in glass jars at the same location of disc deployment at the surface and 2 m. Prior to collection, each container was rinsed three times with ambient river water. Water samples were collected every two weeks for a total of 52 samples. Water quality parameters (temperature, dissolved oxygen, pH, salinity, conductivity, turbidity, ammonia, nitrite, nitrate, and phosphate) were also measured every two weeks at both sampling depths.

2.3. Extraction and categorization of microplastics

After the biofilm masses were recorded, dried biofilm samples were density-separated by combining them with 200 mL of 70 % saltwater solution (1000 mL of vacuum filtered deionized water, 70 g sodium chloride (NaCl)) in a 500 mL separatory funnel, shaking to suspend the solids, and vacuum-filtering through a Buchner funnel with filter paper (gridded Whatman 0.45 μm porosity, 47 mm diameter). Dense organic material settled out of the solution and was transferred into the aluminum container, covered with aluminum foil, and placed in a fume hood to re-dry. The dense material was viewed in its container under a dissecting microscope at 35x magnification for residual microplastics within and under the material. The remaining saltwater solution was vacuum filtered. The water samples collected from the field were also vacuum filtered. After vacuum filtration, the filter paper was placed in an aluminum weigh boat and covered in aluminum foil to dry. These methods were adapted from several other studies [1,28,34–39]. Microplastics were viewed on the filters and in the containers under a dissecting microscope for visual identification using set criteria of 1. no tapering, 2. no visible organic structures, 3. homogeneously colored, and 4. durable and not fragile under poking and prodding [7,38, 40,41]. These criteria should have eliminated observations of organic material and only found the synthetic particles in the samples. The two counts between the filters and the dried dense material were summed for the microplastic abundance value per sample. We acknowledge that visual microplastic identification could potentially lead to errors in quantification. However, the degree of visual identification errors (i.e., anthropogenic versus natural particles) should be consistent throughout the study. This approach allowed for consistent comparison of microplastics across biofilm and water samples.

2.4. Quality assurance

Several steps were taken to minimize microplastic contamination of samples and to quantify and subtract out the method blank. Glass and metal supplies were used to minimize contamination with plastics during and after sample collection. All samples and laboratory equipment were covered with aluminum foil when not in use so that sample exposure to airborne microplastic contamination was limited [1,9,28,42]. To quantify the number of microplastics that settled out of the air onto the samples as they were being processed, a blank Whatman 0.45 μm porosity, 47 mm diameter filter was left exposed on the benchtop next to all sample processing stations to collect atmospheric microplastic contamination. Three liters of the 70 ppt saltwater solution were also filtered to calculate an average number of microplastics in the salt used for density separation. Microplastics were counted using the same criteria for identifying microplastics in the samples [38,40,41,43,44]. The underlying microplastic count was divided by the number of samples processed next to the contamination filter to estimate the blank per sample. The method blank (the number of microplastics one would expect to observe if no microplastics were present in the original sample) was adjusted for sample volume and subtracted from the number of microplastics measured in all samples [45,46]. The method blank included the atmospheric and salt microplastic counts per volume or sample.

2.5. Data analysis

Microplastic abundances were assumed to have a Poisson distribution because this distribution is typical of count data and because microplastic distribution showed the right skew typical of Poisson distributions (Mean abundance per biofilm: 11, median: 8). Other variables were assumed to have a normal distribution because the means equaled the medians and histograms showed no skew. Two hundred sixty Pearson correlation analyses were performed between all biofilm, water quality, and microplastic measurements to assess correlations between physical and chemical water parameters, biofilm growth and richness, and microplastic abundance. The large number of tests performed on the same dataset implies a certain number of false positives for a given p-value. Results with $p < 0.05$ would occur by random chance 5 % of the time, meaning about 13 of the tests may have occurred by random chance. Results with a statistical significance considered at $p < 0.05$ are intended to convey patterns that are likely, but not definitely, indicative of real effects, not as binary options of meaningful or not meaningful.

The total biofilm mass (g), growth area (cm^2), and richness of each biofilm sample were all measured, but none of these variables were significantly correlated ($p < 0.05$) to microplastic abundance, so no further analysis was done. Instead, correlations between functional groups of taxa and microplastic abundance were explored.

