Colonization of plastic pollution in Lake Michigan surface and deep water microbial communities

EEB 447: Surface vs. Deep Group Report

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Abstract

Despite efforts to promote eco-friendly and reusable items in our daily lives, plastics can be found in countless products, many of which make their way into bodies of water across the globe. All plastics that make their way into marine ecosystems are teeming with microbial life, which form biofilms on plastic surfaces that can work to enzymatically degrade natural or synthetic compounds, engage in unique genetic exchange events, and many other specific metabolic interactions that are not yet well understood (Miao, 2018). This study aims to determine how water depth and its associated physicochemical parameters impact the diversity of plastic microbiome communities. We hypothesize that if depth influences the structure and diversity of microplastic biofilms, then the surface and deep will have distinguishable compositions of both types of bacteria and composition of the biofilms, as different environmental conditions are known to select for distinct microbial metabolisms. For our experiments, we compared microbial diversity and composition for biofilms taken from several locations around Lake Erie and Lake Michigan to corroborate findings of plastics acting as vectors for microbes to colonize and proliferate in shallow and deep water biofilms. To investigate our samples, DNA extraction for 16S rRNA sequencing was performed and samples were prepared for flow cytometry, SEM, and confocal microscopy analysis. Our results showed our control data, which was supposed to be "sterile" -- to have much lower phenotypic diversity than any of the samples taken from the lake. From our data, we were able to find which families of bacteria existed on the plastic vs in the water using a 3µl and .22µl filter. This showed us that in each environment (part of Lake Erie), samples taken from the plastic had higher levels of *Proteobacteria* and *Retaria* families of bacteria than what was seen in either of the other two samples from each location. This leads to the conclusion that both of these families are important in biofilm formation on plastics in the lake, though further experiments, including trying to grow biofilms without the presence of these two families to determine their impact on biofilm formation on plastics, must be done to confirm this data. (360 words)

Introduction:

Despite efforts to promote eco-friendly and reusable items in our daily lives, plastics can be found in countless products, many of which make their way into bodies of water across the globe. Due to the slow breakdown of plastics, many of these materials will circulate in coastal or marine environments for hundreds of years (Woods Hole Oceanographic, nd.). Plastics are already known to have detrimental effects on aquatic wildlife, but some of those impacts, such as effects on microbial life, are much harder to assess. Microplastics specifically impact microbes in a number of ways, including creating an environment for prolific gene exchange; however, not all interactions between microbes and microplastics are beneficial. For example, microplastics accumulation in microbes is believed to pose an adverse effect on metabolic activity (Oliveira, 2016). Since most of these specific interactions have yet to be explored, characterizing the relationships between microbes and microplastics will help generate understanding and potential solutions to limit the impact of microplastics on aquatic microbes and on how they conversely impact human life.

Biofilms can colonize the surface of microplastics in aquatic environments: a group of bacteria that grow on surfaces often as a way of resisting environmental extremes. The composition and breakdown of plastics impact the growth of microbes in these biofilms, as many species have been known to metabolize plastic-derived compounds (Tetu, 2020). Plastics can have a range of physical and chemical properties, varying in features such as density, resilience, and breakdown time. Synthetic plastics, such as the petroleum-derived polymer polyethylene, were widely produced for their durability; however, they are much slower to degrade than plastic types which are made of less synthetic materials (Mammo, 2020). After bacteria adhere to plastic surfaces, they secrete enzymes that induce degradation, leading to the release of additives and monomers as the plastic compounds break down. For example, plastic metabolizing microbes have been found to secrete specific enzymes onto plastic surfaces that penetrate and break bonds within the polymers, meaning the chemical properties of the plastic affect its coloniality (Mammo, 2020). We would expect such variations in plastic properties to influence which microbes are able to colonize on its surface or even directly metabolize it, as the enzymatic breakdown speed affects variables such as the number of pollutants released, surrounding dissolved oxygen content, and physical space needed to create biofilms in the niche (Mohanan, 2018).

