ABSTRACT

Title of thesis: QUANTIFYING MICROPLASTICS IN WATER AND SEDIMENT ALONG RIVER-MARSH TRANSECTS IN THE CHOPTANK RIVER

Kerria Burns, Master of Science, 2025

Thesis directed by: Associate Professor William Nardin
Marine Estuarine Environmental Sciences

Plastic began as useful material with many different applications. Due to its widespread production and consumption, its utility and durability are now also contributing to its status as a major environmental and human health contaminant with a long and mostly unknown lifecycle. This thesis first quantifies microplastics in the water and sediment of the Choptank River, a major tributary of the Chesapeake Bay. Microplastics are defined as particles smaller than 5mm that are produced as precursors to larger plastic material or occur when plastic items degrade. We explore the effect of differences in microplastic concentrations due to locations of transects along and positions across the river, seasonality, and interactions with vegetation. In the water column, abundance of microplastics was higher in the marshes flanking the river than the deeper channel at all transects and in all seasons. In the sediment, abundance is higher at subtidal than intertidal sites. The second part of the thesis explores the potentially novel microbial ecosystem(s) generated by the ubiquitous presence of plastic and the possibility of altering metabolic processes, subsequently restructuring biogeochemical flows.

QUANTIFYING MICROPLASTICS IN WATER AND SEDIMENT ALONG RIVER-MARSH TRANSECTS IN THE CHOPTANK RIVER.

by

Kerria Burns

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Thesis introduction

The ingenuity of humanity has led to many revelatory solutions to problems which profoundly improved quality of life and furthered capacity for discovery by removing obstacles and alleviating hardship. One such invention was plastic, the first iteration of which was Bakelite, invented in 1907 by Leo Baekeland (Mercelis 2012). Since then, plastic production has exploded and infiltrated every corner of human life.

Unfortunately, solutions to one problem often involuntarily create problematic by-products. In the case of plastic, the characteristics that make it durable, useful, and convenient, are the same as those which are now creating a global environmental disaster due to its escape from waste management pipelines and subsequent contamination of even the most pristine areas on Earth, including polar ecosystems and the abyssal zone of the ocean (Bergmann et al. 2022; Zhu et al. 2024).

The lifecycle of plastic including its degradation timeline (not to mention into what it degrades) are still in the early stages of investigation (Zhang et al. 2021). Popular media sources present possibilities including “centuries” to “never” (depending on the plastic type and product), but since this time has not yet come to pass, these presentations remain largely guesswork. These timetables are useful though in that they do highlight the recalcitrance of plastic. Unfortunately, even despite these disconcerting predictions, plastic production continues to increase, with no signs of slowing down (Walker & Fequet 2023).

There are many relevant research fields to understanding plastic, all of which address different angles. This leads to some unfortunate disconnects even among studies addressing the same goal, for example, quantifying the presence of plastic in a particular aquatic ecosystem; there exist multiple factors which complicate the formation of consensus or data comparability

(Lusher & Primpke 2023). One such complication is the methodology with which samples are collected and processed (Vered & Shenkar 2021). Though there is a fairly high level of agreement on certain steps, such as density separation, digestion of organic matter in samples, and staining with fluorescent dye, there are many different reagents used for these steps depending on the study (Dong et al. 2023; Aung et al. 2022). The disagreement among researchers about even this baseline goal of monitoring and quantifying (Bank et al. 2021) becomes even more pronounced when considering the many and varied topics related to plastic in the environment. There are issues surrounding movement through the environment (often termed transport and fate) (Wang et al. 2021), interaction with organisms throughout the food web (Tuuri & Leterme 2023), changes to structural integrity and mechanical characteristics (Grause et al. 2020), degradation mechanisms (Singh & Sharma 2008), and overall effects on the environment (of which there are a multitude of different compartments) (Bucci et al. 2019).

Despite these difficulties, it is imperative that the distribution and effects of plastic pollution are understood in order to begin categorizing its contamination footprint. The National Oceanic and Atmospheric Administration (National Centers for Environmental Information) has published an online interactive map of plastic studies all across the world which details sampling method, environmental compartment, units for detection, relevant dates, and publication references. Notably, in the Chesapeake Bay and its tributaries, there are only about 37 published study sites, many of which belong to one study (Barrows et al. 2018), and most of which took place near urban or cosmopolitan areas, mainly on the western shore of the Bay.

This thesis has two major goals. The first is a data-based chapter exploring the nature of microplastic pollution in the Choptank River, a major tributary of the Chesapeake Bay. This represents the only study taking place on the eastern shore of the Bay, and one of only a handful

exploring this issue in rural surroundings. Here we define microplastic existing in a size range from 63 microns (operationally defined based on our collection apparatus mesh size) to 5 mm (the commonly accepted upper cutoff value) and examine its presence in the water column and sediments at three transects along the Choptank River and among the west marsh, channel, and east marsh. We also explore seasonal variations and relations with vegetation. These data provide the means to generate a microplastic "budget" which will inform parameters of a model that can be used to simulate movement of this pollutant and ultimately further understanding of its transport and fate. This baseline information is a first step that will allow more targeted sampling to better resolve distribution patterns and mechanisms such as that discovered by Tasseron et al. (2024).

This information will contribute to understanding of microplastic pollution using the Choptank River as a case study of an estuarine tributary (an understudied environment) with rural surroundings and in different seasons. Knowledge of the watershed is important when comparing pollution signatures in different land use scenarios where the types and sources of plastic may be unique, as well as how degradation and movement unfold. Differences could be due to both hydrological differences based on river bathymetry and geometry, distinct meteorological patterns, and anthropogenic activities and behaviors that vary between agricultural or urban centers, for example.

The second chapter explores the biological consequences of microplastic pollution, first by performing a review and extracting taxonomic information reported in the literature from studies carried out in many different geographic locations, habitats, and environmental compartments. It considers the possibility of plastic as an evolutionary force with the potential to restructure microbial communities on a global scale and subsequently alter their collective

metabolism with implications for climate and major biogeochemical cycles. Complementing the idea of "planetary boundaries" first proposed by Rockström et al. (2009), it explores the potential for alteration of biological trajectories caused by widespread plastic contamination with a spectrum of size categories and chemical properties.

One of the original proposed planetary boundaries within which the Earth must exist for humanity to be sheltered from catastrophe is biodiversity loss (Steffen et al. 2015), which can be categorized as genetic or functional. This thesis seeks to extract reported taxonomic (which is a proxy for genetic diversity) information and extrapolate to ostensible functional significance and thereby inform this potentially more informative component of diversity. Taxonomic categorizations are constantly changing as new information comes to light which is occurring rapidly in the age of massive datasets generated by the 'omics revolution (Mathé et al. 2018). This thesis provides the starting point on a line of thinking about biological categories at their essence and how microorganisms, the mediators between the non-living environment and the rest of the food web, are classified and understood. For example, understanding the relationships between organisms based on their geologic and evolutionary history or functional and genomic characteristics represent different lenses through which to view biological diversity. The goal is to gain a deeper understanding of the configuration of connectivity between different forms of life and how those connections change over time and with mounting, rapid alterations due to human activity; for example, how will the structure of food webs in different ecosystems (e.g., salt marshes, freshwater lakes, or the open ocean) be altered as biodiversity is increasingly threatened by human modification of natural landscapes.

Chapter 1 - Quantifying microplastics in water and sediment along river-marsh transects in the Choptank River

Introduction

The unchecked production and manufacture of plastic concomitant with poor waste management practices in place to properly regulate these substances have led to a global environmental pollution crisis. Only a small percentage of plastic types are recyclable and other disposal methods such as incineration or relegation to a landfill generate air and water pollution in a variety of forms. Before the properties of plastic including its degradation products and environmental lifespan were even superficially understood, it was and continues to be mass produced and society relies on these substances for everything from medical devices to single use beverage or food containers. Its accumulation in every environment on Earth far outpaces our understanding of the implications of this contamination. Different scientific disciplines from toxicology to material science are approaching the issue and attempting to gain a handle on the scope and nature of the problems generated by these novel substances. To begin addressing this grand challenge, there must be an increase in monitoring efforts to understand baseline abundances and distributions of this pollutant in different environments (Kukkola et al. 2022).

Understanding the transport and fate of microplastics may yield a better understanding of how this suite of pollutants moves through and affects the environment. Plastic is exposed to different environmental forces as it is altered chemically and physically including disintegration into smaller components. Monitoring a diversity of environments for microplastic presence and abundance through time, will strengthen our knowledge of where plastic tends to accumulate. In addition, the characterization of attributes such as size and shape (Li et al. 2022) will provide greater detail into how different types of plastic travel from source to sink. The sources of plastic likely to contribute to detection in a given sampling effort should also be considered, whether

proximate or much farther away. It is likely that size and shape tend to influence how far a given particle is likely to travel, for example with fibers (as compared to fragments or particles) moving farther away from their source (Engdahl 2018; Lloret et al. 2021).

There has been a recent shift in the literature from focusing largely on the ocean to include terrestrial and freshwater ecosystems (Rochman et al. 2018). Estuaries are a unique environment in which to study microplastics because they may receive input from the land via precipitation or runoff, atmospheric deposition, and the ocean by way of tidal cycles. One of the few studies to examine plastics in the Chesapeake Bay, Bikker et al. (2020) quantified microplastic pollution in the surface layer of the Chesapeake Bay throughout the watershed, identifying the proportions of different morphologies and plastic types. In contrast with other publications (Wang et al. 2020; Yonkos et al. 2014), this study was unable to correlate population density and land use with microplastic abundance due to complicating hydrological processes within the Bay. In addition, though they recognized wastewater treatment plants (WWTPs) as an important plastic source, the multitudinous WWTPs in the Chesapeake Bay watershed prevented identifying specific land origins of pollution found in the water. However, as expected, increased microplastic presence was related to heavily populated urban areas.

While other studies have quantified microplastics in estuaries (e.g., Zhao et al. 2015; Bakir et al. 2014; Ragoobur et al. 2023), data on microplastic abundance on the eastern shore of the Chesapeake Bay is currently limited. Our study is novel in that it samples across three marsh-river transects along the Choptank River situated within rural surroundings. We explore multiple variables including seasonality, river position (west marsh, channel, and east marsh), and transect location using two data sets: the water column and sediment. It is important to understand both environmental compartments because each is affected differently by meteorological and

hydrological conditions; the water column is likely more influenced by transient conditions or events, while the sediment probably reflects better established patterns of river bathymetry and tidal patterns. In addition, sites were selected due to the presence of intertidal marshes flanking the transects to examine how the presence of vegetation, subtidal and intertidal sites, and location at the river edge and further inland affected microplastic abundance. Salt marshes in general represent an understudied habitat in relation to microplastics research but could potentially act as a sink due to the stabilizing effect and buffering capacity of vegetation on sediments and abiotic conditions present in the marsh (Lloret et al. 2021; Ogbuagu et al. 2022; Stead et al. 2020).

This study investigates the abundance and distribution of microplastics in water and sediment along river-marsh transects in a major tributary of the Chesapeake Bay, the Choptank River. Our study site is important in that rivers are the conduit of microplastics from land-based sources to their accumulation in the ocean, receive plastic from the ocean through tidal cycles, and also represent an understudied environment in microplastics research (Rochman et al. 2018). A data map published by the NOAA National Centers for Environmental Information shows multiple data points in the Chesapeake Bay are from a single study from Barrows et al. (2018), highlighting the need for further study in this area. Though there are other studies taking place in the Chesapeake Bay (e.g., Yonkos et al. 2014; Bikker et al. 2020), our study sites in the Choptank River provide a targeted, higher resolution sampling effort in different seasons in a rural area on the western shore of the Chesapeake Bay. In contrast, Barrows et al. (2018) generated a global dataset to understand large-scale plastic pollution patterns such as higher concentrations of microplastics in the open ocean than in coastal areas.

The forces responsible for the distribution of microplastics in water and sediment of a tributary of the Chesapeake Bay (and estuaries in general) include tidal forces, wind mixing,

turbulence, salinity gradients, location and intensity of the estuarine turbidity maximum (ETM), river bathymetry, precipitation, burrowing organisms, and surrounding land use (Malli et al. 2022). This complex interaction of factors results in observed microplastic abundance and distribution and difficulty isolating a single variable responsible for perceived patterns. In addition, there are multiple sources of microplastic that determine the characteristics of these particles and subsequently their distribution in the environment. For example, some studies report the most prevalent morphology to be fibers, but others, especially in estuarine systems, report fragments and films to be most abundant (Yonkos et al. 2014; Bikker et al. 2020). This variability is likely due to differences in sources of plastic waste and the environment into which microplastics are generated and leached. Sources include derelict fishing gear, tires, wastewater effluent (including fibers generated from washing synthetic clothing), shopping bags, food and beverage containers, agricultural films, or paint, among others (An et al. 2020). For this reason, the surrounding land use in the estuarine or riverine watershed is an important factor when characterizing microplastic pollution in these environments. Kunz et al. (2023) reports a strong positive correlation between urbanization and microplastic concentration, a negative correlation between forest cover and microplastic concentration, and further, a sharp increase in microplastic abundance at the boundary between urban and rural land use surroundings in the Wu River in Taichung, Taiwan. In contrast, Yin et al. (2020) found a greater abundance of microplastic fibers in sediments of East Dongting Lake in rural versus urban surroundings. Rochman et al. (2022) sampled across various locations in North America including the Chesapeake Bay finding microplastics abundances per liter of water at similar levels to our study (generally less than 1 item per liter). In contrast, Yonkos et al. found significantly lower concentrations of plastic per square kilometer than our study (Table 13). This is likely due to differences in sampling, notably

that they sampled only the top 15 cm of the water column and had a significantly higher lower size limit of 0.33 mm as compared to our lower size limit of 63 microns. This could lead to the observed difference between our detected microplastic concentrations.

Our study lays the groundwork for plastic research in this area and demonstrates the need for future work to further discern the preliminary findings presented here. Our study quantified microplastics in water and sediment along three river-marsh transects in the Choptank River. We also explored differences in sediment microplastic content at subtidal and intertidal sites, along with at the river edge and a few meters inland. We sampled for vegetation characteristics at all three transects at the river edge and a few meters inland to relate the presence of microplastics. We hypothesized there would be lower concentrations of microplastics at intertidal versus subtidal sites, and that microplastic concentrations would be positively correlated with vegetation density. We also hypothesized that there would be higher microplastic concentration and higher proportion of fibers at the middle transect due to its proximity downstream of the WWTP.

Materials and Methods

Sample Sites

Three transects in the Choptank River, a major tributary of the Chesapeake Bay, U.S.A, were selected for sampling (Figure 1).

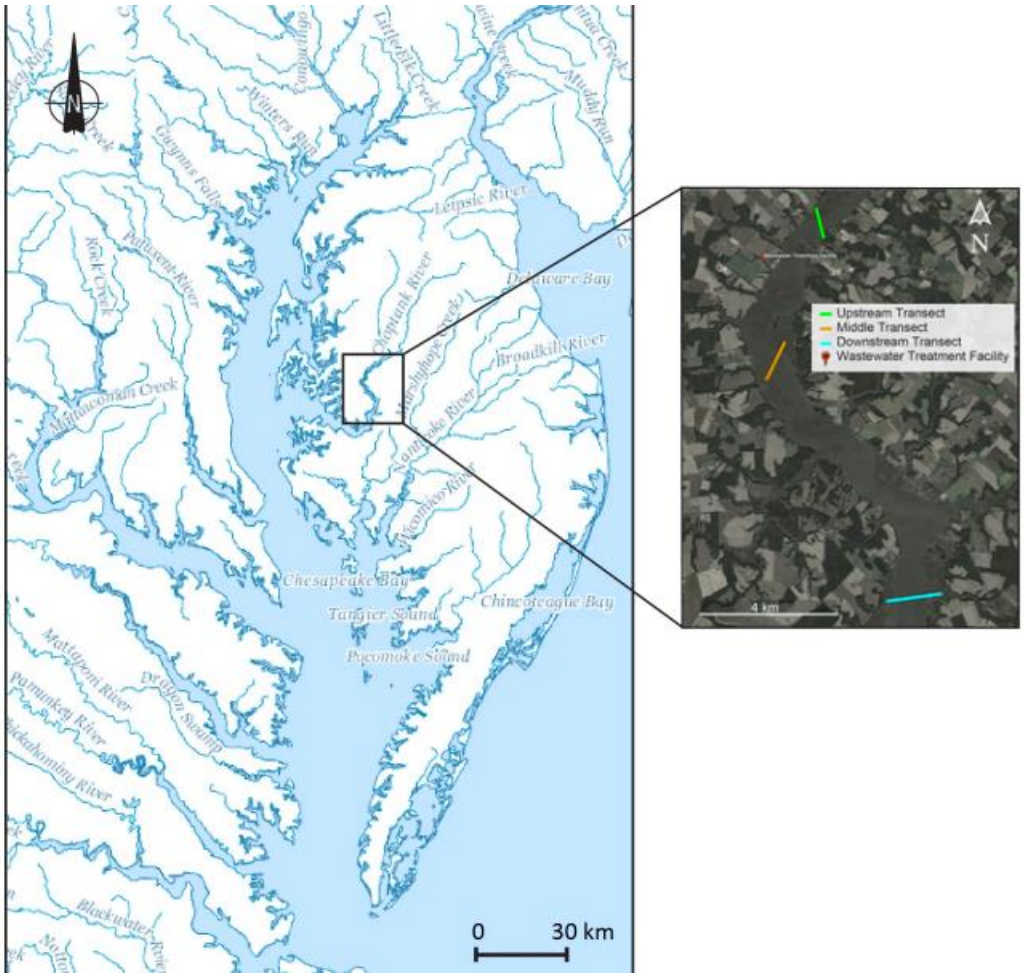

Figure 1. Locations of riverine transect sampling sites within the Choptank River (Chesapeake Bay, U.S.A) sampled for microplastics between May 2022 and July 2023.

The Choptank River (so named after the area's indigenous inhabitants) is a major tributary of the Chesapeake Bay with a length of 114 km and is situated within a watershed spanning 1,756 km^2.

The water column and sediments were sampled for quantification of microplastics. Sampling transects were chosen such that the up river transect is located upstream of a wastewater treatment plant, which is situated on the western shore of the river. All of the transects were chosen so that there were intertidal marshes on both the eastern and western

boundaries. Three stations were identified for each transect, West, Channel, and East, all of which were sampled to collect microplastics from both the water and the sediment. Samples were collected twice in spring, summer, and fall, and once in winter over a period of two years (Table 1).

Table 1. Samples were collected at least twice during spring, summer, and fall, and once in winter, spanning a period of two years.

Date	Season	Year
18-May	Spring	2022
2-Jun	Summer	2022
14-Sep	Fall	2022
21-Oct	Fall	2022
6-Nov	Fall	2022
2-Mar	Winter	2023
31-May	Spring	2023
27-Jun	Summer	2023
31-Jul	Summer	2023

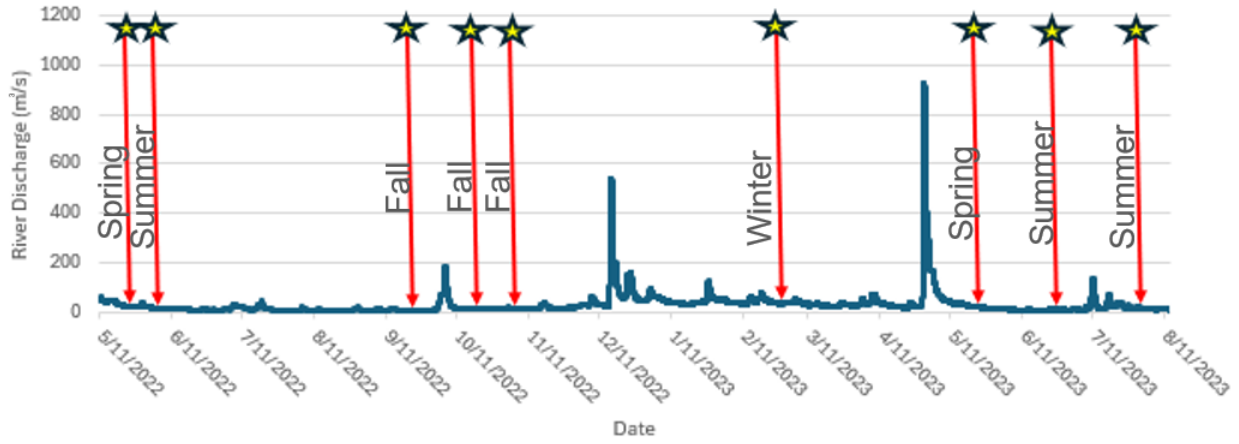

Figure 2. River discharge values around sampling times provided by USGS at site 01491000, the Choptank River near Greensboro, MD. Stars and red lines demarcate sampling efforts, indicating seasonal and river discharge context.