The number of biofilm taxa (n = 20) was too large to consider each as a separate explanatory variable, especially due to collinearity between different taxa. Moreover, the ability of biofilm organisms to aggregate microplastics would most likely be determined by their phenotype rather than their genotype. Thus, we grouped taxa into operational taxonomic units (OTUs) [29,47] and then assessed

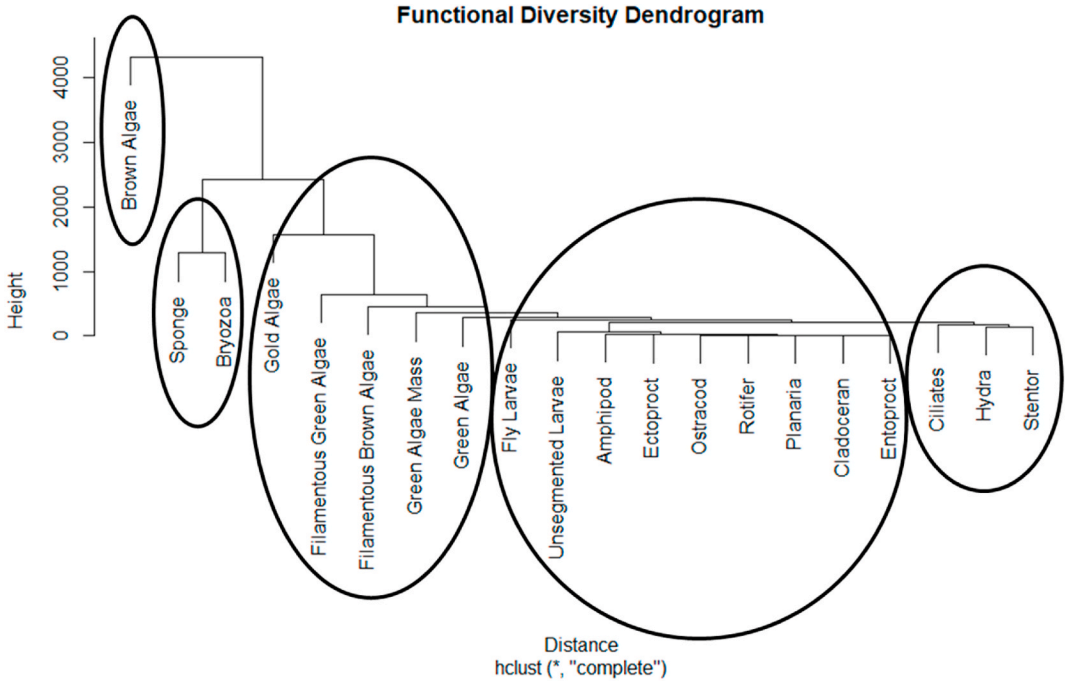

Fig. 4. Functional diversity dendrogram generated using nearest neighbor agglomerative clustering. Clusters are circled. Euclidean distances between taxa were calculated based on their abundance and effects on microplastic abundance, resulting in five OTUs.

correlations between each OTU and microplastic abundance.

Taxa were grouped into OTUs by using agglomerative clustering to generate a dendrogram of all observed biofilm taxa, where the distance between groups was determined by Euclidean distance using the taxa's abundance and modeled effect on microplastic abundance from mixed linear models [29,48]. Taxa that clustered near each other in the dendrogram were considered to comprise an OTU classified by total dendrogram branch length and containing taxa with similar functional traits [49]. Thus, the OTUs were defined from the dendrogram: brown algae (by itself), all other algae (gold algae, filamentous green algae, filamentous brown algae, green algae mass, and green algae), two distinct groups of filter feeders (one comprised of sponges and bryozoans, the second comprised of ciliates, hydra, and stentors), and motile invertebrates (fly larvae, unsegmented larvae, amphipod, ectoproct, planaria, entoproct, cladocera, ostracod, and rotifer) (Fig. 4).

It is important to underline that the dendrogram used to generate OTU categories is based on statistical relationships between areal coverage of a given taxon and microplastic abundance, and it is not equivalent to a phylogenetic tree. In fact, the OTUs do not correspond to a certain level of taxonomic classification, and taxa grouped within the same OTU may not be more similar genetically than those assigned to different OTUs [3,29,30,49–52].