Microbes in biofilms work together to break down plastics, which leads to varying breakdown times based on the composition of the microbial community. There are a variety of different genes, including those for biofilm formation to heat resistance, which are directly involved in allowing bacteria to thrive in a given niche (Oliveira, 2016). Due to this variation, each species of bacteria has a set of genetic and physiological conditions that allow it to thrive most efficiently in a certain environment, causing genes that are more favorable for survival to become more prominent in the community. For example, this might mean a specific temperature and pressure which is only found in the first 40 ft of Lake Superior leads to bacterial species which thrive in those conditions to likely be found in the corresponding microbial community but not in deep water communities.

According to such observations, we cannot expect microbial composition in a body of water to be identical across depths, as the surrounding conditions vary throughout the water column. Deeper water tends to be much colder and devoid of light, which impacts phototrophic microbes, and decreased water temperature has lower dissolved oxygen levels, which influences the prevalence of anaerobic versus aerobic microbes (Lear, 2021). Lower temperatures also decrease metabolic rates, which can be visualized by differences in biofilm size and robustness. In past studies, deep ocean environment plastics were shown to take twice as long to degrade by enzymatic digestion due to decreased temperature and dissolved oxygen when compared to more shallow oceanic environments (Hirai, 2005). In addition to being subjected to higher temperatures, plastics that floated at the surface were subjected to natural photodegradation, which could impact the resulting nutrient pool for uptake by surrounding microbial life. These surface water samples will dissolve faster due to natural elements of sunlight and warmer temperature, and this breakdown of plastics could attract different species of bacteria compared to deep water biofilms (Bhateria, 2016). Additionally, plastic that originally floated on the surface may eventually sink into deeper environments, which would further affect the microbial diversity and plastic-derived communities found at greater depths.

Although most plastic types do not move to the bottom of a body of water, high-density plastics will sink more readily, further impacting which microbial communities are involved in the resulting biofilm proliferation. Processes in deep marine environments that impact the degradation of plastics, how the plastics themselves change in deep water environments, and how these depths impact the interactions of microplastics and microbes have not been well studied (Miao, 2018). All of this information has gaps pertaining to the characterization of marine microbes and how subsequent microplastics biofilms vary with depth. This study sought to determine how water depth and its associated physicochemical parameters impact the diversity of plastic microbiome communities. Because different environmental conditions are known to select for distinct microbial metabolisms, we hypothesize that if depth influences the structure and diversity of microplastic biofilms, then the surface and deep will have distinguishable compositions of both types of bacteria and composition of the biofilms.

Methods:

We compared microbial diversity and composition for biofilms formed in 11 samples taken from seven locations around Lake Erie to corroborate findings of plastics acting as vectors for microbes to colonize and proliferate in shallow and deep water biofilms.

Sample Collection

Water from Lake Michigan was directly sampled from the surface (1 meter) and at a depth of 183 meters, run through a 20 μm filter, introduced to varying types and concentrations of plastics (0.05, 0.005, and 0.0005 g/mL), and stored in sealed glass jars. For both the deep and surface water samples, two groups of plastics were added to these water samples. The plastics used in group one were LLDPE (aged and unaged), PET (aged and unaged), PS (aged and unaged), LDPE, PHA, and

HDPE (aged and unaged) and only LLDPE in group two, all cut to pieces less than 5 mm in length. The plastics were cut in order to increase the surface area to maximize the concentration of plastic leachates, as well as represent the microplastics known to be found in the lake, recreating naturally-occuring conditions. A control sample group of both deep and surface samples without any additional plastics was also maintained. During incubation, the deep treatments were kept at 4 C in a dark room so that the microbial communities could continue to develop in natural deep water conditions, whereas the surface treatments were incubated at 23 C on a 12 h:12 h day-night cycle. On days nine and 16 of incubation, samples were taken for flow cytometry and scanning electron microscopy (SEM), as described below. Additionally on day 16, the pH of each treatment jar was measured. 180 ml of water from each jar was collected and stored at -80C for later DNA filtration -- to find what free DNA existed in the water, not growing with bacteria on the microplastics.