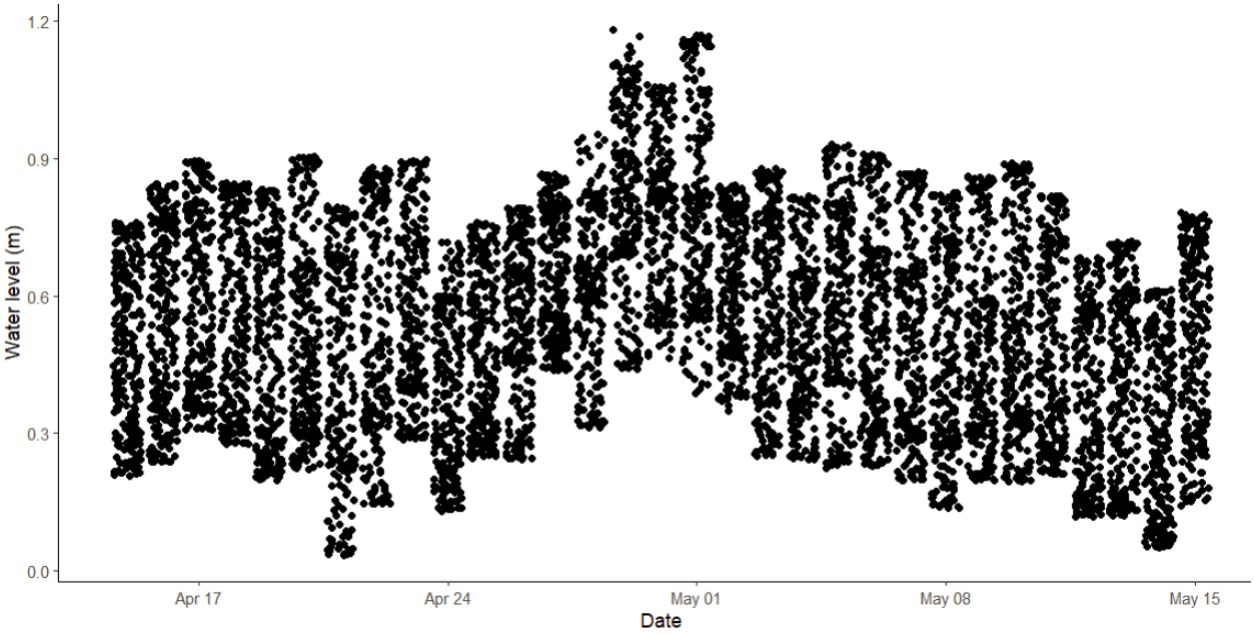

Figure 3. Water level around high discharge event 5/1/23.

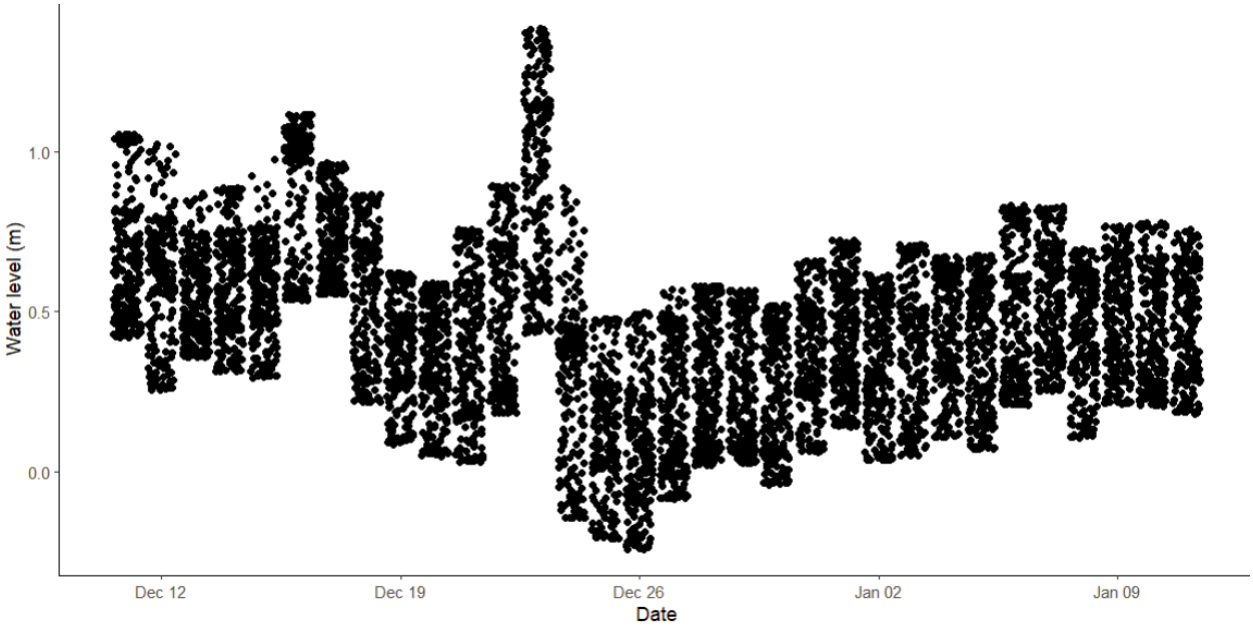

Figure 4. Water level around high discharge event 12/21/23.

Table 2. Characteristics of the USGS station on the Choptank River in Greensboro, MD.

Element	Location
Agency	U.S. Geological Survey
Site identification number	1491000
Site name	Choptank River near Greensboro, MD
Site type	Stream
Decimal latitude	38.99719444
Decimal longitude	-75.7858056
District	Maryland
County	Caroline
Country	U.S.
Altitude of gage/land surface	0.83 m
Method altitude determined	Interpolated from Digital Elevation Model
Altitude accuracy	0.03 m
Drainage area	292.67 square kilometers

Table 3. Seasonally averaged water temperatures during sample collection.

Season	N	Temperature °C (mean ± SD)
Fall	75	19.5±4.44
Spring	47	20.57±0.55
Summer	72	26.76±1.70
Winter	26	9.07±0.26

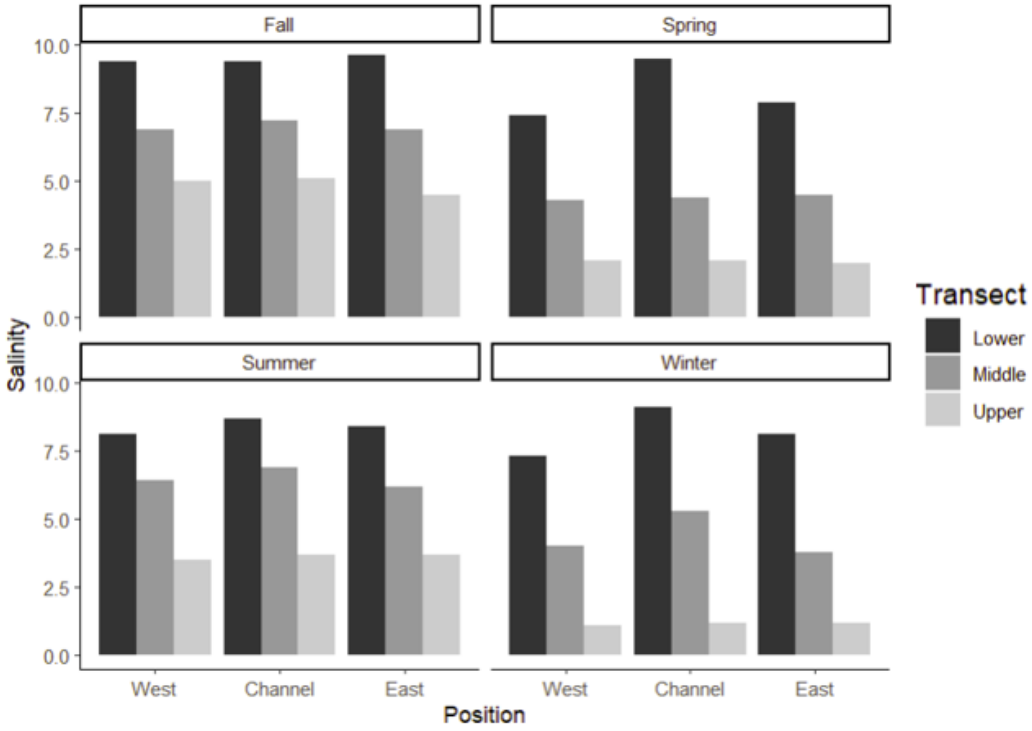

Figure 5. Salinity at each sampling site.

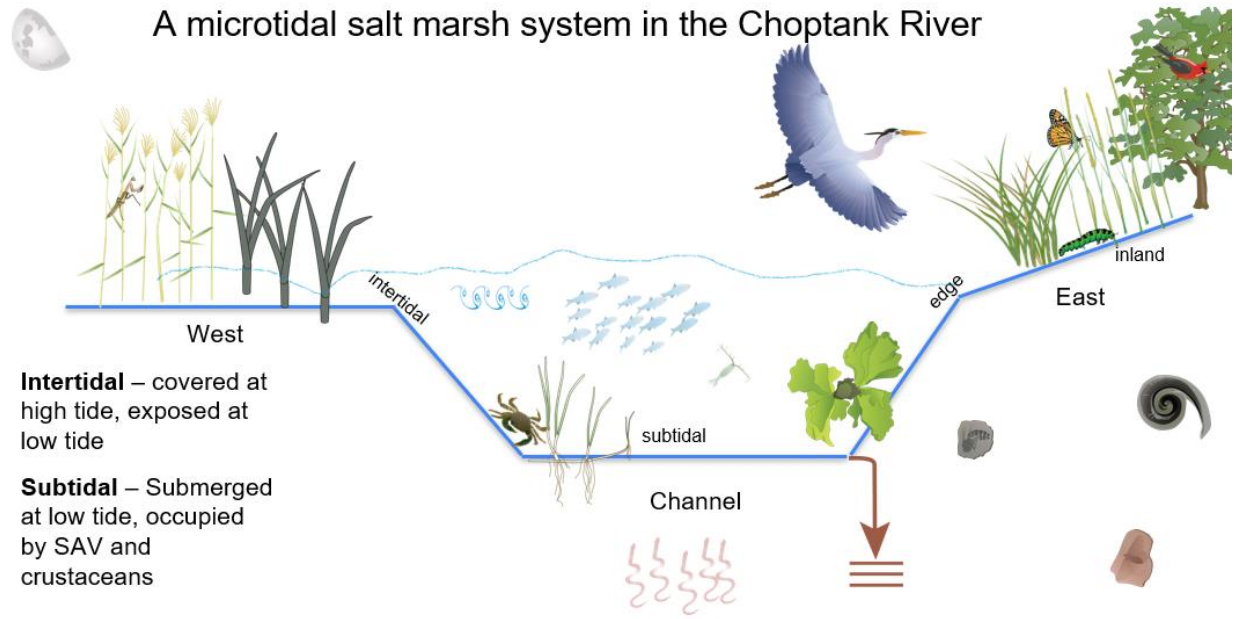

Figure 6. Schematic of our study system.

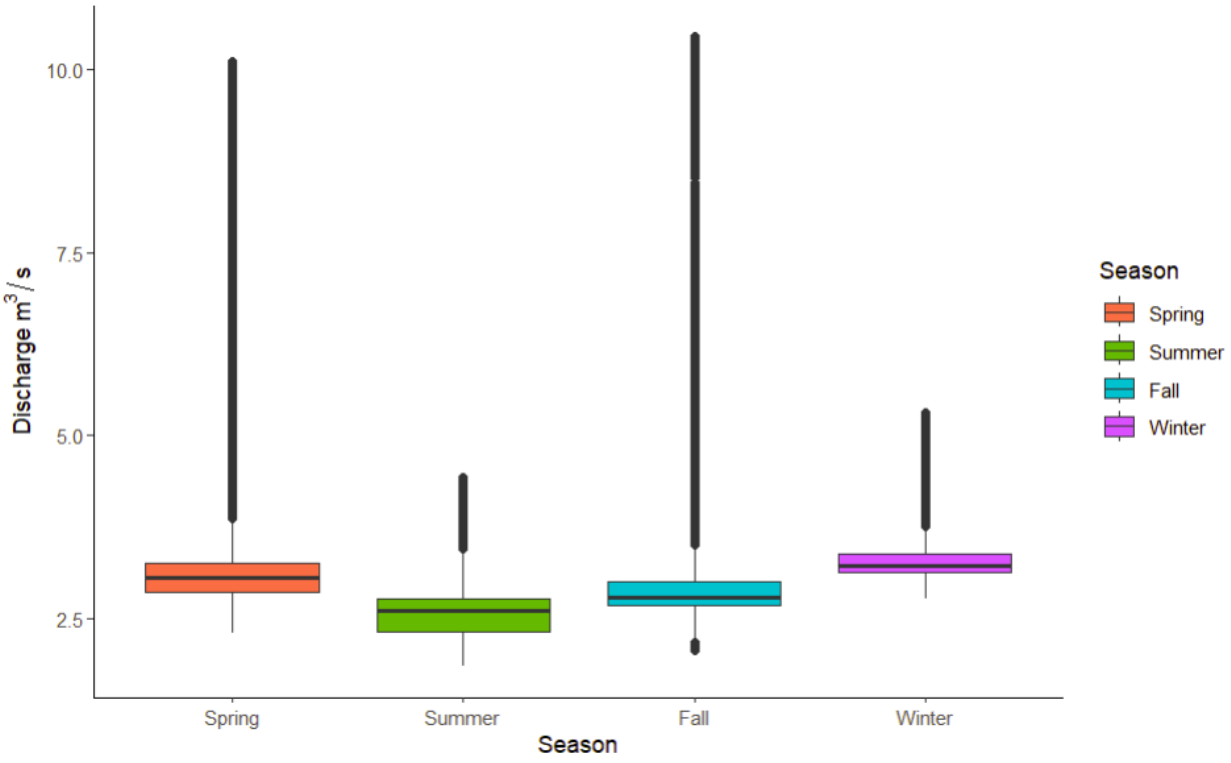

Figure 7. Seasonal flow patterns reported in 15 minute increments for the 2 sampling years.

Sample Collection, Processing, and Quantification

Sample Collection

Water microplastic samples were collected by vertically deploying a 0.5m diameter plankton net fitted with 63µm nylon mesh from the side of a 21 ft. skiff. The mesh size determined the lower size limit for sampling microplastics. Water samples were collected at 0.5 m in either marsh and at variable depth in the channel depending on the maximum depth so as to sample the entire water column. The net was retrieved and water collected in a cod end; the net was rinsed with a hand-pump powered spray bottle into the cod end to maximize material collected. The cod end was then rinsed into a glass jar using the spray bottle until a visual check determined it was clean of debris. Field blanks were collected to quantify microplastic contamination during sample collection on the boat. The spray bottle, plankton net, and cod end were all rinsed using filtered river water into a collection jar to determine the level of contamination from each of these steps in the collection process. Sediment samples were collected with a van Veen grab sampler.

Laboratory Processing

Laboratory protocols were modified from the literature (e.g., Yonkos et al. 2014, Prata et al. 2019) and included density separation using hypersaline solution (1.3 g/mL), digestion of organic matter using 30% hydrogen peroxide, vacuum filtration onto a GF/F, and staining with Nile Red.

Water samples were poured through a 63 micron sieve and rinsed several times until the collection jar was clear of debris. There was a change midway through sample processing. At first, for the digestion step, equal parts DI water and 30% hydrogen peroxide were incubated with the sample which was allowed to digest overnight. Then, a density separation step with

hypersaline solution was performed and allowed to settle out for 24 hours, which was followed by vacuum filtration. Due to high organic matter content in some samples, multiple filters were used to reduce the thickness of debris on each filter. Following revision of methods, the remaining water samples were incubated with full strength 30% hydrogen peroxide for three days, after which they were vacuum filtered onto a GF/F. A test was performed to compare the difference in microplastic quantification between the two methods, and statistical analysis revealed no significant differences, increasing confidence that there was no systematic bias due to the change in processing.

Sediment samples were analyzed for the concentration of microplastics by weight of sediment. In some cases, there was insufficient sample and thus the whole sample was used. The general steps were a density separation followed by an organic matter digestion. Samples were homogenized and added to 1 L flasks. For the density separation, salt water solution was generated using 1 L of ultrapure, tripled filtered, UV sterilized, reverse osmosis (RO) water to which 300 g of table salt was added and stirred with a plastic stir bar until dissolved. This solution was then vacuum filtered through a 0.7µm glass fiber filter (GF/F) to remove contamination. 250 mL of this filtered salt solution was added to each flask and agitated for 30 seconds. After allowing the solution to settle for 24 hours, supernatant was removed using a pipette and stored in a covered beaker. 250 mL of fresh salt water solution was added to the sample, stirred, and allowed to settle for 24 hours, after which the second portion of supernatant was removed with a pipette. For the organic matter digestion, both portions were poured through a 63 micron sieve, the contents of which were then rinsed with 30% hydrogen peroxide into a beaker and allowed to digest for 24 hours. The samples were then vacuum filtered onto a glass fiber filter and stained with 0.5 mL of Nile Red at a concentration of 10 µg / mL and stored in the

dark until photographed. There may have been a higher concentration of Nile Red used on some samples. Six samples were processed per week.

Laboratory blanks were generated to quantify contamination by performing every step in the processing workflow without the addition of actual sample. Non-plastic materials were used when possible. All materials were rinsed with tap and DI water before and after use, and all glassware was cleaned with soap and water, and rinsed with DI water.

Photography

A Canon T3i was outfitted with a Canon EF-S 18-55mm f/3 5-5.6 II SLR lens and a Tiffen Orange 21 filter and blue ring light with a diameter of 60mm and wavelength of 460. Vertical position of the camera was situated such that the entire filter could be photographed in focus (not blurry) and was adapted from Bachiller and Fernandes (2011). The camera was connected to a laptop and image area was viewed in real time. The blue light was turned on at full intensity, room lights were turned off and the door was closed to darken the surroundings, after which the image was taken.

Table 4. Densities of commonly used plastics.

Plastic	Density (g/mL)
PP	0.90-0.91
LDPE	0.92-0.94
HDPE	0.95-0.96
PS	0.02-1.07
PVC	1.16-1.45
PETE	1.38-1.39

Image Processing

The CR2 image files were converted to TIFF format using Photoshop to be analyzable by ImageJ.

Quantification

The TIFF images was opened in ImageJ. MP-VAT and MP-SCALE plugins were downloaded from: Microplastics Visual Analysis Tool (MP-VAT) – A script for simple microplastic analysis. (wordpress.com). MP-SCALE calibrates the image size as a reference to enable size classification of particles. The diameter of the filtration apparatus was 38.5 mm and when visible, the area on which the sample is was selected using the oval tool and set to this size. Otherwise, the entire filter area was selected and set to 47 mm. Following that, the MP-VAT script was run to quantify microplastic particles on the filter, which yields a file containing the number and characteristics of particles detected.

Vegetation Measurement

Vegetation was measured using quadrat within which stem diameter, stem height, and number of stems were quantified. Measurements were taken at the river edge and about two meters inland.

Statistical Analyses

Outliers were identified using the interquartile range method and removed from further analysis due to the likelihood that they are artifacts. 11 samples were removed from the sediment dataset and four samples were removed from the water dataset. Statistical analyses were performed in R and Excel.

Table 5. Outliers removed from the sediment dataset.

Position	Transect	Season	Sample Date	Depth	Plastic/100g
West	Lower	Fall	9/15/2022	subtidal	10.41
West	Lower	Summer	6/27/2023	0.5	24.24
West	Lower	Spring	5/18/2022	inland	42.65
West	Middle	Summer	6/27/2023	0.5	7.21
East	Lower	Fall	10/21/2022	N/A	23.24
East	Lower	Spring	5/18/2022	inland	123.43
East	Middle	Summer	6/2/2022	N/A	5.39
East	Middle	Fall	9/15/2022	intertidal	10.08
East	Middle	Fall	10/3/2023	intertidal	16.3
Channel	Middle	Fall	9/15/2022	N/A	9.58
Channel	Upper	Fall	11/6/2022	5	86.11
N/A	Middle	Winter	3/2/2023	intertidal	17.39

Table 6. Outliers removed from the water dataset.