Best subset analysis [53] identified which water quality and OTU variables were useful in explaining microplastic abundance in biofilms. Three analyses were run; one on all data points; one on surface, and one on 2m-depth data. The best model was defined as the one with the highest adjusted R^2 and the lowest Bayes' information criterion (BIC) and process capability ratio (Cp) values [30,54]. Linear mixed models, which considered biofilm direction and depth, were run on the included variables of the best model, which gave the estimated number of microplastics in the biofilms with which each variable was associated [55]. Statistics were run in R, primarily with the *neighbr* package [48].

3. Results

3.1. Abundance of microplastics in biofilms and the water column

All microplastics found in biofilms were microfibers, although any microplastic type would have been counted had they been observed. Microplastic abundance in biofilms fluctuated throughout the year from a minimum of 2 microplastics on the 2m-down disc

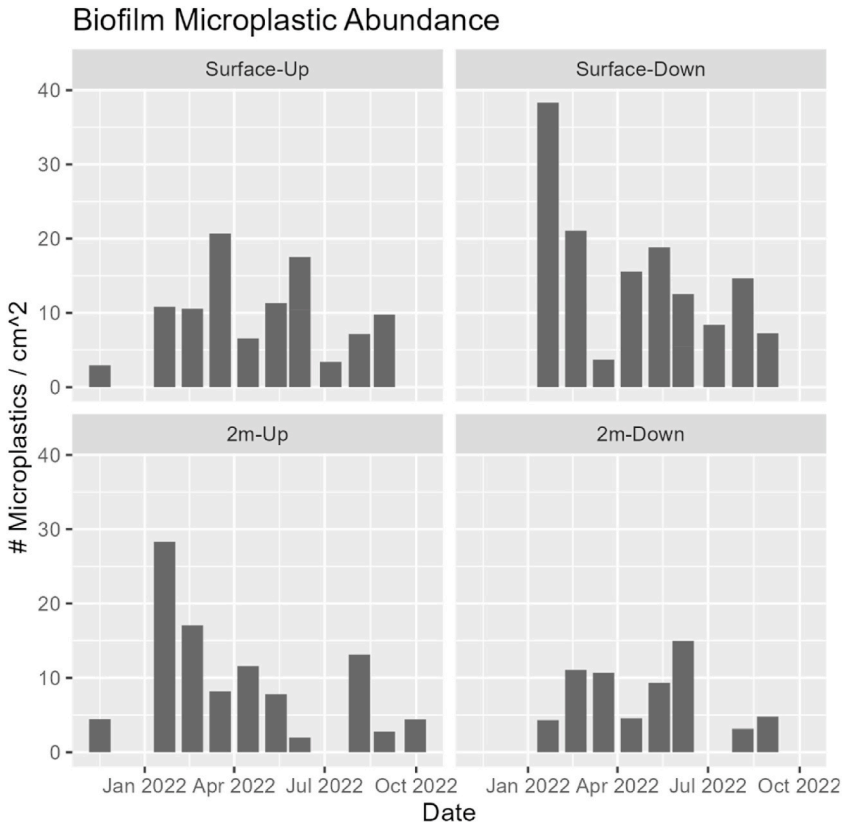

Fig. 5. Biofilm microplastic abundance. Biofilm abundance per biofilm sample, averaged between duplicates and showing only the statistically relevant abundances above the detection limit. Microplastic counts represent the total number observed in each monthly quarter-disc sampling (3 cm^2).

on June 6, 2022, to a maximum of 38 microplastics on a surface-down disc on January 21, 2022 (Fig. 5). Mean microplastic abundance was significantly higher by 2 microplastics at the surface (9 particles) than at 2 m (7 particles) ($p = 0.2692$). An interaction plot showed how depth and direction were correlated with microplastic abundance in biofilms, and, due to the non-parallel slopes of the two effects, showed that depth ($p = 0.00000483$), direction ($p = 0.00296$), and the interaction of both ($p = 0.000000832$) all had statistically significant relationships with abundance. Although there were, on average, more microplastics in up-facing biofilms, the mean difference was only 0.31 microplastics, with an average of 8 particles.