Sample Jar Number Conditions

	SURFACE	DEEP
No Plastic (Control)	37, 38, 39	31, 32, 33
LLDPE *linear low density polyethylene*	46, 47, 48	67, 69
LDPE *low density polyethylene*	49, 50, 51	68, 70, 71, 72

Flow Cytometry

For flow cytometry analysis, a 1 ml sample from each jar was fixed with 5 µm 80% glutaraldehyde and flash-frozen in liquid nitrogen at -80 C to preserve for flow cytometry. Flow cytometry allows for single-cell analytics, therefore defining the chemical and physical properties of our sample. For flow cytometry analysis, samples were stained using SYBR Green.

Scanning Electron Microscopy

For scanning electron microscopy, individual plastic pieces were also taken from each jar, fixed in 1% glutaraldehyde, and flash-frozen in liquid nitrogen at -80C. To prepare plastic samples for scanning electron microscopy, each piece was submerged in PBS to remove debris before being bathed for 15 minutes in ethanol at concentrations of 25%, 50%, 70%, 95%, and two fresh baths of 100%. They were then submerged in a solution of 2:1 ethanol:HMDS, followed by a 1:1

solution, and lastly a pure HMDS solution. After being dehydrated for 12 hours in a desiccator under a fume hood, plastic pieces were affixed onto double-sided carbon tape on an aluminum stud. The pieces underwent another 12 hours of dehydration before being sputter-coated with gold and immediately imaged by SEM at the University of Michigan Microscopy and Image Analysis Laboratory (Duhaime, 2019).

Confocal Microscopy

For confocal biofilm analysis, previously frozen samples were thawed on ice and then submerged in 0.125% glutaraldehyde for 15 minutes in the dark before being flash-frozen in liquid nitrogen again overnight for fixation. Plastic samples were then transferred to a 1000:1 DAPI:PBS solution for staining that had been filtered at 0.22μm to remove all materials larger than the microbial communities being analyzed. After 15 minutes of incubation in the dark, they were transferred back to a 1xPBS solution and stored at 4C until analyzed.

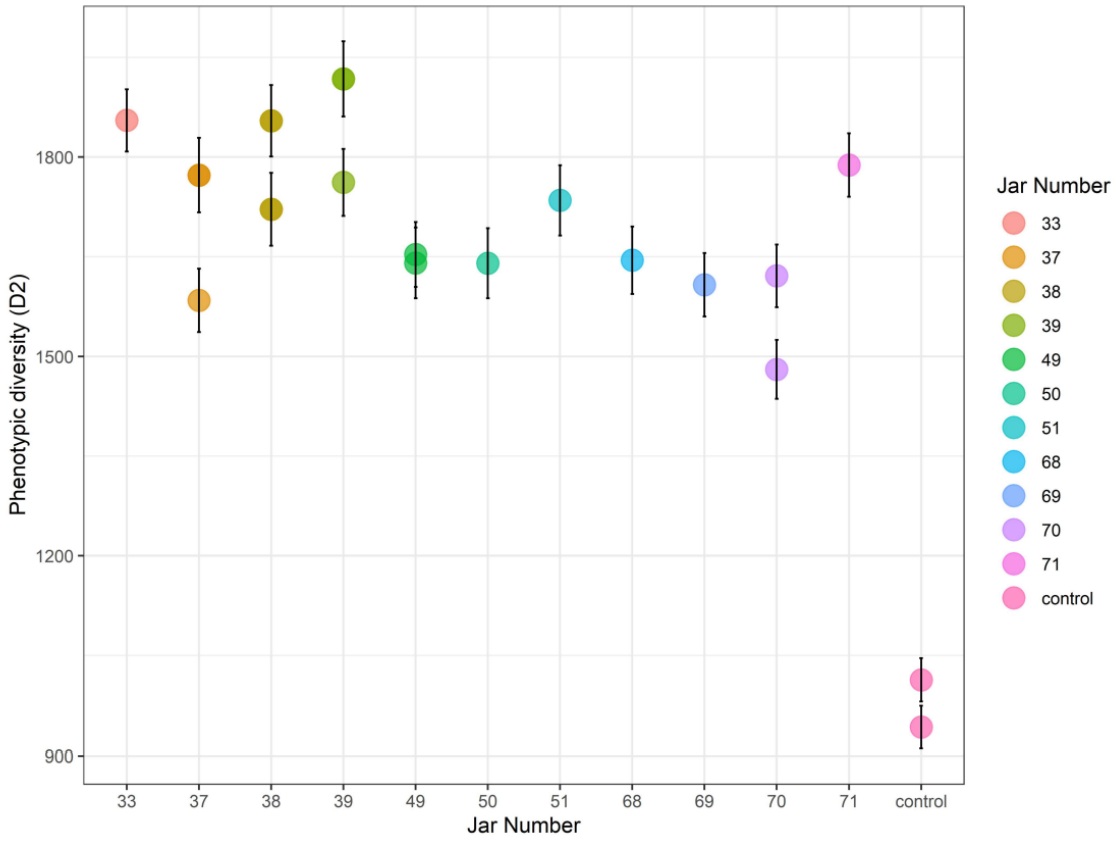
16S rRNA gene amplicon sequence analysis