Position	Transect	Season	Sample Date	Depth	Plastic/L
East	Lower	Summer	6/2/2022	0.5	15.33
East	Upper	Winter	3/2/2023	0.5	17.31
West	Lower	Summer	6/2/2022	0.5	10.76
West	Upper	Fall	11/6/2022	0.5	7.6

Results

Blanks and Recovery

The blank experiments whereby no environmental matrix is analyzed but where laboratory or field procedures are carried out with only the solutions used to treat the samples (i.e. hydrogen peroxide and salt water) show a reasonably low abundance of fluorescent particles, which is reassuring for two reasons. The first is that our laboratory protocols generally avoid significant levels of contamination, and also the MP-VAT automated quantification script performs reasonably well and does not appear to significantly overestimate microplastic on the

basis of fluorescence. Blanks were run on the plankton net and spray bottle in the field, and on the laboratory processing steps in the laboratory.

Unfortunately, the second blank run on the sediment processing procedure yielded 83 particles, which is a troubling level of contamination. One source could be the pipette used to remove the NaCl supernatant for a subset of samples during the density separation step, which was plastic.

Table 7. Recovery for laboratory water sample processing.

Spiking #	Recovered	Type
48	42	Beads
25	24	PVC fragments
21	14	Film fragments

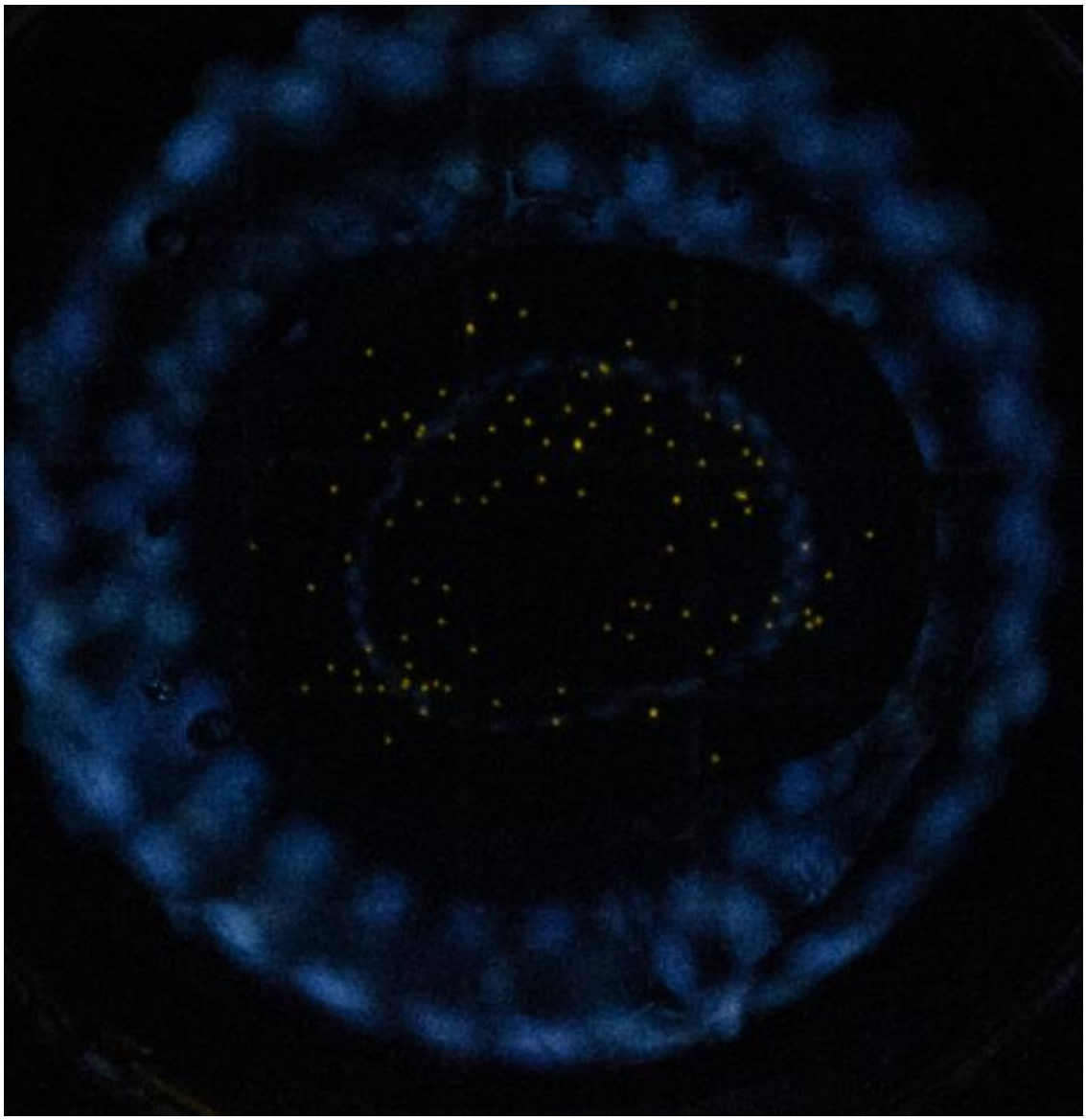

Figure 8. Spiked sample (92 beads) for determination of sediment processing recovery efficiency. Beads are 93 μm.

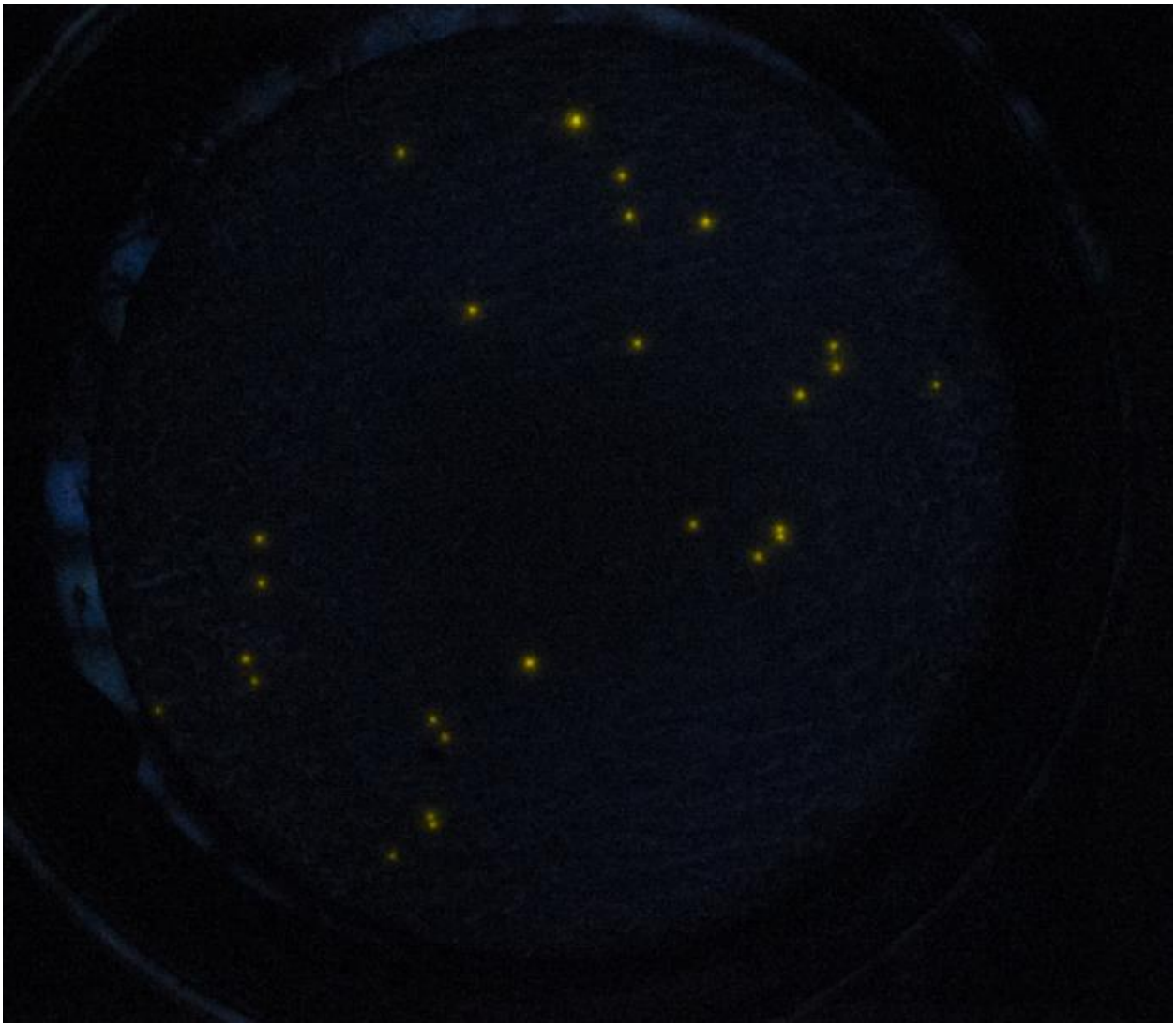

Figure 9. Recovered spiked beads - 26.

Table 8. Field and laboratory blanks quantifying contamination at multiple steps. Blanks were performed using all materials and procedures in the absence of environmental sample.

Date	Type	Microplastic Count
12/23/2024	Sediment Lab	84
10/11/2024	Sediment Lab	13
2/11/2025	Sediment Lab	4
6/7/2024	Water Lab	27
6/11/2024	Water Lab	6
6/27/2024	Water Lab	1
7/8/2024	Water Lab	10
7/15/2024	Water Lab	1
6/27/2023	Net Blank	63
5/31/2023	Spray bottle	4
6/27/2023	Spray bottle	8

Water Samples

Plastic concentrations are highest in the marshes flanking the river. There does not appear to be an effect of season on this pattern, though we may want to include data such as precipitation events or tidal cycles to determine if these processes generate significant variation in plastic concentrations in any area of the river or marsh. Though not significant, the middle transect has a lower mean abundance than either the upper or lower.

Contrary to most studies that found fibers to be the most abundant morphological type, we found fragments and particles to be generally more abundant. This could be due to the classification algorithm encoded in the MP-VAT software.

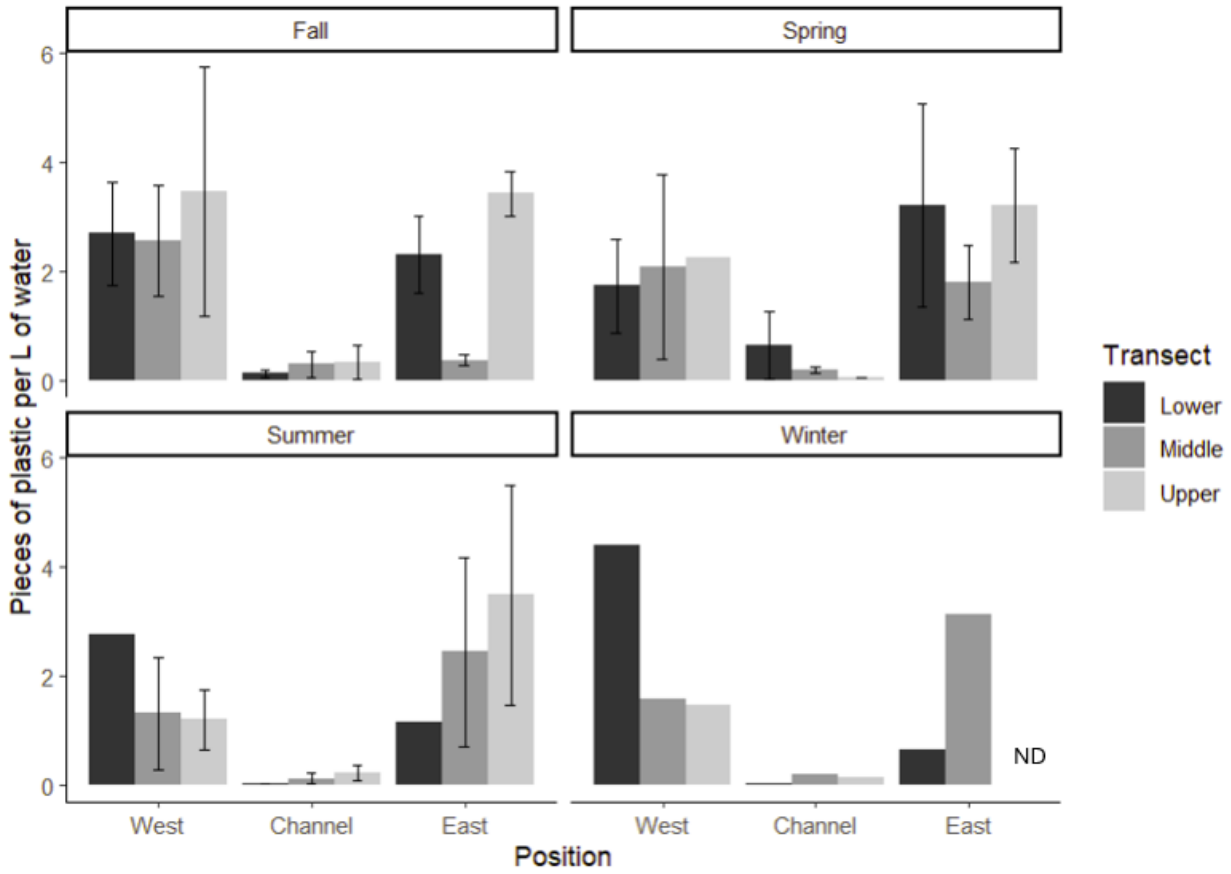

Figure 10. Microplastic abundance in the Choptank River water column summarized by season, position, and transect. Bar height is the mean of replicates and standard error is reported where n > 1. P-values for season, position, and transect, respectively - 0.4975, 9.131e-09, and 0.7962.

Table 9. Summary statistics of water samples.

Transect	Position	Reps	Pieces/L (mean ± SD)
Lower	West	7	2.67±1.38
	Channel	9	0.212±0.41
	East	6	2.14±1.64
Middle	West	8	2.01±1.51
	Channel	9	0.19±0.18
	East	8	1.59±1.47
Upper	West	6	2.17±1.82
	Channel	7	0.21±0.23
	East	6	3.37±1.46

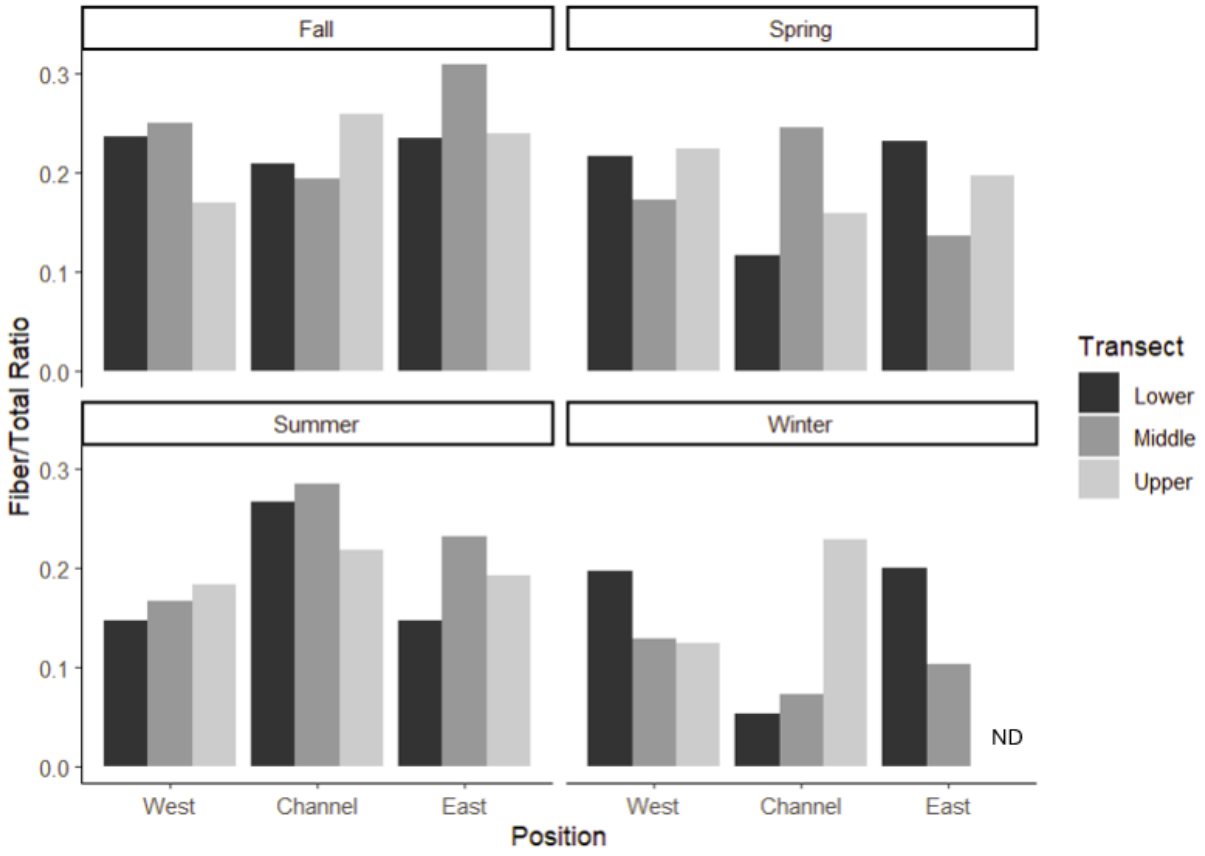

Figure 11. Fiber to total plastic ratio in the water column. The ratio appears lower during winter at the lower and middle transects in the river channel. P-values for season, position, and transect, respectively - 0.5559, 0.7233, 0.7219.

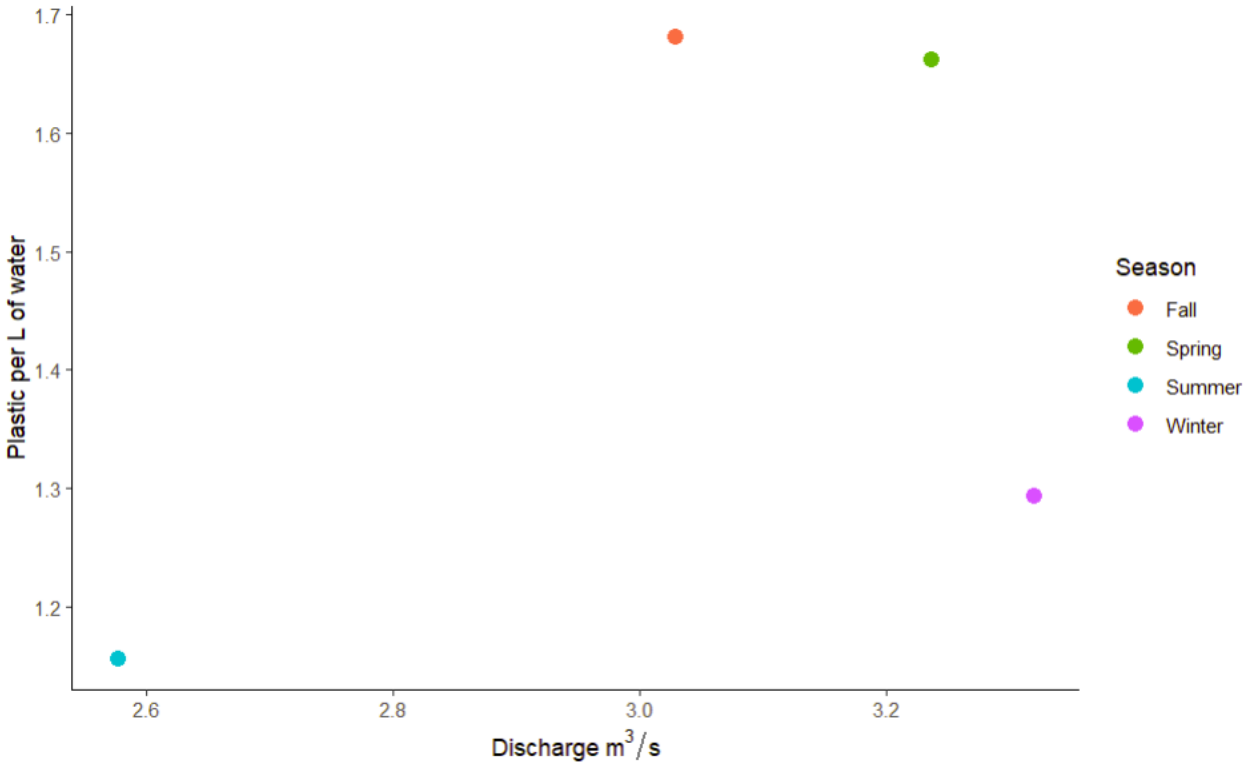

Figure 12. Relationship between plastic abundance in the water column and seasonally averaged river discharge.