Microplastic abundance in the water column fluctuated throughout the year from a minimum of 2 particles found at 2 m in August 2022, to a maximum of 66 microplastics per liter at the surface in January 2022. Water column microplastic abundances were significantly higher at the surface (11 particles/L) than at depth (8 particles/L) ($p = 0.2749$) by an average of 3 microplastics.

3.2. Taxonomic group influence on microplastic abundance

There were significantly higher richness values in surface biofilms (n = 6) compared to 2 m biofilms (n = 5), but richness itself had no significant correlation to microplastic abundance in the biofilms. There was no significant richness difference between up- and down-facing biofilms. Since biofilm microplastic abundance also significantly differed between depths, the effects of each OTU at each depth (surface and 2 m), as well as all together, was further analyzed.

Upon individual OTU analysis, only three taxa had significant correlations to microplastic abundance in biofilms. Brown algae had a positive significant association with microplastic abundance across all three tests (all discs, just surface, and just 2 m) ($p = 0.0451$). The other algal OTU, composed of gold algae, filamentous green and brown algae, green algal masses, and unicellular green algae coverings, showed that, across both depths combined, only the unicellular green algae taxon ("green algae") had a significant correlation to microplastic abundance ($p = 0.00000388$). Considering only the surface data, green algae again showed a positive correlation to microplastic abundance ($p = 0.000178$). There were no significant correlations between any algal taxon and biofilm microplastic abundance at 2 m. The filter feeder and motile invertebrate OTUs had no significant correlation to microplastic abundance.

3.3. Water quality correlations to biofilm assemblages and microplastic abundance

We observed that both biofilm assemblages and microplastic abundance in biofilms varied seasonally. Algae dominated from November–May, while filter feeders were more abundant in June–October (Fig. 6). Water temperature, which showed the expected pattern of seasonal variability, was positively correlated with biofilm richness ($p = 0.000000045$), and negatively correlated with microplastic abundance ($p = 0.0036$). Richness ($p = 0.0000011$) and temperature ($p = 0.00000036$) were positively associated with larger biofilms by area (cm^2, measured from the entire disc). Smaller, less diverse biofilms, which were predominantly composed of

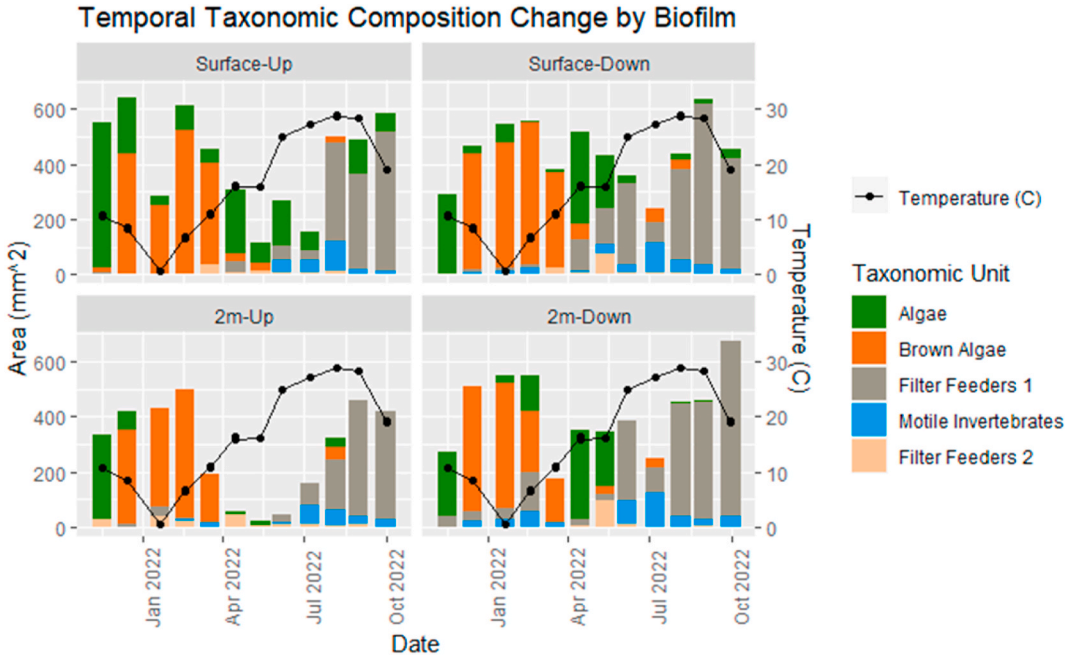

Fig. 6. Taxonomic group assemblage of each biofilm over time (See Fig. 4 for OTU classification). Winter months show a dominance of brown algae, while summer months moved more towards filter feeders and other invertebrates. The first filter feeder OTU consists of sponge and bryozoans, while the second consists of hydra, stentors, and ciliates.

algae, contained more microplastics, leading to an inverse correlation between biofilm area and microplastic abundance.