To prepare to extract DNA from our samples, materials were treated with ATL and Proteinase K to lyse cells. Filters that had been previously folded inwards to be stored had been flipped inside-out to ensure enough material was collected (Duhaime, 2019. Using the Qaigen DNeasy PowerBiorilm DNA extraction kit (cat. No. 24000-50), samples were eluted in 50 μL AE buffer and kept at 4 C until submitted for sequencing.

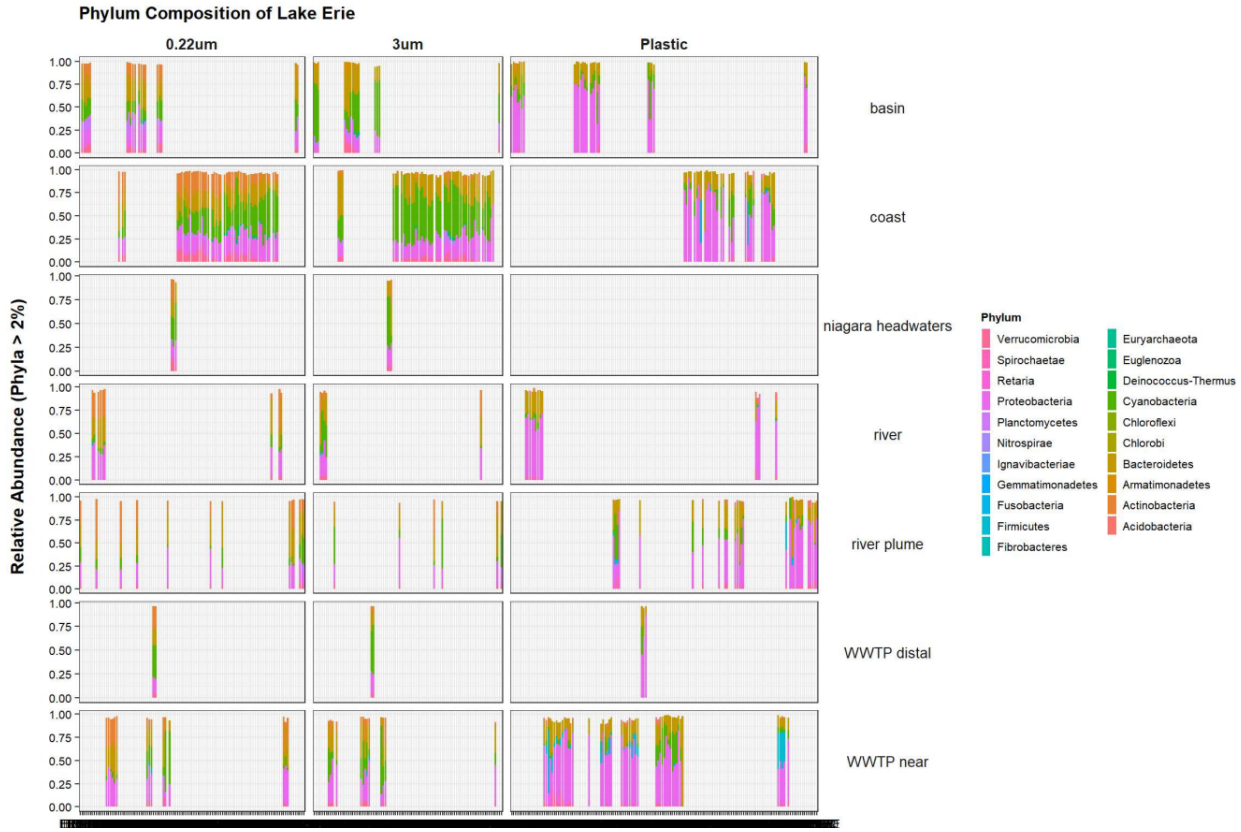
DNA sequence data was generated by amplifying the 16S rRNA-encoding gene using a modified set of dual-index 16S EMP primers that trigger the V4 region developed by Walters et al (Walter, 2016) then sequenced using the Illumina MiSeq system at the University of Michigan Microbiome Sequencing Core. Sequencing data was processed using the mothur software package and analyzed using the phyloseq package in R.