Sediment Samples

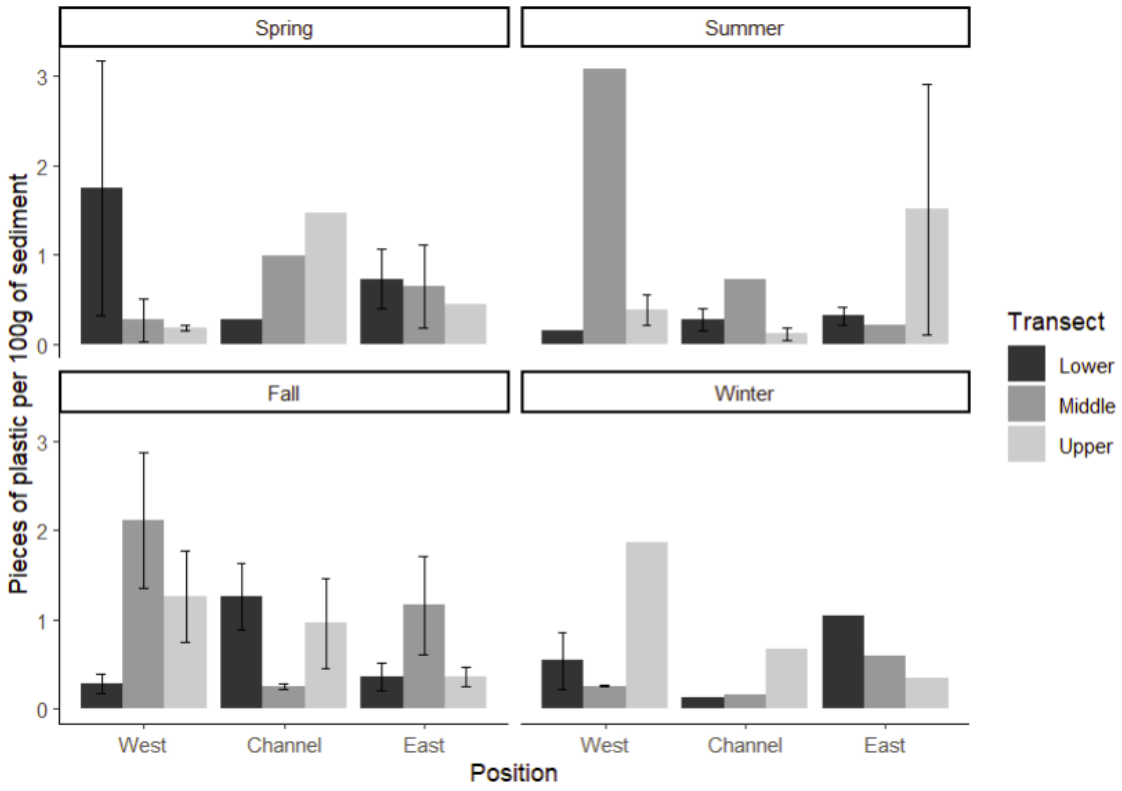

Figure 13. Microplastic abundance in the Choptank River sediment summarized by season, position, and transect. Bar height is the mean of replicates and standard error is reported where n > 1. P-values for season, position, and transect, respectively: 0.6126, 0.9702, and 0.5847.

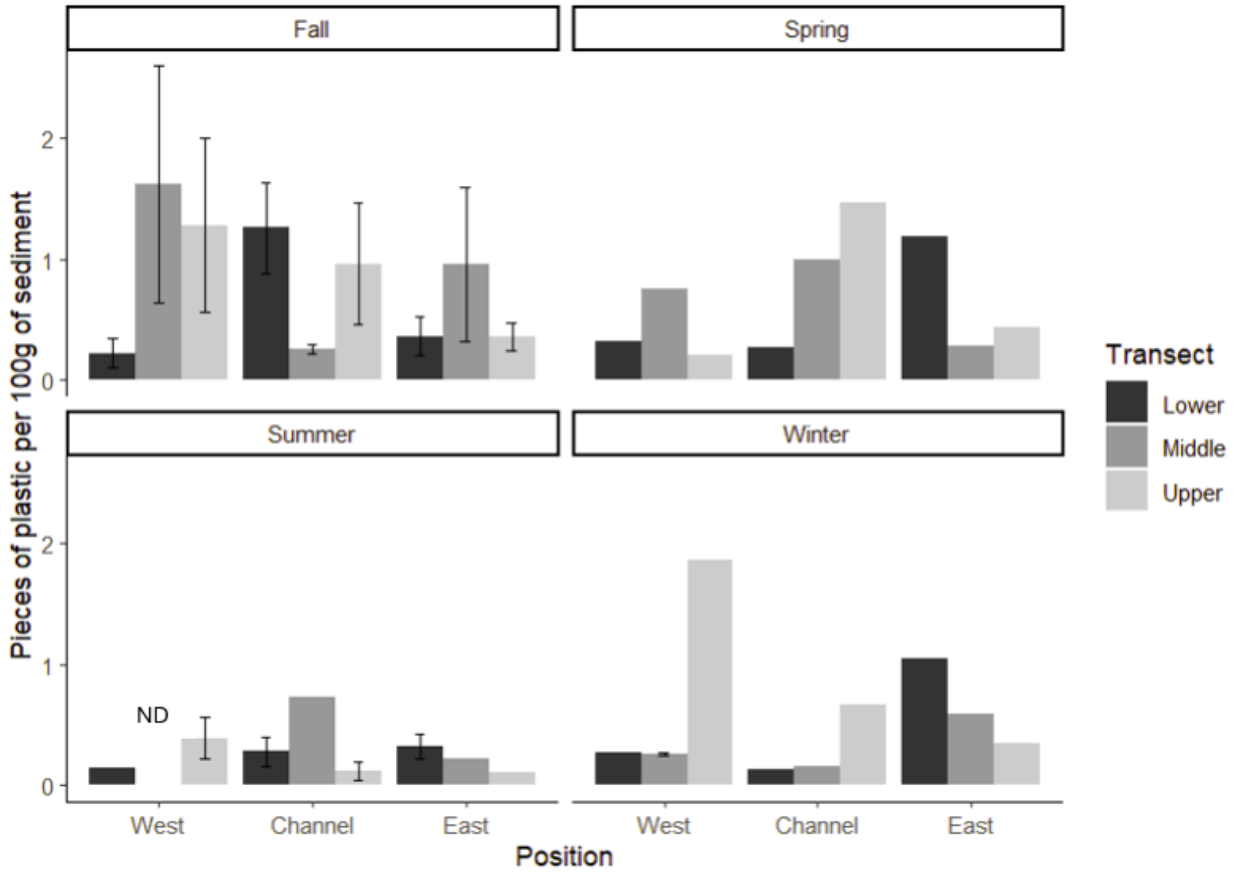

Figure 14. Microplastic abundance in the Choptank River sediment summarized by season, position, and transect. Inland, edge, subtidal, and intertidal sites removed. Kruskal Wallis test for seasonal significance, p = 0.072, transect, p = 0.4964, and position, p = 0.9627.

Table 10. Summary statistics of sediment samples with intertidal, subtidal, inland, and edge observations removed.

Transect	Position	Reps	Pieces/100g (mean ± SD)
Lower	West	7	0.24±0.18
	Channel	7	0.68±0.66
	East	6	0.60±0.42
Middle	West	5	0.90±0.97
	Channel	5	0.48±0.36
	East	6	0.66±0.78
Upper	West	7	0.96±0.96
	Channel	6	0.72±0.62
	East	6	0.33±0.17

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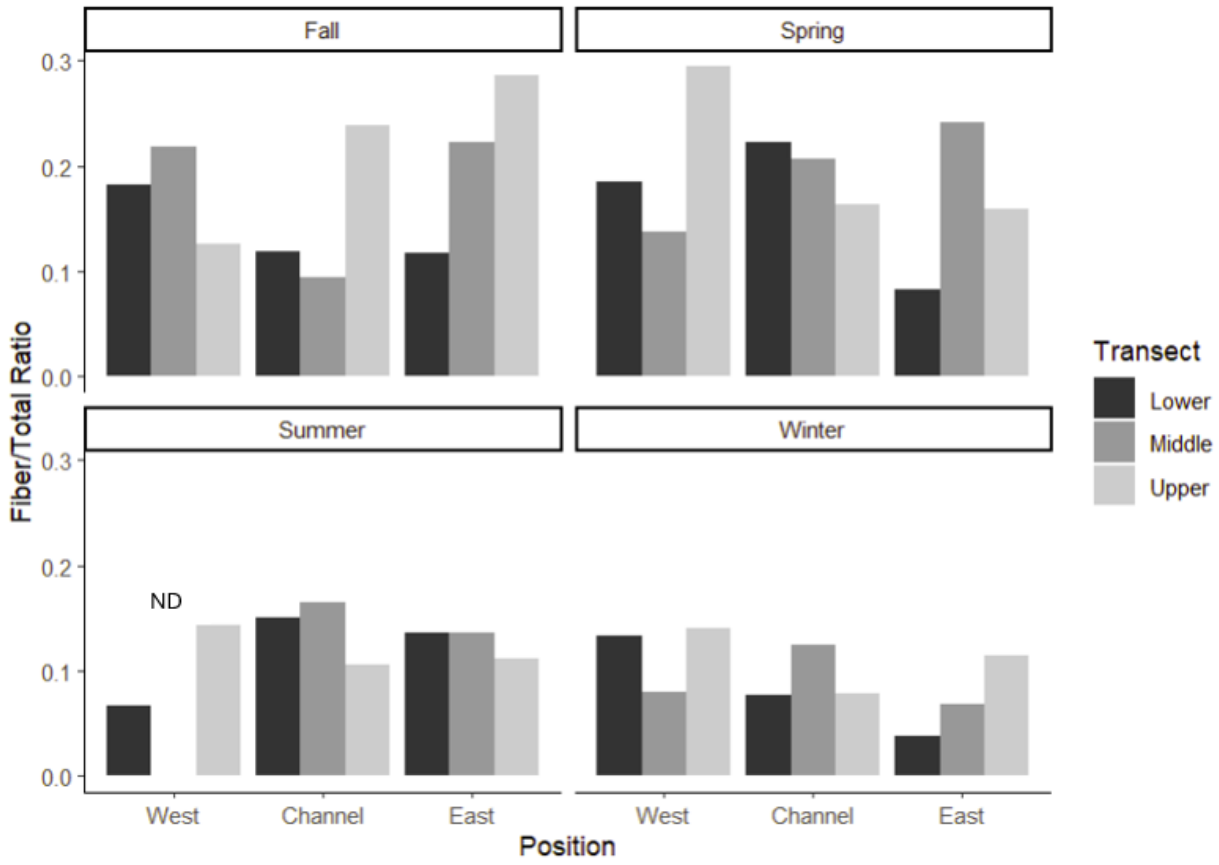

Figure 15. Fiber to total plastic ratio in the sediment. The fiber/total ratio is lower in summer and winter (Kruskal Wallis test p-value = 0.0098). Dunn's post-hoc test shows that fall and spring (unadjusted p = 0.003), spring and summer (p = 0.0101), and spring and winter (p = 0.0021) differ from one another.

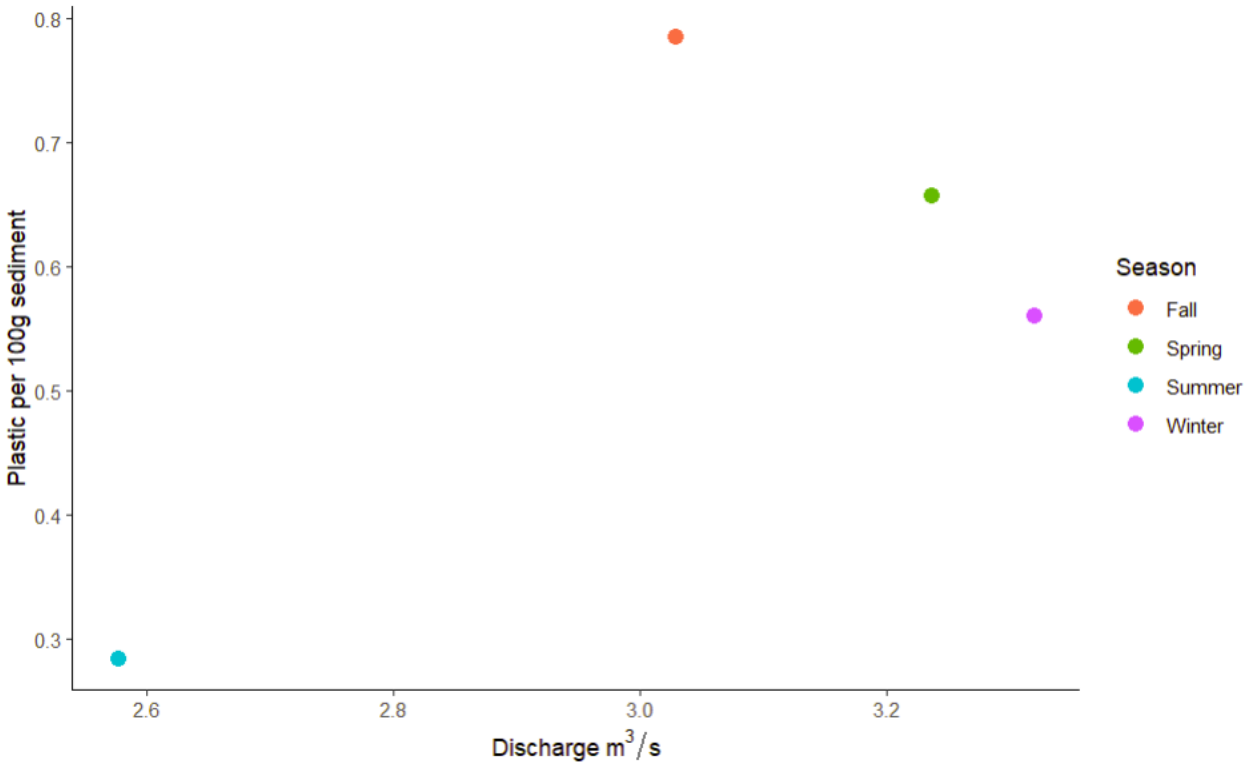

Figure 16. Relationship between plastic abundance in sediment and seasonally averaged river discharge.

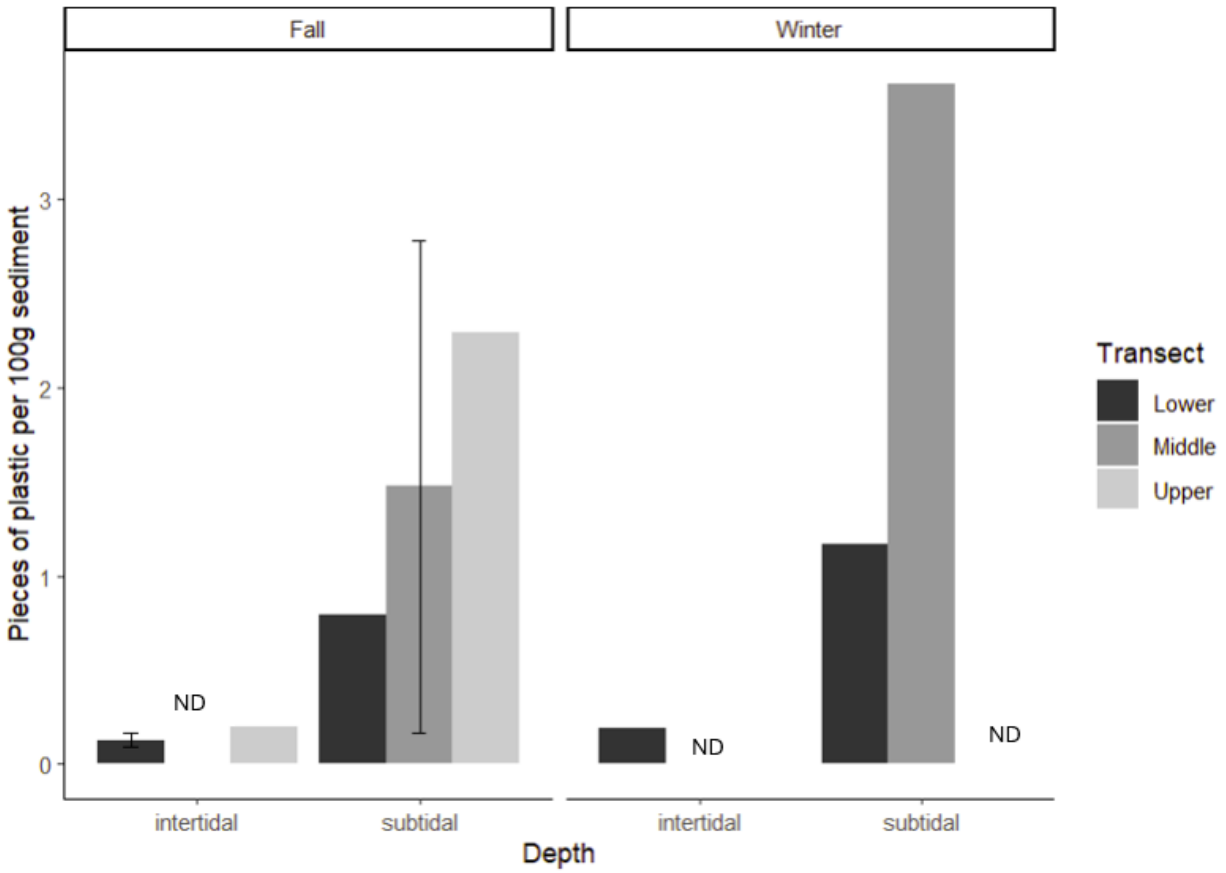

Figure 17. Microplastic abundance at subtidal and intertidal sites in the Choptank River summarized by season and transect. There is significantly less plastic at the intertidal site as determined by performing a Kruskal Wallis test with a p-value of 0.03.

Table 11. Summary statistics of sediment samples collected from intertidal and subtidal marsh positions.

Depth	Reps	Pieces/100g (mean ± SD)
Intertidal	4	0.16 ±0.05
Subtidal	6	1.80 ±1.31

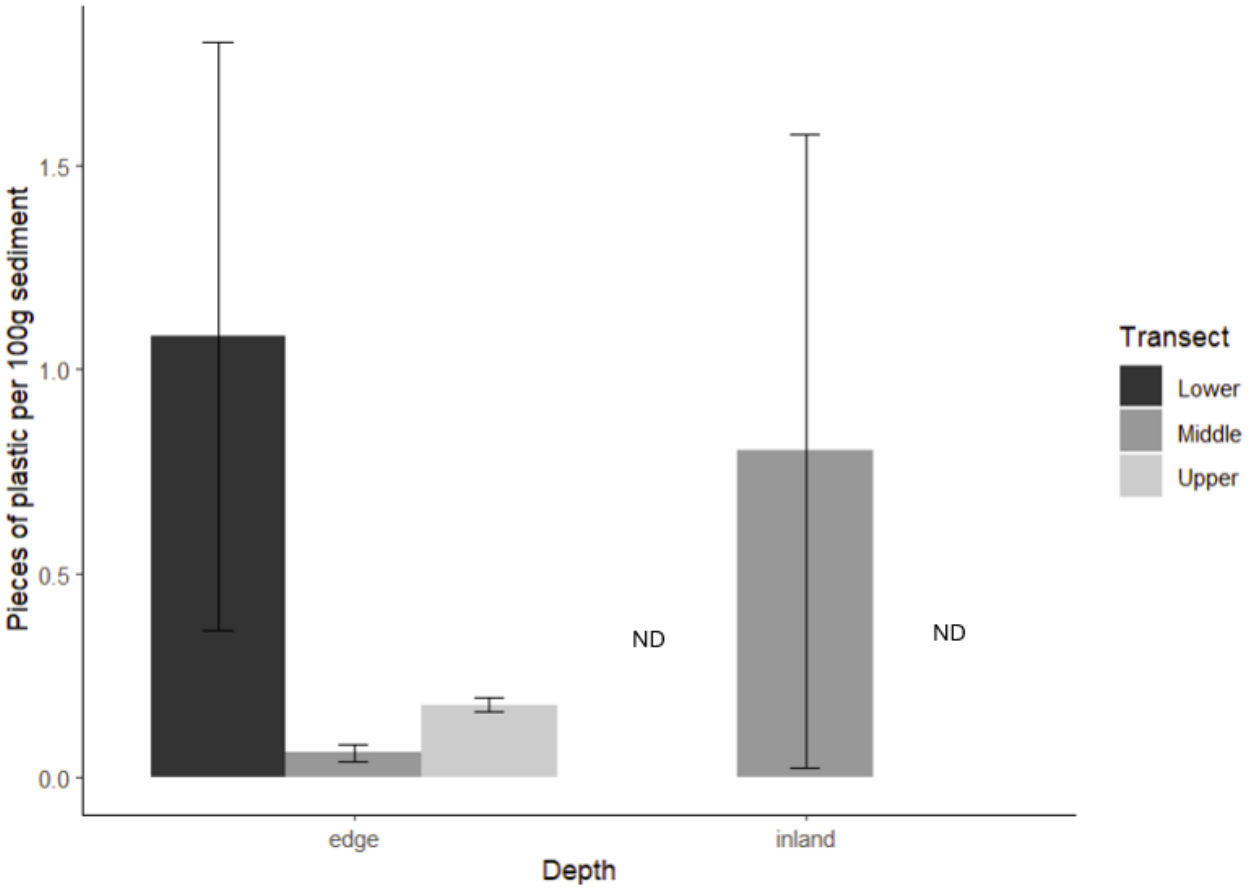

Figure 18. Microplastic abundance at the river edge and inland - collected on 5/18/2022. There is not a significant difference between the edge and inland sites as determined by a Kruskal Wallis non-parametric test with a p-value of 0.8972.