4. Discussion

Microplastic abundance in the biofilms and the water column was significantly higher at the surface than at the 2 m depth. We had hypothesized that depth would influence biofilm microplastic abundance indirectly, through its correlations with water quality or biofilm characteristics. However, variables related to water quality and biofilm characteristics were not significantly correlated with biofilm nor water column microplastic abundance. Thus, it seems likely that surface biofilms contained more microplastics simply because the water to which they were exposed had a higher microplastic concentration. It is worth noting that different polymers and sizes have different settling and aggregation rates in freshwater environments [56], so only certain types of microplastics may have been observed in the aggregations of certain biofilm assemblages, but only microfiber presence in the water samples indicates this may be unlikely at this research site.

Biofilm microplastic abundance was highest during colder months, when biofilms were dominated by unicellular algal coverings composed of green and brown algae. In contrast, during warmer months, biofilms contained more invertebrates, especially filter feeders, and less algal covering by area. Indeed, the areal extent of OTUs corresponding to algal coverings was positively correlated with biofilm microplastic abundance, while that of OTUs corresponding to invertebrates had a negative correlation.

Algal OTUs may have been associated with higher biofilm microplastic abundance because of microplastic entanglement or adhesion to the sticky polysaccharide mucus layer of algal surfaces [39,57,58]. Meanwhile, filter feeders and motile invertebrates fulfill other roles in the biofilm that could lessen the number of visible microplastics. Invertebrate assemblages may remove visible microplastics through several means. Tube-dwelling organisms, like bryozoans or fly larvae, may incorporate microplastics in their tubes, and filter feeders and grazers may ingest microplastics from the biofilm via filter feeding or grazing on algae on which microplastics have attached [18,22,40,57,58]. Although most microplastics studies have been conducted on marine organisms, there is evidence that freshwater amphipods, snails, worms, water fleas, and ostracods ingest microplastics as well [20], so grazing and filter feeding may decrease the number of microplastics visibly aggregated in larger biofilms in warmer months. This study, however, did not focus on the ingestion of microplastics, so if the microplastics were inside the tubes or bodies of invertebrates, we would not have measured them. Nor did this investigation include the effect of microplastics on the health or mobility of invertebrates which possibly ingested them.

The field site being an active marina may have played a role in microplastic distribution. Various activities of human visitors to National Harbor, wastewater discharges from boats, litter, and stormwater runoff from surrounding impervious surfaces may have resulted in more microplastics entering the harbor at the water's surface, providing more opportunity for them to settle into surface biofilms rather than the biofilms at 2 m.

As environmental conditions, biofilm assemblages, and microplastic abundances fluctuate throughout the year, biofilms do not appear to be a permanent sink for microplastics in the water column. Instead, abundances are tied to seasonal patterns of temperature and dominant taxonomic groups, which vary in their ability to aggregate microplastics. In this study, the base algal coverings of green and brown unicellular algae were associated with higher microplastic aggregation, which may have been due to a sticky polysaccharide mucus layer aggregating microplastics. Biofilms may aggregate microplastics during colder months, when the biofilms are dominated by sticky unicellular algae, and then release them in warmer months as invertebrates become more dominant. However, it is important to note that this study design could not distinguish the effects of season from those of disc deployment time. For example, discs sampled in November 2021 had been deployed for 1 month, and those sampled in June 2022 had been deployed for 8 months. The differences between them could be because June is a warmer month with longer days, or because the biofilm had been growing uninterrupted for a longer time. More research is needed to distinguish between these two effects.