Results:

Phenotypic Diversity of Lake Michigan water samples calculated from flow cytometry using SYBR stain: Jar 33 contains no plastic, but is a deep lake sample. Jars 37, 38, and 39 contain no plastic, but are surface water. Jars 49, 50, and 51 contained low density polyethylene in surface lake water. Jars 68, 70, and 71 also contained low density



polyethylene, but in deep lake water. Jar 69 contains linear low density polyethylene in deep lake water.



Lake Erie Phylum Composition from 16S rRNA: Between all locations, 0.22um and 3um samples show the most similarity in composition compared to Plastic samples. Between all sample types, Niagra Headwaters showed very little identifiable Phyla.

Discussion:

Our results showed our control data, which was supposed to be "sterile" -- contained much lower phenotypic diversity than any of the samples taken from the lake. This verifies that our data from lake water has much more microbial content than sterile water. Jars 37, 38, 39, and 70 all had two data points for diversity from two flow cytometry samples from the same jar; this confirms that the samples were similar, but not the same as one another, as samples under the same conditions will grow different biofilms and utilize different bacterial species.

When comparing samples without plastic in deep versus surface lake water, the diversity of phenotypes from flow cytometry were similar. The same similarity in diversity was seen between LDPE-containing surface versus deep water. Thus, our change in water sample depth did not see an impact on microbial phenotypic diversity. To prove that this was the case we would need to repeat the experiment and use another gene which is highly conserved such as the *rpoB* gene to determine if the 16S rRNA gene was too conserved and thus did not allow us to see a true picture of the microbial phenotypic diversity.

However, LDPE jars of both surface and deep lake water had lower phenotypic diversity than surface and deep lake water jars without any plastics. The sample containing LLDPE from deep water showed similarly lowered diversity to the LDPE samples. This shows that regardless

of depth, low density polyethylene and linear low density polyethylene inhibits the phenotypic diversity of microbes in lake water. However, especially with the linear low density polyethylene, more trials will be needed to confirm this relationship. More trials with no plastic in deep lake water samples are also needed.

Due to time and resources, our methods did not allow us to draw conclusions about the structure of the biofilms. That said, we were able to find which families of bacteria existed on the plastic vs in the water using a 3µl filter and then a .22µl filter from samples taken in multiple locations in Lake Erie. This showed that plastic-based samples had higher levels of *Proteobacteria* and *Retaria* families of bacteria than what was seen in either of the other two samples from each location. This leads to the conclusion that both of these families are important in biofilm formation on plastics in the lake. More experiments, including trying to grow biofilms without the presence of these two families to determine their impact on biofilm formation on plastics must be done to verify this data.

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