Table 12. Summary statistics of sediment samples collected from the river edge and inland.

Depth	Reps	Pieces/100g (mean ± SD)
Edge	8	0.60±1.08
Inland	2	0.80 ±1.10

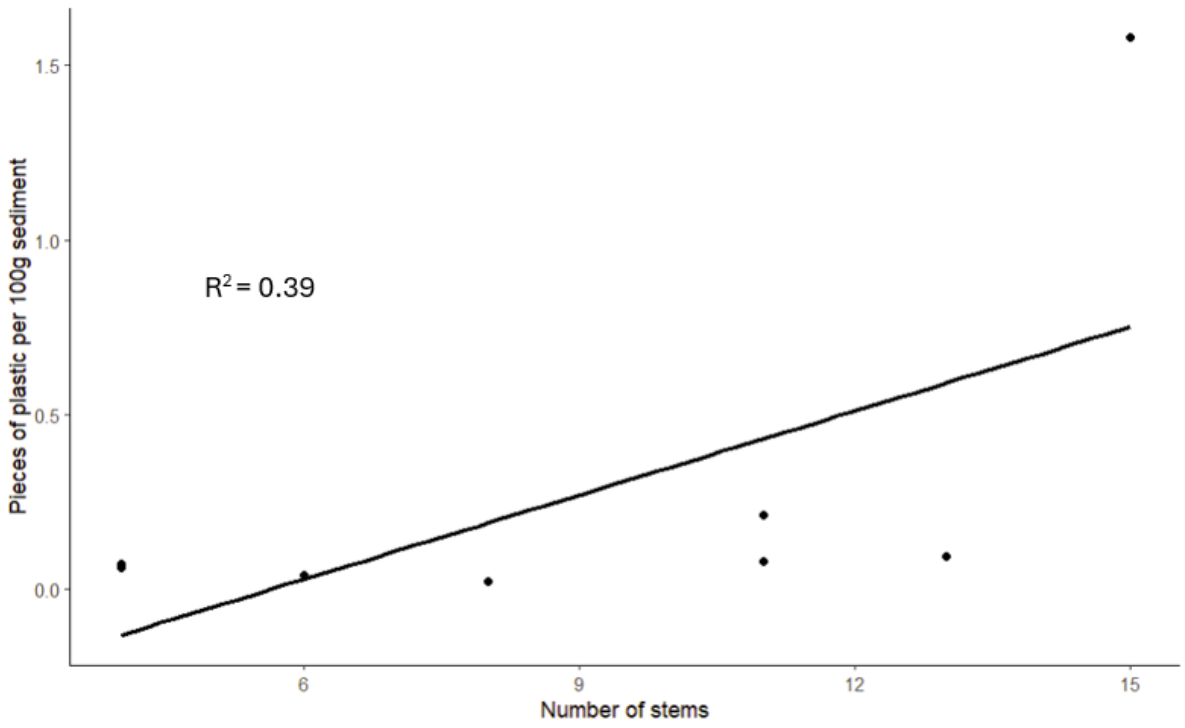

Figure 19. Correlation between sedimentary plastic content and the number of stems. Correlation is not significant as determined by a p-value of 0.09779.

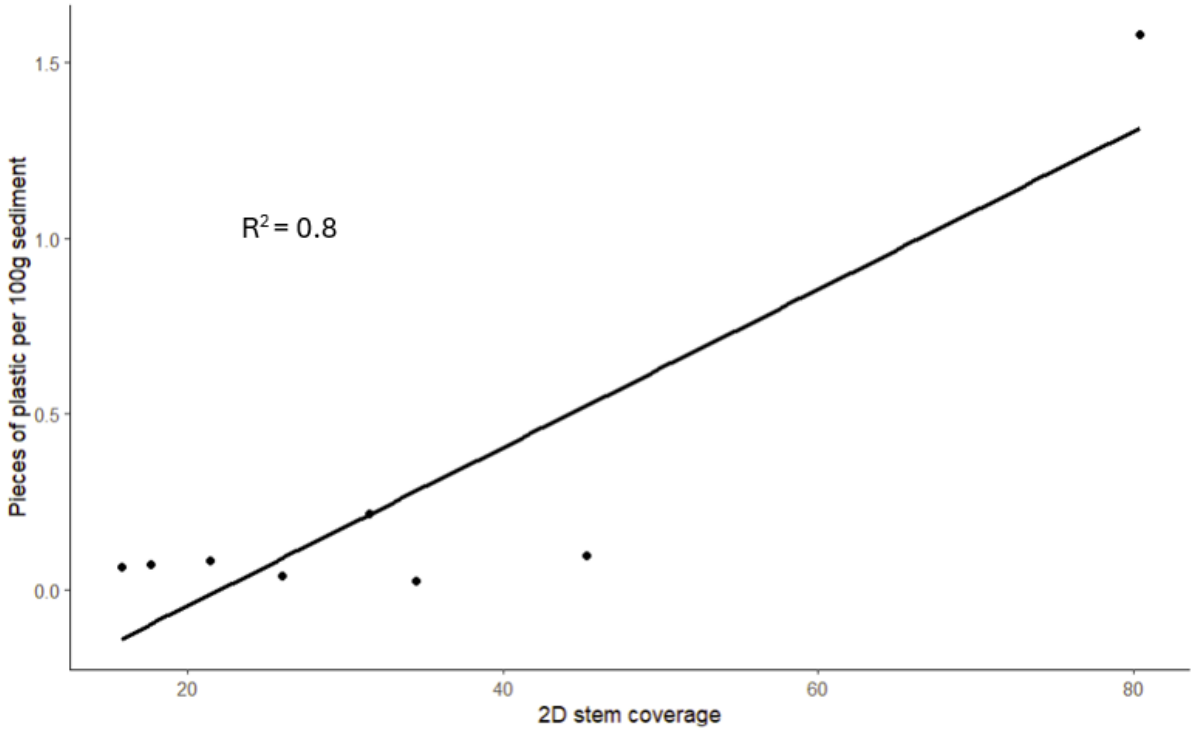

Figure 20. Correlation between sedimentary plastic content and calculated 2D stem coverage. Correlation is significant as determined by a p-value of 0.002837.

Table 13. Watershed characteristics and tabulated microplastic concentraitons per square kilometer. Choptank River values are from this study, the remaining river values are from Yonkos et al. (2014). Note the discrepancies in sample size and depth sampled.

Watershed characteristics	Choptank River	Patapsco River	Magothy River	Rhode River	Corsica River
population	18834	899000	32350	4300	3500
watershed area (km^2)	1756	1637	92	67	97
population density (people/km^2)	10.73	550	351	64	36
urban/industrial (%)	9	28	5	0	3.1
suburban/residential (%)	0	26	54	12	10.4
agricultural (%)	58	18	0.5	16	60.4
forested (%)	33	17	32	68	24.4
microplastic concentration (items/km^2)	1092401946	155373	112590	67469	40851
Depth sampled (m)	0.5-7	0.15	0.15	0.15	0.15
Lower size limit (cm)	3.30E-05	0.033	0.033	0.033	0.033

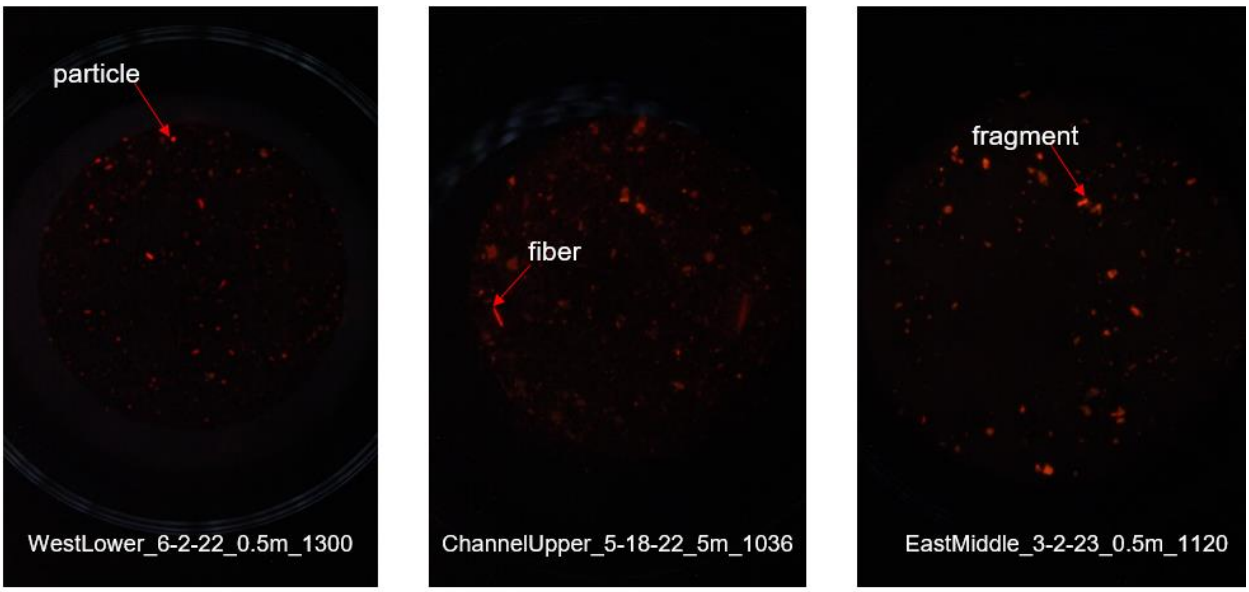

Figure 21. Example of MP-VAT image input and sample IDs containing position, transect, date, depth, and time collected. Particle, fiber, and fragment correspond to microplastic morphology categorizations.

Discussion

Despite the variation in physical and chemical characteristics of microplastics (e.g., density, shape, presence of additives), it may be informative to consider their transport and fate within the paradigm for natural sedimentary particles (Paduani 2020; Harris 2020). A possible complication from consideration of microplastics as natural sedimentary particles is their mostly unknown degradation process and into what types of components they disintegrate. That is, the snapshot captured at the time of sampling may become an unreliable explanation as time goes on and degradation proceeds. Which types of byproducts will accumulate as contamination levels rise and which will exist only transiently represents a critical knowledge gap. Studies that leverage seasonal or yearly sampling and characterize size, shape, and chemical composition are addressing the question of how microplastic pollution changes over time in different ecosystems (Covernton et al. 2019; Range et al. 2023).

The automatic quantification program MP-VAT allows for a characterization of shape based on a deviation from circularity, and an approximation of size based on the measured diameter of the filter which provides calibration for the particle quantification and characterization output (Prata et al. 2020). This is important because these characteristics will determine how plastic moves through the environment and interacts with other materials and organisms including the formation of aggregates that are physically and chemically distinct from plastic particles (de Haan et al. 2019). Interestingly, López et al. (2021) modeled microplastic movement in the Chesapeake Bay and found that particle density, but not size, influenced transport and fate with the vast majority of particles being deposited on land, very few leaving the Bay, and even fewer being suspended in the estuarine water column. Our results tend to agree with this finding, in particular the lower abundance in the river channel as compared to the surrounding marshes, and the higher abundance inland than at the river edge.

The greater abundance of microplastics in water samples collected from the marshes flanking the river (Figure 9; Table 7) is reasonable considering the greater flux of energy and water through the channel at a given time, which would tend to dilute any particles, including microplastics. Additionally, the trapping capacity of vegetation in marsh habitats has the potential to be a sink for sedimentary debris of all kinds (Almeida et al. 2023). Other studies (e.g., Pinheiro et al. 2022; Trusler et al. 2025; Yao et al. 2019) have also found elevated concentrations of microplastics in drier, more heavily vegetated areas of the saltmarsh than the wetter, less colonized areas.

The apparently reduced occurrence of microplastics in the river channel could also be due to the fact that plastics could be concentrated in a small volume of the total water column, for example the top 0.5 meters. A future research direction along this vein could be to preserve the

vertical structure by sampling discrete depths in the water column to better understand the spatial distribution of microplastics. Bagaev et al. (2018) employed this method and found significantly higher concentrations in the top 0.5 meters and near bottom of the water column as compared to intermediate depths. Other studies that employed this technique when sampling sediment have found reduced numbers of microplastics with increasing depth (Li et al. 2020; Uddin et al. 2021). This reflects the increasing use of plastic from past to present and this trend will likely continue as production and use continue unabated.

The paucity of microplastics detected at intertidal sites compared to subtidal sites (Figure 16; Table 9) could be explained by the higher energy of the intertidal habitat including sunlight and the movement of water which could cause chemical and mechanical abrasion leading to polymer breakdown or movement out of the intertidal zone. It also shows that the intertidal zone is likely not a long-term plastic sink but is instead a transition zone whereas the subtidal sites are less exposed to forces causing degradation and movement of microplastics and may allow particles to settle and be retained in this area.

Higher abundance of microplastics inland than at the river edge (Figure 17), though not statistically significant, could be explained by the greater movement of water at the river edge. This higher energy area will be subject to tidal changes, wind-induced water motion, and mechanical forces of water at the river edge. Further inland, plastics could accumulate and maintain their position since there is less disruption caused by the dynamics of the river. Future studies should sample these areas to determine if the suggestion of a pattern suggested by our data is a robust signal. Other studies (Pinheiro et al. 2022; Trusler et al. 2025) have reported this finding as well.

We sampled vegetation at the river edge and about two meters inland to establish a correlation between sedimentary microplastics and various plant characteristics. Of the variables measured (including stem height, stem diameter and number of stems), we found the number of stems and two-dimensional coverage (a measurement derived from calculation of stem volume using stem diameter and the equation for volume of a cylinder multiplied by the number of stems) to be the most significant contributors to the presence of microplastics. This is in line with our expectations based on the stabilizing effect of vegetation. Of the two, two-dimensional coverage demonstrated a higher correlation with sedimentary microplastics (Figure 19) and statistical significance than stem density (Figure 18). We did not factor stem height into our analysis because in our study system, the Chesapeake Bay, tidal amplitudes are not sufficient to completely submerge vegetation, even at high tide. However, stem height may be an important variable in other systems where vegetation is submerged or in this system as sea levels rise. Another important factor for the retention of sedimentary microplastics is the type and configuration of plant root systems. For this reason, species identification combined with knowledge of root and stem morphology is another variable that should be included in future studies. The fact that the trend is mostly attributable to a single outlying point could be explained by the fact that the effect of vegetation on the trapping of microplastics is more pronounced at higher densities of vegetation, and thus the relationship may not be linear. That is, the trapping effect of vegetation may not be observable until the density of vegetation reaches a certain threshold.

Temperature (Table 2) and salinity (Figure 5) will also affect the deposition of microplastics to the sediment. In the dry season, salinity will be higher; this could explain the lower abundance of microplastics in the sediment in summer compared to spring and fall. Fewer

microplastic particles reach the benthos because the density of the water is higher. The patterns of microplastic abundance in the sediment likely reflect longer term, more stable patterns of precipitation, wind, settling and tidal forces than the patterns of microplastic abundance in the water column which are probably more subject to transient changes. For example, there is a greater correlation between seasonal discharge averages and plastic in the sediment (Figure 15) than in the water column (Figure 11).

More frequent, higher intensity flow events during the fall and spring (Figure 6) coincide with greater abundance of microplastics in the sediment (Figure 13), and a higher proportion of fibers (Figure 14). There could be flushing of land-based plastic into the river which settles into the sediment during these two seasons. That this pattern is not observed in the water column could be explained by the greater, continuous flux of water through the channel so that high flow events may lead to only transiently elevated concentrations of microplastics in the main channel. Sampling directly after high flow events could enable correlation between discharge and microplastic abundance and establishment of a timeframe for how long higher concentrations linger in the water column of the river channel.

Though there was not a direct significance by season on microplastic abundance in the water column (Figure 9) or sediment (Figure 13), there were relationships between average seasonal discharge and especially frequent high discharge events (Figure 11; Figure 15). Microplastics were more abundant in the sediment during fall and spring (the wet season), both of which, though comparable in mean to summer and winter, had a much greater incidence of high flow events. Also, the correlation between seasonally averaged discharge and sediment microplastic abundance shows that the relevant seasonal variable could be river discharge. The lesser correlation between seasonally averaged discharge and water column microplastics could

be attributed to the greater flushing rates and more frequently altered composition of the water column.

One difference between processing of water and sediment samples is that the entire sample of water is processed and photographed for quantification of microplastic, whereas the sediment sample is mixed with saltwater which ostensibly separates the plastic from the environmental matrix. The saltwater supernatant is subsequently collected for analysis. In addition, there is much less organic material in the final sediment samples for this reason, though in the process of removing the salt water supernatant, a small amount of sediment may be retained. This remaining sediment could be stained with Nile Red and mistaken for microplastic depending on its composition and characteristics. This could explain the presence of outliers with significantly higher estimates as compared to the other samples.

The use of sodium chloride for the density separation means that plastics with a higher density than the hypersaline solution (1.3g/mL) will not be detectable using our methods. In order to maximize microplastic recovery, we homogenized sediment samples prior to processing and used two iterations of density separation, where the sample and salt water were mixed to hopefully liberate as many plastic particles as possible from the sediment matrix.

In general, the deposition and retention of microplastics in sediment is favored by low-energy conditions (Ghani et al. 2013). However, high flow events may mobilize plastic particles and flush them into the river where we detected them in the sediment. This highlights the need to distinguish between discrete high energy events as compared to a continuously higher energy environment. The former, based on our findings relating higher discharge events in spring and fall to higher abundance of microplastics in the sediment during these seasons, appear to contribute to accumulation of microplastics in the sediment. The latter appears to counter this

effect, as demonstrated by our findings of lesser microplastic abundance in the river channel, at intertidal sites, and at the river edge as compared to inland.

Vertical wind-induced mixing is an important physical process to consider because the Bay is relatively shallow–this could lead to reduced microplastic abundance found in the surface layer and thus this study (Bikker et al. 2020) must be complemented with others that sample the entire water column, as well as the sediment in surrounding marshes and in the benthos. Surface microlayer, surface, water column, benthos, sediment surface microlayer, core preserving vertical structure of sediment should be sampled and monitored over time and through different flow regimes, as well as after discrete meteorological events with high wind speed and large amounts of precipitation (Range et al. 2023).

The interaction between marine tides and freshwater discharge leads to the formation of the estuarine turbidity maximum (Liu et al. 2023) which is an important structure when considering the transport and fate of microplastics. Settled plastic particles may become resuspended in this highly turbulent area where flocculation and aggregation, with organic matter and other sedimentary materials, could occur, changing the shape, size, and density of the particles. Alternatively, advective transport downstream could be disrupted by the frictional boundary between freshwater and saltwater and reduce further transport out of the region. The ETM will be affected by changes in tidal amplitude and river discharge, moving upstream or downstream depending on the relative strength of these forces which will in turn influence the movement of microplastics.

Rising sea level and associated tidal amplitude is likely to affect transport, distribution, and fate of microplastics in several ways (Chen et al. 2024). One is that sites previously submerged only during high tides or high flow events are likely to be underwater during average

conditions. This could result in complete submersion of vegetation in the salt marshes of, for example, the Chesapeake Bay. Plant communities are likely to be affected by associated increase in salinity and water levels (Ge et al. 2015). Due to the particle trapping capacity of vegetation, this could alter the storage of microplastics in the marsh if plant communities decline or their associated morphologies (diameter, stem density, root configuration) are altered. Saltwater intrusion into the marsh could also disrupt sediment (Zhang et al. 2024), resuspend microplastic particles that had settled and potentially redistribute them to another area of the estuary, marsh, or out to sea (Yin et al. 2022).