5. Conclusion

This year-long study examined how seasonal variations in water quality and biofilm community composition affected microplastic entrapment. Of the factors we investigated, two appeared to significantly correlate with biofilm microplastic abundance: (1) depth and orientation of the substrate within the water column, and (2) areal extent of algal vs. invertebrate OTUs in the biofilm. These results suggest that biofilm microplastic abundance is influenced jointly by the number of microplastics available in the water column, and the ease with which the biofilm surface can trap them.

Both the supply of microplastics in the water column and the dominance of different OTUs in biofilm assemblages vary seasonally, but for different reasons. Many human and natural factors contributing to microplastic concentrations in rivers – including how heavily different areas are used by humans, the amount of rainfall and runoff, volume and microplastic concentration of wastewater effluent, and temperature fluctuations that influence estuarine circulation and microplastic buoyancy – exhibit seasonal variation. Likewise, changes in light, temperature, and nutrient inputs can drive regular seasonal variability in the types of organisms comprising biofilms. More research is needed to discern the underlying causal mechanisms behind the seasonal variability observed in this study.

Observations of seasonal changes in biofilm assemblages over the course of this study appeared to follow the classic pattern of biofilm formation: settling algae, periphyton growth, and finally invertebrate colonization [15]. In colder months, biofilm assemblages consist mostly of algal taxa, and as the weather warms, filter feeders and other invertebrates become more prevalent after having died back in colder months. Algal taxa are associated with the most effective aggregation of microplastics, while no other OTU in this study had a significant association with microplastic abundance. This may be due to microplastic adhesion enabled by the mucus layer in algal groups. Biofilm microplastic abundances fluctuate throughout the year as seasonal assemblages change, showing that biofilms

may not be a permanent sink of microplastics from the water column.

This study found that biofilms present in freshwater systems, such as the Potomac River, can trap microplastics from the water column. The findings confirm the presence of microplastics in the Potomac River at the study site, but show that they are not evenly distributed at all times of year. More microplastics are found in biofilms under cold, biologically sparse conditions than warmer, more biodiverse time periods, suggesting that complex interactions of physical and biological variables, both of which vary seasonally, may drive microplastic transport and fate in the Potomac River. This project is relevant to emerging contaminant issues and can contribute to the body of recent literature as natural sinks of microplastics, especially over time, and their interactions with biofilms are still being discussed.

Data availability statement

Open data access in the American University data repository at Barnes, Joseph (2023). Biofilm and Water Quality Measurements Effects on Microplastic Abundance. American University. Dataset. https://doi.org/10.57912/23609544.v1.

CRediT authorship contribution statement

Joseph Barnes: Writing - review & editing, Writing - original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Barbara Balestra:** Writing - review & editing, Writing - original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Karen L. Knee:** Writing - review & editing, Writing - original draft, Methodology, Formal analysis. **J. Adam Frederick:** Writing - review & editing, Writing - original draft, Resources, Methodology. **Natalie Landaverde:** Methodology, Investigation. **Jesse Meiller:** Writing - review & editing, Writing - original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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

Images of each of the taxonomic groups as grouped into Operational Taxonomic Units (OTU). Each image is a scale of approximately 6 × 11 mm.

Brown Algae

Fig. A.1. Brown algae as a unicellular base algal covering from December 15, 2021.

Filter Feeders

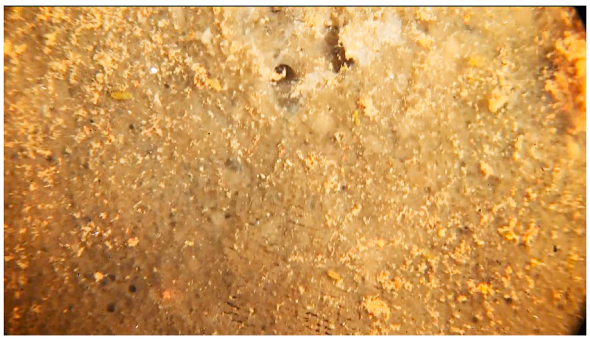

Fig. A.2. Sponge visible as a gray covering with flow holes taken on August 30, 2022.