There is likely an interaction between multiple sources of plastic and transport mechanisms that lead to the distribution of microplastics. This presents a complication when trying to attribute observations to a single variable. The challenge exists in understanding the mechanisms underlying the patterns we observe. Trends could be obscured by intermittent high flow events, increased river discharge, intense precipitation or wind, significant changes in tidal amplitude, or pulses of plastic leakage into the environment.

Contamination of different environmental compartments means that multiple habitats and types of organisms will be exposed to microplastics with consequences for their health, physiological function, and reproduction (Lima et al. 2014; Rebelein et al. 2021). Ambient microplastics concentration may correlate with presence in organisms (Cai et al. 2023). Even within the life cycle of one organism, there could be multiple points at which contact occurs, for example in oysters with a free-swimming larval stage and benthic adult stage; this type of organism could encounter both suspended and buried microplastics at different points in time (Bringer et al. 2021).

There were several methodological lessons learned from our study. The first was addressed in the methods (the change in processing water samples). Another was inconsistencies in the timeframe between staining with Nile Red and photographing. However, we do not expect this to result in systematic error as select samples were reimaged and rerun through MP-VAT and there was no significant change in the results. Another was the sometimes high level of microplastics detected even in blanks. It is unclear from where this contamination originated. There also may have been a higher concentration of Nile Red used to stain a percentage of samples. We do not expect this to significantly alter the results as it is likely the lower limit that is important. Other researchers (pers. comm.) have tested published organic digestion methods and found they did not perform as expected. These challenges highlight the need to streamline the processing of microplastics samples to facilitate comparability and consensus.

Though most (e.g., Barrows et al. 2018), but not all (Wang et al. 2020), studies report fibers as the most abundant morphological category, we did not find this to be the case. This could be due to the particulars of our methodologies, for example the use of a plankton net which could retain fibers so that while they may be present, we were detecting them in quantities less than their true abundance. There were variations in the proportion of fibers, especially in the sediment, which were elevated in relation to high discharge events in spring and fall. However, the proportion of fibers was slightly higher overall in the water column than the sediment. This may indicate that fibers tend to remain buoyant and travel further in the water column from their source rather than settling to the sediment (Engdahl 2018; Lloret et al. 2021).

Understanding degradation mechanisms and the products they yield is important in describing the fate and transport of microplastics because as the properties of plastic change, the way it interacts with other substances and organisms is subsequently altered. For example, the

production of different functional groups following UV exposure or bond cleavage via hydrolysis can cause adsorption of nutrients or heavy metals, for example (Nguyen et al. 2023). This has implications for the leaching and transfer of possibly hazardous materials or organisms and thus affects human and ecosystem health. This highlights the complexity of plastic pollution in that it may indirectly foster the occurrence and prevalence of other issues such as proliferation of harmful organisms or other types of contamination.

Our study provides critical data that contributes to the literature examining the role of estuaries and salt marshes in the transport and fate of microplastics through this environment. Future work will capitalize on the insights, including methodological and logistical challenges, gained during this study to better inform microplastic monitoring efforts. Our study is the first to quantify microplastic pollution in the Choptank River, contributing to understanding of this contaminant within a major tributary of the Chesapeake Bay.

Chapter 2 - Taxonomy, metabolism, and ecological significance of plastic pollution: a review of biological trajectories in an increasingly contaminated world

Introduction

Plastics are derived from hydrocarbons, the remains of ancient organisms, such as coal, oil, and natural gas. These same materials are incinerated to generate energy which leads to an alteration of the global carbon cycle when carbon dioxide, methane, or other thermally active substances are released into the atmosphere (Andres et al. 2012). The significant fraction of plastic debris that escapes the waste management pipeline and enters the environment also offers the potential to affect the carbon and other biogeochemical cycles by way of interaction with microorganisms, which are the link between the living and nonliving components of the environment and that mediate global biogeochemical cycles through their metabolism. Therefore, the potentially unique microbial habitat presented by (micro)plastic pollution may alter these cycles if plastic-colonized ecosystems have distinct metabolic capacities from those found in the absence of plastic pollution (Arias-Andres et al. 2019). This review focuses on the biological carbon pump and specifically the potential alterations caused by plastic, especially by way of its interactions with microorganisms responsible for key processes in the carbon cycle. There is a shift occurring in the literature where two key issues once treated as separate, climate change and plastic pollution, are now recognized as linked (Ford et al. 2022). Both involve the liberation of inert carbon and release into the atmosphere, ocean, and terrestrial environments (Amaral-Zettler et al. 2020).

Plastic affects numerous links in ecological networks due to the variety of sizes, morphologies, densities, polymer types, presence of additives, the degradation of which leads to its existence in every conceivable environment. This fulfills one stipulation for considering

plastic pollution as a planetary boundary threat: its persistence and effects on multiple scales (Steffen et al. 2015). At the same time, ecosystems are stressed by regime changes (whether continuous or abrupt) and alterations to discrete meteorological, biological, and physical events brought forth by anthropogenic forcing (Möllmann & Diekmann 2012).

Humans have emerged as a force of global change commensurate with other geologic events occurring over much longer timescales. The liberation of carbon from inert reservoirs and subsequent input of carbon dioxide to the atmosphere that has occurred in the last two hundred years has surpassed levels found in the past ~12,000 years (Nehrbass-Ahles et al. 2020). The stability of the Holocene age, the most recent in the Quaternary Period of the Cenozoic Era, is the state of the Earth from which modern human civilization evolved. In terms of climate, biodiversity, and biogeochemical cycles, it is the only known set of global conditions certain to be amenable to humanity in its current configuration (Richardson et al. 2023). Agriculture and urbanization rely on known environments to continue supporting large, complex human communities. Understanding current, rapid changes by looking to the past is one approach to acknowledging possible trajectories of the Earth system. Importantly, the Holocene age is in its infancy compared to the emergence of microbial forms of life 3.7 billion years ago (Alegado & King 2014). Thus, understanding the microbial communities found on plastic and their evolutionary history in relation to past conditions and coexisting biota may provide insight into the types of organisms likely to thrive in an increasingly plastic world.

The idea of a “Plastisphere" has been proposed due to the pervasive and recalcitrant nature of this pollutant, which suggests formation of a unique microbial ecosystem due to the presence of this novel substrate (Zettler et al. 2013). The functions of these ecosystems will be explored, and the production of metabolic byproducts, some with direct climate relevance such

as nitrous oxide or carbon dioxide (Cornejo-D'Ottone et al. 2020), will be examined. In addition, there has been experimental and observational evidence of direct effects on cellular processes such as the photosynthesis of microbial as well as larger vegetated species (Tetu et al. 2019). Using the communities of microorganisms hypothesized to flourish in the presence of an increasingly plastic-polluted, warming, and biologically deteriorating planet, hypotheses about how the increasing dominance of certain groups, and diminishing others, will alter global conditions will be generated. Predicting such shifts in microbial communities is imperative to understand how these changes could contribute to widespread, abrupt, and possibly irreversible ecological state changes (Barnosky et al. 2012).

Starting at this smallest scale of the food web, further literature examining the effect of plastic on and their role in the microbial loop and biological carbon pump will be discussed. Potential disruption or alteration of these systems will be considered, and potential ramifications of this will be explored. Other publications have already put forth evidence of combined effects on organisms with biogeochemical cycles such as oxygen dynamics resulting from disruption of the food web (Kvale & Oschiles 2023). Other authors (e.g., Arias-Andres et al. 2019) have already emphasized the importance of studying plastic pollution from a microbial ecology perspective.

Methods

Taxonomy

Web of Science search for keywords "plastic" and "microbial community". This is distinguished from "plastic" and "microbes" which yielded mainly literature on biodegradation rather than the taxonomic groups inhabiting plastic as a substrate and habitat for complex multi-

domain communities. This demonstrates the focus on the possibility of degradation by microorganisms rather than plastic as the base for a novel ecosystem. Some papers were selected based on title and abstract content, and some were reviewed in greater detail, including analysis of papers in the reference section of selected publications.

Taxonomic information generated from genotypic or phenotypic identification measurements was extracted from publications and compiled, with an emphasis on presence of organisms at varying levels of biological categorization (e.g., phylum, genus). The location of the study, type of environment, environmental compartment, and type of plastic was also extracted. A separate table details, if present in the publication, the microbial community present in the surrounding, ambient environment, for comparison of the biological community with and without the presence of plastic. Taxonomic level is reported, to discern the biological resolution present in the publication.

Metabolism

Beginning from the taxonomic groups identified in selected publications, metabolic tendencies and the ecological role of these groups was systematically explored, and publications reporting metabolic functionality were also included in the review. Phylum-level classifications were selected for the limited scope of this review.

Regions of study

Consideration of different regions is important when exploring possible plastic-specific microbial communities for several reasons (Figure 1). One is that different locations (open ocean, semi-enclosed seas, urban rivers) have different proximity to potential plastic sources and

also plastic types. It is likely that there will be plastic at various stages of degradation that are distinct between these locations. Also, the characteristics of these regions including meteorological, hydrological, geographical, geological, biochemical, and anthropogenic patterns gives them their distinct physical features which will determine their ambient conditions including salinity, nutrient status, presence of oxygen, and food web configuration, which will give them unique microbial communities. A complication in trying to understand the composition of an ostensible "Plastisphere" is whether the organisms detected on plastic at a given time and location originated in the location sampled, or whether they became plastic-associated at an earlier time or different place and simply traveled on the particle.

Results and Discussion

Taxonomy of the Plastisphere

As plastic moves through the environment, it alters the physical and chemical conditions found through the pathway from source to sink, experiences surface characteristic alteration due to degradation, and accumulates the microorganisms and amalgam of (in)organic matter that comprise the biofilm (Lobelle & Cunliffe 2011). The mechanisms underlying distribution of the components of the biofilm including their temporal trajectory is one complication in predicting how plastic pollution is likely to alter biological communities (Sauer et al. 2022). Understanding how microplastic pollution could interact with other anthropogenic stressors and significantly alter processes such as biogeochemical cycles (another planetary boundary) requires linking taxonomic identification of colonizing microorganisms with an understanding of their ecological function through examining their metabolic tendencies (Lausch et al. 2016).

A microbial biofilm is the term used to describe the particle-associated ecosystem composed of microbial communities embedded in a matrix of cellular secretions that form on substrates or aggregate together. They can be composed of prokaryotes (Bacteria and Archaea), eukaryotes (plants such as algae, animals such as bryozoans, protists, or fungi), and viruses. There are three major stages of a biofilm: attachment, growth, and dispersal (Sauer et al. 2022). Each stage corresponds to changes in gene expression or phenotype of the colonizing organisms, such as the formation of appendages (e.g., cilia or flagella) which alter the surface properties of Bacteria for example and change interactions with the substrate on which the biofilm forms. This relates to plastic debris in the environment because communities of microbes inhabiting a biofilm have different characteristics than free-living microorganisms, for example having enhanced resistance to antibiotics or being sheltered from nutrient limitation or other harsh conditions present in the surrounding environment (dos Santos et al. 2018). The formation of a biofilm consisting of microorganisms and their metabolic byproducts generates a matrix within which a unique ecosystem can emerge that is distinct from configurations arising from free-living strategies demonstrated by many microorganisms**.** The initial steps in the formation of a biofilm is species- and environment-specific, while the development and configuration of the mature biofilm reflects a generalized biofilm-forming process shared by distinct organisms with different physiological activities and needs (Stanley & Lazazzera 2004).

Du et al. 2022 offers a thorough review on the taxonomy of the plastisphere; however, this publication reports “species”, but actually identifies different biological categories (order, family, genus, species, etc.). From “cyanobacteria” to “Flavobacteriaceae”, this review will address the differing resolution possible when reporting at the phylum vs. family level, for example, and how this could affect consideration of specific metabolic and cellular processes.

The results will be compiled so that higher level classifications can be extracted from reported organisms. Du et al. 2022 also does not reflect on phylogenetic relationships between plastisphere members or describe the geological conditions from which they emerged. In addition, there are rough categories of "autotrophs" and "heterotrophs", as well as fungal components of plastisphere communities (which are generally underexplored (there is often an emphasis on prokaryotes)), along with some information about the metabolism and cellular functions of certain organisms, such as which light-harvesting strategies are used in the plastic-associated organisms vs. those living in the surrounding environment. The current review will explore in greater detail what is known about the physiology of plastic-associated organisms in different environmental contexts (e.g., coastal habitats, oligotrophic ocean gyres). Also, as De Tender et al. 2015 points out, the immediate surroundings may not be representative of ambient microbial communities due to the dynamic nature of aquatic ecosystems and dispersal patterns of microorganisms, especially as association with plastic particles may aid long distance movement.

There is disagreement in the literature about how communities inhabiting plastic differ in biodiversity metrics such as richness and evenness. Dudek et al. 2020 reports significantly lower richness and evenness (and consequently, biodiversity) than the plastisphere community. Some studies report the presence of dominant groups on plastic as compared to the surrounding environment (e.g., Delacuvellerie et al. 2019), while others calculated greater evenness in community composition on plastic (e.g., Zettler et al. 2013). Li et al. 2020 segregated analyses of microbial community composition into "HM" and "LM", corresponding to relatively higher and lower concentrations of microplastics in the sampling sites (riverine sediment). For one diversity index (Chao1), alpha diversity in the HM group was less than in the LM group, though other metrics such as Shannon or Simpson diversity did not show a significant difference. This

contradiction could be the result of temporal differences not addressed (e.g., community assembly successional patterns (Rodriguez-R et al. 2015)), in which case a deterministic pattern could be present, or alternatively an indication of the contribution of stochasticity to the formation of plastic-associated (or in fact, any) microbial communities. Studies such as Dussud et al. (2018a) that carry out controlled mesocosm experiments can document the succession of biofilm formation on plastic polymers and compare them to microbial communities in the surroundings and provide insight into time-dependence of community composition. This suggests that metabolism of the plastic as a source of carbon may be occurring. The resolution of this contradiction has important implications for a discussion of plastic as an evolutionary force, where the relative influences of the random versus deterministic processes could influence the predictability of biological trajectories given increasing plastic loads. Studies such as Xu et al. 2019 examining potential temporal successional signals encourage further attention be paid to the possibility of such patterns.

Some studies (e.g., De Tender et al. 2015) report significant overlap between plastic-associated communities and the surroundings, but with different relative abundances. Also, due to the vagaries of methodology, the actual abundance of organisms considered central to a plastic community, may actually be quite low (De Tender et al. 2015). However, the many studies corroborate the findings of Zettler et al. 2013 that plastic represents a unique habitat niche for microorganisms (distinct from the surrounding environment), supporting the validity of the "Plastisphere", a distinct layer of the biosphere generated by the accumulation of plastic.

De Tender et al. 2015 is unique in that it explores the possibility that differences in community composition among different MPL samples could be the result of snapshots of biofilms at different points in time. To address this possibility, they performed an experiment

which controlled for exposure time, finding classes of Proteobacteria to be dominant initial colonizers, giving way to Bacteroidetes dominance as time progressed. Kettner et al. (2019) specifically sought to quantify eukaryotic biodiversity and reported the presence of more of eukaryotic kingdoms in the water samples as opposed to wood (which contained mostly fungi (phylum: Ascomycota or Ciliophora)) or plastic-attached substrates. Network analyses by these authors demonstrate an interesting finding where Eukaryotes and Bacteria were more connected on plastic substrates, while Bacteria tended to cluster with other Bacteria on wood substrates. The networks in general were decentralized and sparsely connected, possibly indicating the ecological novelty of plastic as a microbial habitat, whereas well-established ecological niches may be highly centralized and connected, illustrating the possibility of plastic to upend the stable ecosystems which characterize the Holocene epoch. This generates an interesting question about how the biofilm ecosystems on different substrates are assembled, which can be addressed by examining phylogenetic relationships among members and considering what is known about their ecological roles. Kettner et al. (2019) also discusses the possibility of eDNA attachment to plastic particles (or attachment at a smaller, earlier life stage such as larvae) as a potential explanation for detection of larger eukaryotic organisms.

Notably, the identity of the members of the plastic-associated ecosystem tends to be distinct from its surroundings, for example, the water column as demonstrated by Zettler et al. 2013. Vaksmaa et al. 2021 collected plastic from the environment (rather than retrieving experimental plastic that was deployed intentionally) and found there to be substrate-specific communities (PE differed from PP or PS), which was evident using different metrics, both order and Operational Taxonomic Unit. They also discovered that biofilms were composed mainly of the domain Bacteria (vs. Archaea or Eukarya), that microbial composition was heterogeneous,

and that certain organisms grouped, showing this by way of microscopy and cell staining. Frére et al. 2018 found significant overlap in OTUs in surrounding seawater and on a plastic substrate, but there were more OTUs found only on plastic than there were in the seawater. For those OTUs found only on plastic, a significant majority (~95%) did not differ on different types of plastic (they were specific to plastic, but not to type of plastic). This study differs from Zettler et al. 2013 in that no difference in evenness was found between plastic and seawater, at least in December. A study conducted by Huang et al. (2019) examined bulk soil community composition and found that it did not differ in LDPE-altered soil as compared to the control, but the Bacterial community on the microplastic itself was significantly lower in species richness, evenness, and diversity in comparison to both. The LDPE-altered soil and the control generally tracked one another through time while the biodiversity metrics on the microplastic tended to have a similar pattern through time, albeit at a much lower value. The abundance of certain groups increased dramatically with time since incubation, for example, the families of pathogenic Bacteria Nocardiaceae or Campylobacteraceae. This is analogous to the high prevalence of Vibrio in aquatic systems, supporting the hypothesis that microplastic may act as a vector or otherwise favorable habitat for pathogenic microorganisms.

Amaral-Zettler et al. 2015 reported differences in Bacterial community defined by biogeographical location, but not by polymer types (apart from PS being distinct from other types), suggesting that a given plastic community may be mainly a function of the location in which it is situated. A negative correlation between latitude and Bacterial species richness was observed for two water depths and the plastic community. The presence of plastic seems to increase the variability in microbial ecosystem structure, as compared with more consistency in the seawater in which it is found. This could indicate the presence of specialized niches

generated by the microplastic habitat leading to communities distinct from those that would otherwise be present in the absence of plastic.

Taxonomic identification of the following persistently detected plastic-associated phyla were reported: Cyanobacteria, Pseudomonadota (Proteobacteria), and Bacteroidetes (Table 1). These are the phyla most commonly reported in the studies reviewed and comprise the greatest proportion of plastic-associated communities. Other, less frequently reported phyla include Actinobacteria and Acidobacteria.

Cyanobacteria

Zettler et al. 2013 found Cyanobacterial genera Phormidium & Rivularia on the plastic but not in the water column, while Prochlorococcus were the major photosynthetic Bacteria in the water column. The pathogenic genus Vibrio was, in comparison to its usual distribution, much more concentrated on plastic debris (possibly attributable to a fast growth rate), especially because it is usually a significant minority of the composition (Zettler et al. 2013). Corroborating visual identification, 16S rRNA sequence analysis also revealed a predominance of Bacteria, showing one dominant phyla to be Cyanobacteria (Vaksmaa et al. 2021). Bryant et al. 2016 reports Cyanobacteria in the North Pacific ocean gyre. Mughini-Gras et al. (2021) examined the microbial community in the Rhine River, with almost 100% of mapped reads attributed to Bacteria, one phylum of which was Cyanobacteria. Delacuvellerie et al. (2019) reports Cyanobacteria in the Mediterranean Sea. Dussud et al. (2018) reports Cyanobacteria in the Mediterranean Sea.

Pseudomonadota (Proteobacteria)

Corroborating visual identification, 16S rRNA sequence analysis also revealed a predominance of Bacteria, showing one dominant phyla to be Proteobacteria (Vaksmaa et al. 2021). Bryant et al. 2016 reports Alphaproteobacteria and Gammaproteobacteria in the North Pacific ocean gyre. Mughini-Gras et al. 2021 examined the microbial community in the Rhine River, with almost 100% of mapped reads attributed to Bacteria, one phylum of which was Proteobacteria. Frère et al. 2018 reports Alphaproteobacteria and Gammaproteobacteria (including Vibrio) in the Bay of Brest. Li et al. (2020) found both HM and LM groups consisted mostly of Proteobacteria. Basili et al. (2020) reports Proteobacteria in the Mediterranean Sea. Delacuvellerie et al. (2019) reports Proteobacteria in the Mediterranean Sea. de Tender et al. (2015) reports Proteobacteria, specifically Gammaproteobacteria with the family Vibrionaceae in the Belgian part of the North Sea. Dudek et al. (2020) reports Proteobacteria, specifically Alphaproteobacteria and Gammaproteobacteria in the Caribbean Sea. Xu et al. (2019) reports Proteobacteria, specifically Gammaproteobacteria and Alphaproteobacteria in China's coastal seawaters. Dussud et al. (2018) reports Proteobacteria, specifically Alphaproteobacteria in the Mediterranean Sea. Dussud (2018a) reports Proteobacteria in the Mediterranean Sea, specifically Alphaproteobacteria (which became more abundant on polymers as time passed) and Gammaproteobacteria. Kirstein (2018) conducted a seawater incubation study and found three classes of Proteobacteria including Alphaproteobacteria and Gammaproteobacteria. Erni-Cassola et al. (2019) reports Proteobacteria in the Mediterranean Sea, including Alphaproteobacteria and Gammaproteobacteria. Xu et al. (2023) reports Proteobacteria, specifically Alphaproteobacteria and Gammaproteobacteria in the Dongshei River in Beijing, China.

Bacteroidetes

Corroborating visual identification, 16S rRNA sequence analysis also revealed a predominance of Bacteria, showing one dominant phyla to be Bacteroidetes (Vaksmaa et al. 2021). Mughini-Gras et al. 2021 examined the microbial community in the Rhine River, with almost 100% of mapped reads attributed to Bacteria, one phylum of which was Bacteroidetes. Frère et al. 2018 reports Bacteroidetes in the Bay of Brest. Li et al. (2020) found the LM group had higher Bacteroidetes. Basili et al. (2020) reports Bacteroidetes in the Mediterranean Sea. Delacuvellerie et al. (2019) reports Bacteroidetes in the Mediterranean Sea. de Tender et al. (2015) reports Bacteroidetes in the Belgian part of the North Sea. Dudek et al. (2020) reports Bacteroidetes in the Caribbean Sea. Xu et al. (2019) reports Bacteroidetes in China's coastal seawaters. Dussud et al. (2018) reports Bacteroidetes in the Mediterranean Sea. Kirstein (2018) conducted a seawater incubation study and reports Bacteroidetes. Xu et al. (2023) reports Bacteroidetes in the Dongshei River in Beijing, China.

Other Plastisphere members

Individuals belonging to multiple genera of diatoms were present in the plastisphere community and identified by genomic sequencing and SEM imaging. Multiple other groups of protists (e.g., cryptophytes, dinoflagellates, haptophytes, among others) were also identified in the plastic-associated community (Zettler et al. 2013). Hydrocarbon-degrading Bacteria were reported in significant numbers by Vaksmaa et al. (2021), in both early and late-stage biofilms; diatoms too were also a significant component of the eukaryotic community–archaea were found sparsely and did not appear to be substrate-specific. Bryant et al. 2016 examined plastic-associated communities in the surface waters of the North Pacific Gyre (location of the Great Pacific garbage patch) and found bryozoans to be significant colonizers, and associated with them were diatoms and variously shaped unidentified cells possibly representing Bacterial

colonizers. Genomic data corroborated the presence of bryozoans as primary eukaryotic colonizers, while diatoms contributed less than 1% of mapped reads. Carson et al. 2013 related physical parameters corresponding to water conditions, location, biological communities, and polymer characteristics to measures of microbial diversity. The most commonly found organisms were Bacillus (Bacteria) and pennate diatoms. Coccoid Bacteria, other diatom shapes, and other protists such as dinoflagellates, coccolithophores, and radiolarians were present in small quantities. Delacuvellerie et al. (2019) reports Verrucomicrobia in the Mediterranean Sea. de Tender et al. (2015) reports Actinobacteria and Actinomycetota on beach pellets in the Belgian part of the North Sea. Dudek et al. (2020) reports the eukaryotic phylum Bacillariophyta in the Caribbean Sea. Kettner et al. (2019) took place in the Baltic Sea and is one of few studies to focus on eukaryotic Plastisphere membership and reports multiple phyla within the kingdom metazoa including Chlorophyta, Cryptophyta, and Picozoa. Kirstein (2018) conducted a seawater incubation study and found multiple eukaryotic phyla including Arthropoda and Nematoda.

Explanations and implications

One of the most commonly reported phyla is Proteobacteria, and specified further are classes Alphaproteobacteria and Gammaproteobacteria. This doesn't imply specificity to plastic as Proteobacteria (also known as Pseudomonadota) are abundant, prolific and found in many environments from the human body (Rizzatti et al. 2017) to hydrothermal vents (Corre et al. 2001). The genus Vibrio belongs to the class Gammaproteobacteria which is of particular interest due to its pathogenic nature with respect to human health. Some studies (e.g., Zettler et al. 2013; Frère et al. 2018), but not others (Vaksmaa et al. 2021) report enrichment of Vibrio on plastic debris. Zettler et al. (2013), the first to introduce the idea of the "Plastisphere" and report on its

community composition generated a Venn diagram showing greater overlap between plastic-associated communities as compared with the surrounding seawater.

Many studies report microbial communities on plastic from the Mediterranean Sea (a well known area of plastic accumulation) or other semi-enclosed bodies of water (e.g., Erni-Cassola et al. 2020; Dussud et al. 2018), while others sample from the open ocean, often in the gyres where plastic debris is known to accumulate due to physical processes. This is important when considering microbial communities because depending on the plastic source, how long it has been in the environment, and extent of degradation the properties will be altered which will mediate formation of the biofilm. Since distribution patterns and long-term sinks are not well known, it is not clear in which locations microbial communities may be permanently altered rather than temporarily restructured. The Plastisphere may evolve as a function of polymer weathering as degradation proceeds to alter chemical properties (such as the addition of functional groups) and surface characteristics, both of which mediate interactions between microorganisms and particles (Erni-Cassola et al. 2019).

Most studies conclude that the plastic-inhabiting biofilm is dependent more on ambient conditions and the environment in which sampling took place than substrate type (e.g., Basili et al. 2020; Miao et al. 2020; Amaral-Zettler et al. 2015). When substrate type was found to be significant, it was usually between natural substrates and plastic, rather than between different types of plastic (Miao et al. 2020), with the exception of polystyrene being reported to have a unique biological community (Amaral-Zettler et al. 2015).

The higher contribution of prokaryotes to overall community assembly could be the resulting of sampling method bias or could represent a true pattern of the Plastisphere.

There are potential candidates capable of breaking down plastics and these are those organisms that are often described as the "core" biome, those that are found colonizing plastic across locations and on different types, with a focus on Bacteria because these are the largest contributor to biomass in the ocean (Roager & Sonnenschein 2019). Roager & Sonnenschein 2019 report the following groups as possible plastic degraders due to their prevalence in the plastisphere, evolutionary history, and metabolic capacities: Proteobacteria, Bacteroidetes, Firmicutes, and Cyanobacteria.

It is important to note that there is significant overlap between the phyla found inhabiting plastic and the surroundings in which the studies took place, including Proteobacteria (Delacuvellerie et al. 2019; Dussud et al. 2018; Dussud et al. 2018a; Xu et al. 2023), Cyanobacteria (Zettler et al. 2013; Dudek et al. 2020; Dussud et al. 2018a), and Bacteroidetes (Dussud et al. 2018a).

Metabolism of the Plastisphere

There is increasing attention being paid to the potentially unique metabolism of the plastic-associated community. For example, Messer et al. 2024 generated a schematic overview of metabolic functions of genera identified in a heterotrophic plastisphere. This publication outlines specific processes that are involved in various cellular activities including motility, metabolism, stress response, and energy acquisition. In this way, taxonomy is linked to function and is explored by way of identifying expressed proteins. The novel contribution proposed by this review is linking taxonomy, and functional ecological significance across different habitat types from coastal marshes to oligotrophic ocean gyres, as well as considering possible evolutionary trajectories given increasing plastic load and other compounding stressors.

This section aims to examine which organisms may be likely to flourish under the conditions predicted with increased warming and pollution due to the peculiarities of their metabolism. The question is framed in the context of evolutionary history and how past geological changes have led to massive alterations in extant biological systems. The overall goal is to understand their metabolism not in the context of plastic degradation, but their role and contribution to biochemical cycles. Plastic pollution is framed as an evolutionary stressor commensurate with other geologic changes which led to significant alterations to lifeforms existing and dominating at particular times in Earth's history, and the goal is to begin to understand how this forcing will alter biological trajectories and postulate potential communities which will emerge as they exploit multiple interacting environmental changes.

Why might plastic pollution be expected to change microbial metabolism on a global scale? These high molecular weight polymers generate a continuum of plastic waste products, at varying stages of degradation, may contribute to labile, semi-labile, and refractory pools of carbon and matter. A sterile, newly discarded plastic product is recalcitrant, but as its surface and integrity degrades, it may generate particles with which microorganisms can interact. The lifetime of any type of plastic in the environment is poorly understood, and it may be a worthwhile question to consider when these polymers cease to be recognizable as the products they once were. Understanding the polymer generation process, including the components of which they consist, is imperative towards understanding how these materials will break down, and to what category (labile, semi-labile, or refractory) they are likely contributing to most. This has implications for which groups of microorganisms can exploit increasing contamination, whether it be attachment to refractory particles which accumulate a biofilm and function as a

nutrient-concentrating mechanism, or whether it be direct use and metabolism of labile or semi-labile plastic waste products.

There is evidence of multiple components typically found in an ecosystem including autotrophy (photosynthesis), heterotrophy, symbiosis, and predation, showing that the plastic substrate supports a diverse array of organisms with distinct and complementary functions (Zettler et al. 2013).

Nitrogen cycle regulation

Denitrification is one important metabolic pathway in terms of climate due to the production of nitrous oxide, a greenhouse gas, and other NOx compounds. In anoxic environments, use of nitrate as an electron acceptor (Shen et al. 2015) yields reduced nitrogen species such as nitrous oxide. Seeley et al. 2020 examined the impact of different types of plastic pollution in salt marsh sediment on nitrogen cycling, particularly nitrification (successive addition of oxygen atoms) and denitrification (removal of oxygen atoms eventuating inert dinitrogen). Taxonomic identification was carried out using 16S rRNA genotyping, and PCR was used to quantify genes involved in nitrogen processing, combining knowledge of the microbial community with its functional significance. Different forms of inorganic nitrogen were quantified and showed variation between incubation with different polymer types and through time, concomitant with changes in nitrogen-associated gene expression and microbial community composition. Changes to the nitrogen cycle - increasing paucity of oxygen could favor denitrification (an anaerobic process) because nitrate can replace oxygen as an electron acceptor in anoxic environments. At the same time, nitrate may become depleted because nitrification requires the presence of oxygen. Again, the presence of plastic could have cascading

effects–potential increases in heterotrophy could deplete oxygen, which will alter the nitrogen cycle as described above.

Heterotrophy

Opportunistic bacteria may encounter nutrient concentrations on plastic particles which could increase net heterotrophy, resulting in fluxes of carbon from the seawater to the atmosphere, a potential link between plastic pollution and climate change. Commonly found phyla such as Proteobacteria (Pseudomonadota), a Gram-negative Bacteria, exhibits a wide variety of metabolic tendencies including heterotrophy.

Autotrophy

Stress-resistant characteristics present in Cyanobacteria such as buoyancy regulation and specialized cell mechanisms such as N-fixation by heterocysts (Yema et al. 2016) or resistance to cold and desiccation via akinetes allow Cyanobacteria to exploit and promote the otherwise detrimental conditions present and developing in many water bodies (O'Neil et al. 2012; Kudela et al. 2012). For instance, Cyanobacteria can thrive in both eutrophic, turbid waters and at the air-water interface, between which they migrate via modulation of gas vesicle buoyancy and carbohydrate weighting (once they've photosynthesized for some time, they sink); under a dense surface bloom that shades the epilimnion, Cyanobacteria can still thrive at the expense of other species.

Particle-associated vs. free-living

Another metabolic category to which microorganisms can be assigned is whether they exist particle-attached or free-floating. It is likely that within a single organism, different

attachment styles are preferred during different times in the life cycle, and could be considered facultative, obligate, or constitutive. In those that may vary their strategy throughout the life cycle or when experiencing ephemeral conditions, there may be changes in gene expression that could be captured using transcriptomics. There could possibly be regions of the genome implicated in attachment strategies that are dormant but could be increasingly activated as the concentration of microplastic increases, providing areas of concentrated, often limiting nutrients such as nitrogen or phosphorus, which would be especially impactful in the oligotrophic ocean gyres. In addition, attachment-related changes to gene expression or physiology may coincide with biofilm-forming changes to gene expression or physiology. An interesting question could be: will different organisms come to the fore as they possess the genomic and physiological machinery to exploit the attachment and biofilm-related lifestyles, or will there be transcriptomic and physiological changes within already present microorganisms as they increasingly encounter the novel environment produced by plastic pollution.

Xu et al. (2023) reports lower diversity in the particle associated vs. free living microbial fractions, and lower diversity in the particle-associated vs. microplastic-associated communities. There was lower species diversity but higher phylogenetic diversity on the microplastic particles as opposed to natural substrates, and as incubation proceeded, Bacterial diversity decreased.

Plastic degradation

Though plastic may represent a novel evolutionary force to which microorganisms may quickly adapt, it does not necessarily follow that they will evolve to use plastic as an energy or carbon source into which they could incorporate plastic into biomass and/or “degrade” (i.e. cleanup) and therefore mitigate or resolve plastic pollution. For example, the oxygen crisis of the

Proterozoic did not yield microorganisms which disposed of the oxygen, but rather generated an entirely new domain of complex, multicellular life, the eukaryotes. Though this is perhaps a possibility, the uncertainty regarding microbial evolutionary trajectories following increasing contamination levels makes it vital to understand which taxonomic and functional groups will flourish with increasing plastic pollution. It is also possible that, counter to the possibility of microorganisms "cleaning up" plastic (similar to some hydrocarbonoclastic Bacteria "cleaning up" oil spills (i.e. transforming a detrimental substance into one that is inert)), the types of organisms that are able to capitalize on the presence of plastic pollution may generate a positive (with negative consequences) feedback loop by way of their metabolism by for example, generating greenhouse gas waste products, liberating toxic additives by altering the composition of plastic as it moves through the environment, or outcompeting beneficial organisms, thereby reducing their positive (from the perspective of maintaining favorable Holocene climatic and physical conditions) metabolic functionalities such as sequestering carbon dioxide. However, some hydrocarbon-degrading Bacteria are hypothesized to represent a possible unique signature of the plastic-associated community composition, since plastics are derived from hydrocarbons (though they have additives depending on their function to make them more suitable and usable for humans). These include the Cyanobacterial groups Phormidium and Pseudoalteromonas, and the Alphaproteobacteria group Hyphomonadaceae. For hydrocarbon-degrading microorganisms which could ostensibly metabolize components of plastic, or use it as a carbon source–into what will the plastic be transformed? Understanding which groups are enriched on plastic at different stages of its decomposition will enable an understanding of what the long-term environmental effects of plastic will be through knowledge of their metabolism.

Oceanospirillales and Alteromonadales are hydrocarbonoclastic Bacteria present on plastic debris across different environments indicating that their evolutionary history may predispose them to an ability to take advantage of plastic pollution. Dussud et al. (2018a) also found hydrocarbonoclastic Bacteria to be enriched (~30%) on plastic polymers compared to ~4% in seawater.

Ecosystem shifts, thresholds, and biological response - combined forcings

Increasing extreme events such as precipitation (Reichwaldt et al. 2012), drought, and temperature (Arandia-Gorostidi et al. 2017) may exacerbate currently developing, aquatic-specific issues in near coastal or inland ecosystems (e.g., estuaries or lakes) such as eutrophication and the associated development of turbidity, which will threaten resilience (Pecl et al. 2017; Howarth et al. 2011) of ecosystems and the species of which they consist (Loreau et al. 2013; Loreau et al. 2001)—there is a state space within which a given organism (or ecosystem) may exist and thrive (Colwell et al. 2009), and adaptive ability in any particular case depends on the relation between the rapidity (and magnitude, and character) of the disturbance and the time necessary for adjustment (e.g., Hung et al. 2014). Current, human-induced changes are occurring more rapidly than previous shifts of similar magnitude, and it is for this reason that current trends are of such concern (Hansen et al. 2007). To say nothing of their cumulative, nonlinear interactions, the trajectory of any given factor (e.g., Larsen et al. 2014) remains difficult to predict (Persaud et al. 2015). As the state space of numerous environmental variables continues to shift, an increasing number of organisms will find themselves not only outside of their optima, but approaching and often surpassing the boundary beyond which persistence is impossible (Lepš et al. 2011; Litchman et al. 2007; Demirel et al. 2002).

Plastic represents one of many human-induced ecological changes that is putting pressure on ecological configurations as they currently exist. Another such change is warming-induced sea-level rise, which is causing changes to tidal ranges, thereby disrupting the patterns to which organisms in the coastal zone are accustomed (Idier et al. 2017). For example, increased sea level will cause increased tidal amplitude, so that areas that were once only submerged during high tides or storm surges are now always underwater. This will alter sedimentary ecosystems and their associated organisms such as those that burrow and provide aeration as do macroinvertebrates or bivalves. This will in turn alter biogeochemical cycling such as possibly increasing low-oxygen areas, as well as changing the physical properties of the sediment. This is important, as coastal sediments receive plastic debris input from both the ocean (flood tides) and terrestrial (ebb tides) sources, and may represent an important plastic sink. Recent publications (Waldschläger et al. 2022; Paduani 2020) have put forth frameworks for understanding plastic transport by comparing them to paradigms for considering natural sedimentary particles (i.e. silt, sand, clay, and gravel) in an attempt to understand their transport. Characteristics such as size, shape, and density, as well as tendencies to interact with other particles or organisms (dependent on surface charge or hydrophobicity, for example), are those emphasized to be most important in determining movement. In addition, physical processes in the environment will affect movement, such as turbulence, diffusion, currents, eddies, or wave action. Which of these processes is most important for movement of sedimentary particles (whether they be natural or synthetic) depends on the characteristics of a particular environment: depth, width, bathymetry, distance to the ocean, tidal energy, presence and characteristics of vegetation, and fetch, for example. Coastal systems may therefore be especially vulnerable to these combined stressors (Doukakis 2005).

Ecosystems may be trending toward a tipping point due to accumulating circumstances and then be pushed by a discrete event so that the shift is falsely attributed to that event (Hughes et al. 2013). Plastic could be one such destabilizing force which tends to disrupt the ecological communities which have developed over long periods of time through coevolution in the relatively stable conditions of the Holocene. Studies could reveal indicators that could be used to understand how multiple anthropogenic stressors are reorganizing ecological communities in a short period of time.

For example, alterations to global ocean currents due to increasing heat absorption along with the transport of massive amounts of plastic pollution, most of which is microscopic, may restructure biological communities responsible for nutrient and carbon cycling, possibly causing a feedback that further stretches the stability of the climate system (Ford et al. 2022). Downwelling, low-nutrient zones such as ocean gyres which are dominated by the highly-abundant Prochloroccus and Synechococcus, are also plastic pollution hotspots. How might the input of plastic to these areas alter the composition of these communities? Larger, heavier types of phytoplankton with higher nutrient needs (and silica) that dominate in upwelling zones might also experience changes to their once favorable environments due to the combined effects of multiple stressors.

The deep chlorophyll maximum is a key component of both the microbial loop and biological carbon pump, and has the potential to be altered by plastic pollution. It could change in depth, pigment abundance and proportions, or the taxonomic groups of which it consists. This is a measurable structure directly related to metabolism, notably photosynthesis, where the location and composition will alter the fixation of inorganic carbon into biomass, heterotrophic respiration of that biomass and consequently alter the processing of carbon and nutrients.

Alteration of the deep chlorophyll maximum by plastic pollution represents another instance of the potential for destabilization of established ecological niches which could interact with other stressors such as increasing temperatures and resultant stratification. This will also alter the severity and distribution of gradients that regulate passive motion and the dispersal of organisms and nutrients that rely on diffusive forces for their movement. Different organisms prefer the DCM to be present at different depths (Latasa et al. 2017), supporting the idea that variability in this structure could alter ecosystem members.

Ocean gyres, defined by their low-nutrient, oligotrophic characteristics, also happen to be hotspots for accumulation of marine debris, the majority of which are some type of plastic. This may create an artificial concentration of nutrients and substrate on which microbial ecosystems can become structured (He et al. 2020). This could potentially alter the oligotrophy that is created based on the physical processes which structure these low-nutrient regions. In this way, accumulation of plastic debris and resultant emergence of particle-associated microorganism communities could fundamentally change these historically low-production ocean deserts. This could also increase respiration rates which could lead to further production of climate relevant greenhouse gasses as respiration waste products. Plastic may act to concentrate nutrients and organisms so that distinct microbial communities and unique biogeochemical cycling are present in otherwise low-activity, oligotrophic environments such as the open ocean (Zobell 1943).