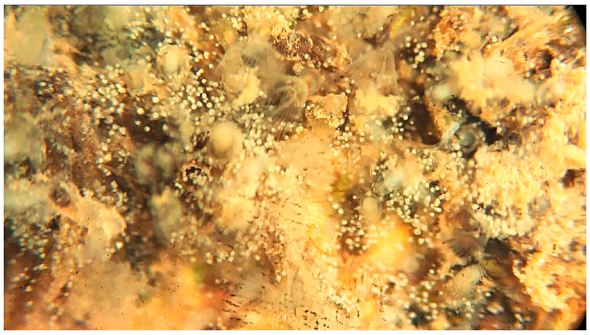

Fig. A.3. Bryozoan biofilm assemblage seen as the white wisps taken on June 6, 2022.

Algae

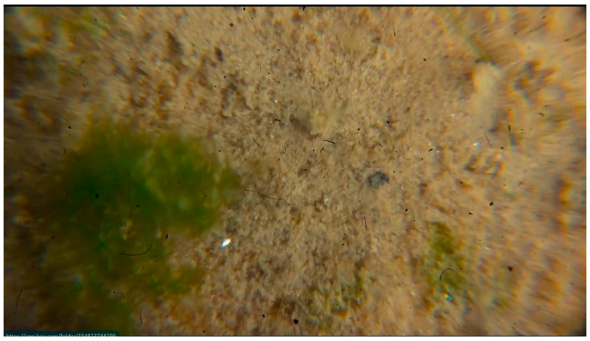

Fig. A.4. Green algae mass comprised of a spherical 3-dimensional structure from January 21, 2023.

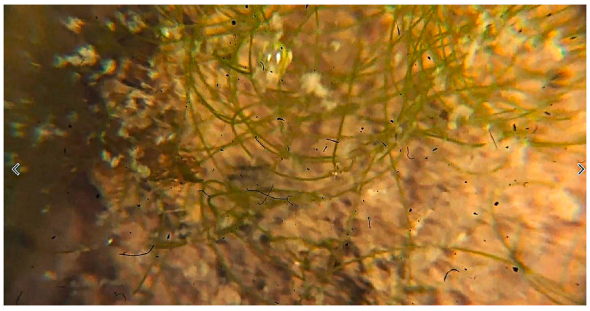

Fig. A.5. Filamentous green algae as individual strands from January 21, 2022.

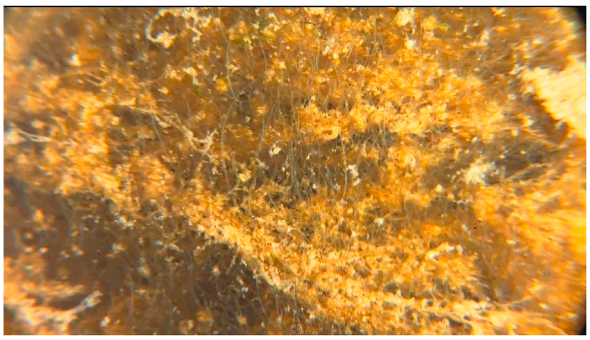

Fig. A.6. Filamentous brown algae as individual strands taken on April 15, 2022.

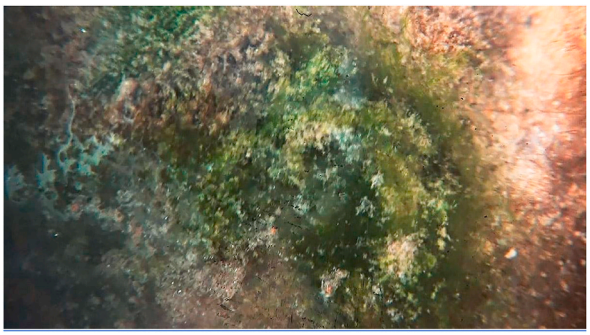

Fig. A.7. Green algae as a unicellular base algal covering taken on December 15, 2021.

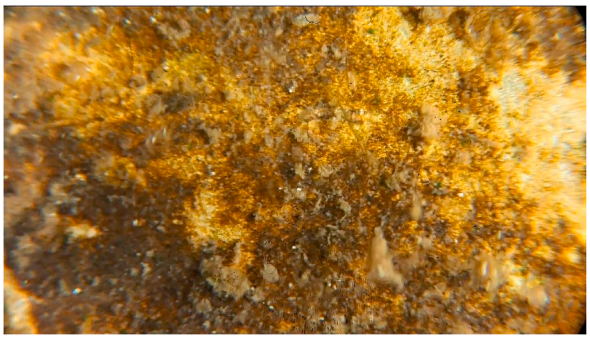

Fig. A.8. Gold algae as a base unicellular coating taken on April 15, 2022.

Motile Invertebrates

Fig. A.9. Evidence of fly larvae (burrows) from May 13, 2022.

Fig. A.10. Amphipod burrowed in the biofilm, taken on August 30, 2022.

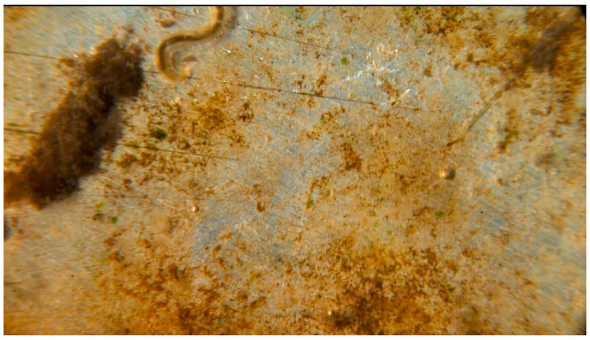

Fig. A.11. Unsegmented larvae at the top left shown as a translucent worm, taken on March 18, 2022.

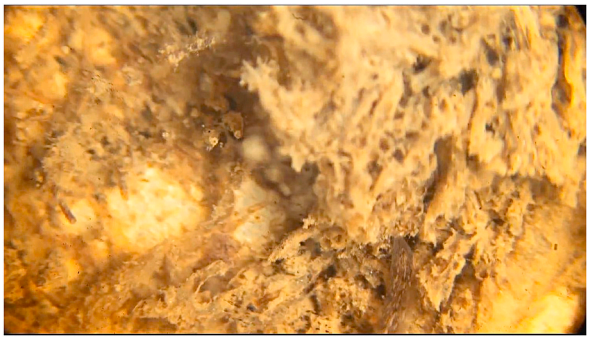

Fig. A.12. Planaria seen in the bottom right, near-translucent brown with a white patch pattern, taken on August 30, 2022
* Ectoprocts, entroprocts, cladocera, ostracod, and rotifers were incapable of having clear pictures for the appendix due to their speed, camera focus, or lighting causing blurriness.

Filter Feeder 2

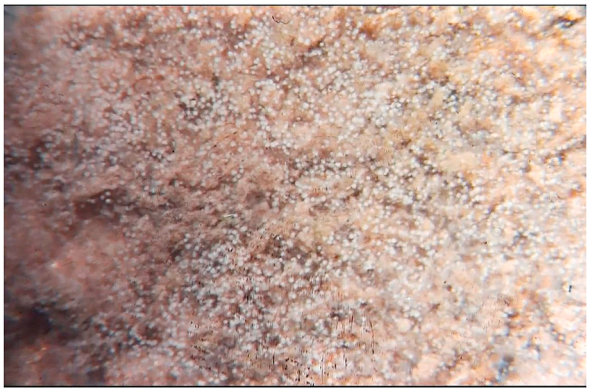

Fig. A.13. Ciliates seen as the small white dots around the photo, taken on January 21, 2022- changed to February 18, 2022.

Fig. A.14. Hydroids seen as tube-looking organisms in the middle, taken on August 30, 2022.

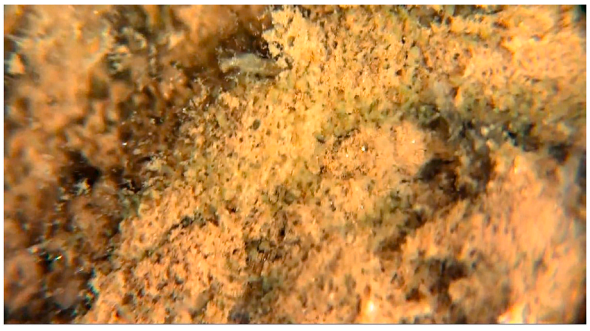

Fig. A.15. Stentors present along the left ridge as horn or bugle-shaped green-tinted organisms, taken on July 7, 2022.

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