Increased temperatures will potentially alter enzymatic activity, with implications for metabolism but also climate (e.g., Dimethylsulfoniopropionate (DMSP) to dimethyl sulfide (DMS), which forms cloud condensation nuclei (CCN) and changes atmospheric albedo). Production of cloud condensation nuclei generated from dimethylsulphide produced by haptophytes and the associated changes to the sulfur cycle is another instance of a linked effect

whereby plastic pollution could cascade through global conditions such as climate by way of altering microbiological trajectories. Antimicrobial compounds such as the Vibrio eliminating andrimid (the production of which decreases with temperature) could be degraded at higher temperatures, acting in concert with other variables to change the checks and balances on populations of certain species and changing the composition of entire communities. For example, the pathogenic Vibrio species responsible for cholera infection in humans is a microbe with a strong affinity for particle-association (Main et al. 2015). In the highly competitive, resource replete environments characteristic of particles in the otherwise oligotrophic ocean, anti-Vibrio compounds (such as andrimid) are produced by competing microorganisms (Long et al. 2005). The production of this compound decreases with temperature–this is an instance of multiple, compounding stressors acting in concert to favor the success of *Vibrio cholerae*, perhaps indirectly. The increasing presence of plastic creates preferable habitat for the lifestyle of this pathogenic microbe, while the check on its success is inhibited by reduction of production of the antimicrobial compound due to increasing temperatures. Vibrio may also simply be an opportunistic colonizer.

Temperature can affect microorganisms in different ways. Metabolism including intracellular and extracellular processes are temperature-dependent, with enzymes and proteins becoming inactive or degraded at very high temperatures. Alternatively, metabolism may become more efficient with modest temperature increase, due to higher energy making collisions between atoms and compounds more likely, or surpassing the activation energy needed for a reaction to occur. Alternatively, temperature changes may create physical differences such as stratification or the amount of a gas dissolved in water, which in turn will favor certain

organisms able to, for example, navigate vertically a stratified water column in order to access nutrients, light, and inorganic compounds such as carbon dioxide.

Evolutionary trajectories - mechanisms of change

There is expectation of plastic to be a novel particulate habitat and for this reason a possible evolutionary force. Mutations and genetic drift may provide the starting point from which deterministic selective forces may proceed, either generating new functionalities and possibly taxonomies, or favoring certain currently existing modalities. Plastic pollution exists in an almost unfathomable spectrum of materials including variable sizes and chemical properties. Once plastic particles fall below a certain threshold (usually termed nanoplastic), they are almost impossible to detect (Adhikari et al. 2022). So not only will there be larger substrates (macroplastic but also, from the perspective of microorganisms, microplastic) that will either interact directly with macrobiota or provide an attachment opportunity for microorganisms, but there is also the possibility and likely the probability of smaller plastic pieces crossing cell or tissue membranes. This further complicates the task of predicting how plastic pollution will affect biological trajectories. Strategies to encapsulate, remove, or render harmless these tiny plastic remnants could emerge quickly due to the short generation times and vast diversity of microbial solutions to all variety of problems given their deep evolutionary histories. Alternatively, organisms with large genomes (possibly corresponding to functional redundancy) such as dinoflagellates may have the genetic blueprints on which to rely when encountering the novelty of plastic pollution.

Horizontal gene transfer is a major driver of microbial evolution; microplastics may provide a substrate on which to concentrate this process (Arias-Andres 2018). Given this complication of vertical evolution, what would a graphical representation of microbial evolution

look like, and what role would a novel substrate such as that provided by microplastic play in shaping it? Does plastic pollution increase the influence of genetic drift on structuring populations (because microbes closest to a surge of plastic particles or the (in)organic amalgam of the biofilm associated with them will be favored, even considering an entirely motile population (i.e. those that are closer will get there faster)). Other authors have considered how current trends may alter biological trajectories–modeling studies such as Heneghan et al. 2024 predict a greater proportion of biomass to be made up of prokaryotes in global oceans under predicted climate shifts such as warming. This could be attributable to the fact that organisms with deep evolutionary history such as Cyanobacteria, or even Bacteria in general which have been exposed to and adapted to billions of years worth of environmental changes may be more likely to survive given current, rapidly unfolding, compounding stressors.

Ecological significance of plastisphere metabolism

Galgani & Loiselle (2021) explore the need to understand interactions between multiple stressors including rising temperatures and ocean acidification by examining the effect on the carbon cycle of introducing large quantities of plastic into the ocean. The interplay between increases in sinking velocity due to accumulation of mass (organisms and POM or DOM) may be offset or countered by the increase in production of organic matter fueled by the use of microplastic as a habitat and carbon substrate facilitating Bacterial heterotrophy (Conan et al. 2022). This also may represent a cycle causing sinking and resuspension out of the surface microlayer and euphotic zone (Rummel et al. 2017). This dynamic is important because it may clarify whether increasing concentrations of microplastic will ultimately sequester carbon to depth, or act as a source of carbon to the atmosphere due to increased rates of Bacterial respiration at the air-water interface. Higher Bacterial metabolism could also lead to oxygen

depletion in areas highly polluted with plastic in a way similar to that caused by eutrophication (Kvale et al. 2023).

In general, degradation of plastic begins mechanically or chemically (e.g, cleavage of bonds and generation of new functional groups via absorption of energy caused by UV light (photooxidation), thermal oxidation, or hydrolysis) which reduces the molecular weight of plastics so that the resulting oligomers are amenable to biodegradation via microorganisms (Oberbeckmann & Lorenz 2020). Biodegradation can culminate in the production of water or carbon dioxide which can then be assimilated into biomass (Harrison et al. 2018). Plastics upon degradation produce different hydrocarbon gases such as methane, ethane, propylene, and ethylene when exposed to the solar radiation or microbial degradation, which are important for other stressors such as climate change (Wani et al. 2023).

Plastic is likely to affect the biological carbon pump both by introducing particles of variable buoyancy (especially as compared to particles of natural origin such as fecal pellets) and interacting with the organisms comprising the pump. The latter is why plastic contamination is the reason plastic is likely to affect multiple nutrient, carbon, and oxygen dynamics. The link between taxonomic changes due to the novel habitat provided by plastics and biogeochemical cycles including the biological carbon pump is differences in metabolic processes of organisms throughout the food web and comprising multiple points and links throughout the biological carbon pump. As particles are colonized, respired, remineralized, and degraded, complex interactions between autotrophy, heterotrophy, symbiosis, parasitism, and viral affects offer the opportunity for plastic to disrupt or alter multiple links in this ecological network responsible for the biological transport of inorganic carbon throughout the water column and atmosphere.

Degradation varies with polymer type and environmental context such as aquatic (salinity, temperature, depth, nutrient availability), or terrestrial (beach, soil, agricultural land, urban area) habitat, which will also affect which microbial communities are naturally present at the inception of degradation, or proximity to plastic source. Microorganisms secrete enzymes while attached to plastic particles which can cleave the polymer into oligomers with different characteristics, notably a greater susceptibility to biodegradation (Santo et al. 2013). Microorganisms degrade MPs using oxygen as an electron acceptor in the case of aerobic biodegradation (Yoshikawa et al. 2016) and the metabolic byproducts leached from the breakdown of plastic can enter and impact the carbon cycle in the ocean (Rogers et al. 2020).

Accumulation of the "ecocorona" consisting of the biofilm and extracellular polymeric substances increases the density of microplastic particles which if sufficient can lead to sinking, perhaps to the benthos, and incorporation into the sediment where it becomes largely immobile (Rummel et al. 2017). This also removes plastic from the euphotic zone which will lead to reduction in UV-induced degradation and also preclude the persistence of photosynthetic organisms which rely on sunlight.

Quorum sensing alters population-level transcriptomes in the presence of relevant substances, potentially upregulating certain functions such as hydrolysis or phosphatase activity. The processes, if sufficiently enriched, could alter metabolism of carbon and thus its distribution in the ocean (decreasing the amount of carbon sequestered at depth, or increasing the labile fraction of carbon by upregulating microbial functions which intercept it and recycle it back into the active pool, including as a heat-trapping gas in the atmosphere).

Planetary boundaries

Plastic, when considered in the framework of planetary boundaries, belongs to the "novel entities" category. This category remains poorly quantified due in part to its generality–from nuclear waste to genetically modified organisms, setting a limit not to be transgressed is not straightforward (Richardson et al. 2023). This review then gathers information and puts forth hypotheses about how plastic, as a novel microbial habitat, may alter evolutionary trajectories, in an attempt to understand how this novel entity (which in itself comprises many types of compounds, polymers, and chemicals) may play a role in shaping Earth when it is no longer safely contained within waste management pipelines. The effect of plastic on the ocean could also be analogous to another planetary boundary, land use change, principally deforestation and resultant effects on primary productivity, because of its impact on phytoplankton communities and changing their role in net primary productivity. This then illustrates another link between the planetary boundaries of land use change, novel entities, and climate change, as well as biogeochemical cycles of nitrogen and phosphorus. This review then culminates in viewing the effects on multiple boundaries driven by evolutionary changes to microbial communities resulting from plastic pollution.

The novel entities planetary boundary to which plastic belongs is unique in that by its nature, the global forcing it represents is entirely unknown. Whereas other categories are better understood, for example, the relationship between greenhouse gas emission and temperature increase, the suite of pollutants collectively termed "novel entities" are still poorly understood. This is why it is important to begin compiling information to generate hypotheses about possible trajectories of biological systems as they are affected by and interact with the other changes underway.

Another consideration is the rapidity and projected increases in accumulation of plastic waste in the environment. This brings into focus a discussion on the processes of evolution and the timescales at which they operate–an interesting basic science question is whether plastic will cause microbial organisms to evolve (and how is this defined–speciation, or is it a change in the proportions of extant groups?) more quickly by the nature of their interaction, as compared to larger organisms for which the effects of plastic might be more predictable and uniform (e.g., smothering, starvation). This raises another question: the differences between micro-level environmental variation and the environments we tend to group by (e.g., coastal, marine, boreal forest, desert).

Lavers et al. 2022 present the argument that plastic pollution and climate change are both components of the planetary emergency and must be addressed in tandem. For example, plastic generates GHG emissions at every stage of its life cycle, and they predict that plastic will likely outpace the contribution of the transportation sector by 2050 if trends continue as predicted. The review also highlights the abuse of human rights inherent in the plastic industry, where affluent countries or areas export the toxic, often carcinogenic petrochemicals to lower income, underserved areas, who are much more likely to report crisis-level conditions.

In particular, the community of St. James Parish, Louisiana is known as “Cancer Alley” for the astronomical rates of illness of all varieties. Once a flourishing agricultural area where one could step outside and breathe the fresh air, the current reality is that every lungful of contaminated air, every sip of contaminated water, is causing illness in every member of the community, including children and the elderly. This situation is not unique, in that the toxic constituents of plastic are leaching into the environment (disproportionately affecting low

income communities) at every step of their life cycle from production to its fate once it has served its purpose.

Conclusion

An outstanding scientific question is understanding how compounding stressors are likely to alter biological trajectories, with a focus on biosphere-dominating, biogeochemical-regulating microorganisms in all three domains of life. In terms of basic science questions, there are issues regarding how biological categories are generated and maintained with respect to their evolutionary origins and the geologic conditions from which they emerged. A consideration is how these categories classify functionality and specifically the relationship between organisms and their interaction with the environment. It addresses questions such as: how do microbial ecosystems on plastic assemble? Does this process occur mostly at random, at the whim of environmental conditions in a given instant in time and space, or are there patterns which repeat that reveal themselves if the system is considered from a particular angle (e.g., function as opposed to taxonomy)? With increasing biological resolution (e.g., species vs. phylum), does increasing specificity offer increasingly useful information with regard to cellular functioning? In terms of application, this review seeks to understand how the microorganisms at the base of every food web may alter how energy and matter flows upwards and outwards in the presence of plastic. Understanding these microorganisms and their metabolisms may offer the possibility of interventions that could potentially alter evolutionary trajectories into a more favorable configuration.

It is difficult at this time to fully comprehend how plastic may be altering both taxonomic composition and functionality of resident microbes. One complicating factor is that for a given

species, there may be different ecotypes found depending on where in state space they exist at a given time (Lowry 2012). For example, globally distributed Cyanobacteria genera Prochloroccus and Synechoccus may have variable physiology along nutrient gradients so that though they may be detected by their genetic signature, their metabolic contribution may be unknown without further laboratory categorization, which may not be feasible.

Another consideration is that the true extent of microbial diversity, its geological origin, phylogenetic relationships, and functional capacity for systems not polluted with plastic (which at this point, is a statement devoid of meaning) remains largely unknown and uncategorized. The methods used to detect microorganisms include microscopy (which provides a more general visual illustration), amplicon sequencing, or ribosomal DNA could also invite ambiguity and deter firm conclusions. In addition, there are multiple paradigms of how organisms should be classified and gene or protein annotations are often messy and uncertain. Further, the names of organisms and groups of organisms change frequently, for example the replacement of "Proteobacteria" with the name "Pseudomonadota", which is still a topic of debate among researchers.

This review seeks to summarize how plastic microbial communities in various habitat types differ in their metabolism and evolutionary history, how this depends on ambient physical conditions or substrate and the resulting changes to the functioning of the plastic-associated ecosystem. This is important when considering how the increasing prevalence of plastic pollution will affect biochemical processes performed by microbial organisms such as transformation of matter and nutrients, including their possible contribution to climate change in conjunction with what is underway due to incineration of fossil fuels for energy. Finally, this

review explores the role of plastic pollution as a global forcing and planetary boundary threat as previously defined (Persson et al. 2022).

Figure 22. Global map of study sites reporting plastic-associated taxonomic information.

Table 14. Taxa reported to be associated with plastic at the level of phylum

Citation	Phylum	Study Site	Publication Date
Zettler et al. 2013	Bacillariophyta	North Atlantic Subtropical Gyre	2013
	Cryptophyta		
	Cyanobacteria		
	Dinoflagellata		
	Proteobacteria		
Vaksmaa et al. 2021	Acidobacteria	Island of Elba, Mediterranean Se	2021
	Actinobacteria		
	Bacteroidetes		
	Cyanobacteria		
	Planctomycetes		
	Proteobacteria		
Frère et al. 2018	Bacteroidetes	Bay of Brest (Brittany, France)	2018
	Cyanobacteria		
Bryant et al. 2016	Bacteroidetes	North Pacific gyre	2016
	Bryozoa		
	Cyanobacteria		
	Proteobacteria		
Mughini-Gras et al. 2(	Bacteroidetes	Dutch portion of river Rhine	2021
	Cyanobacteria		
	Proteobacteria		
Basili et al. 2020	Bacteroidetes	Mediterranean Sea (Ancona & N	2020
	Proteobacteria		
Delacuvellerie et al. 2	Bacteirodetes	Mediterranean Sea	2019
	Cyanobacteria		
	Proteobacteria		
	Verrucomicrobia		
de Tender et al. 2015	Actinobacteria	Belgian part of North Sea	2015
	Actinomycetota		
	Bacteroidetes		
	Proteobacteria		
Dudek et al. 2020	Bacillariophyta	Caribbean Sea	2020
	Bacteroidetes		
	Proteobacteria		
Kettner et al. 2019	Chlorophyta	Baltic Sea	2019
	Chytridiomycota		
	Cryptophyta		
	Picozoa		
Xu et al. 2019	Bacteroidetes	China coastal sea	2019
	Proteobacteria		
Dussud et al. 2018	Bacteroidetes	Mediterranean Sea	2018
	Cyanobacteria		
	Proteobacteria		
Dussud et al. 2018a	Proteobacteria	Sewater incubation experiment	2018a
Kirstein et al. 2018	Actinomycetota	Incubation experiment	2018
	Arthropoda		
	Bacteroidetes		
	Chloroflexota		
	Ciliophora		
	Gastrotricha		
	Nematoda		
	Nitrospirae		
	Planctomycetota		
	Porifera		
	Proteobacteria		
Erni-Cassola, G., Wrig	Proteobacteria	Mediterranean Sea	2020
Xu et al. 2023	Bacteroidetes	Beijing, China - Dongshei River	2023
	Chloroflexota		
	Proteobacteria		

Appendix

An experimental setup aimed at quantifying commonly used plastic degradation rates was deployed on the Horn Point Laboratory campus in Cambridge, MD. Six types of plastic, with ten replicates of each type were affixed to a wooden board and placed in different locations to measure degradation rates. A control board was placed atop the AREL building, and boards were deployed at subtidal and intertidal sites in the Choptank River. After 60 weeks, there was negligible mass loss for all six types of plastic. This experimental setup highlights the long degradation times of all commonly used types of plastic, and demonstrates the need for measuring differences in other plastic properties to quantify mass loss, such as surface alteration or changes in tensile strength.

Figure A1. Control board deployed on top of building with six types of plastic: PP, PVC, LDPE, HDPE, PS, and PET.

Figure A2. Boards deployed at subtidal and intertidal sites in the Choptank River.

Figure A3. Boards deployed at intertidal and subtidal sites in the Choptank River.

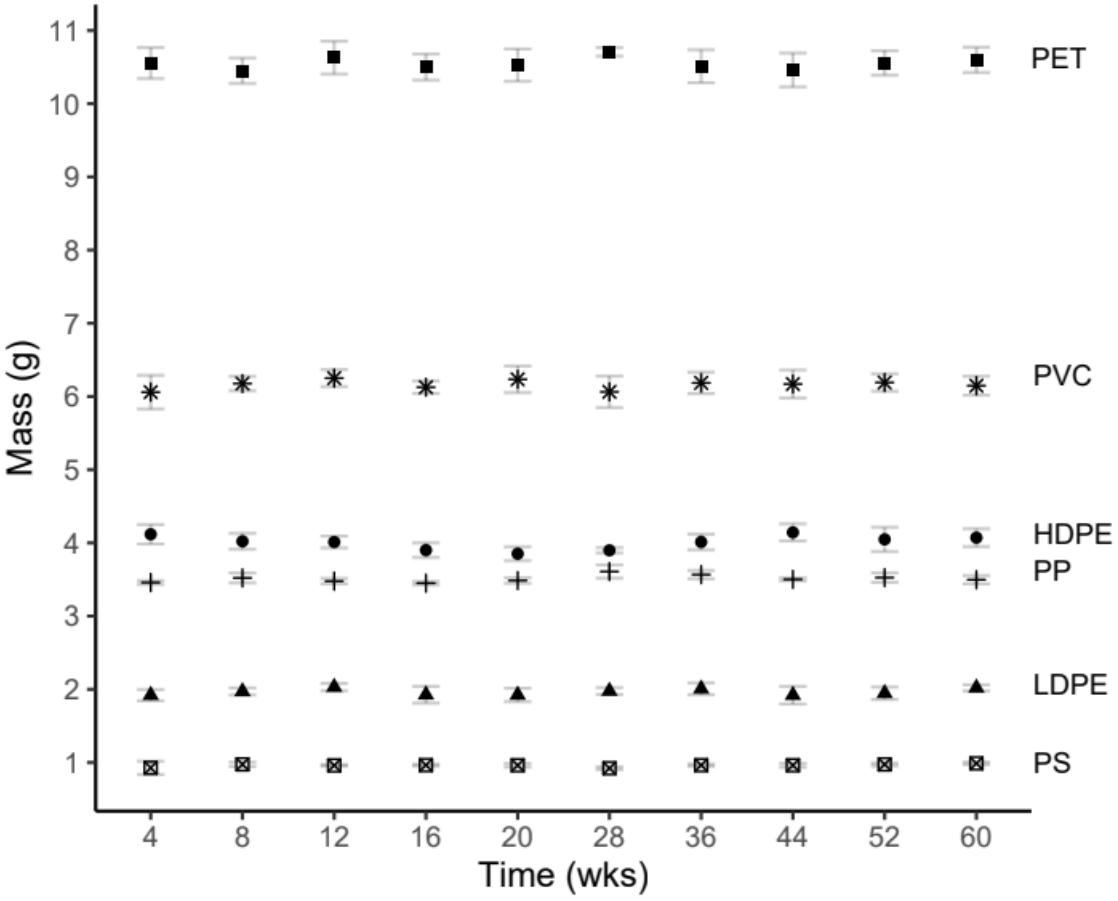

Figure A4. Average plastic mass does not decline over 60 weeks regardless of polymer type.

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