7-22-2021

Effects of Microplastic on Freshwater Benthic Ecosystems: toxicity and impacts on biogeochemical cycling

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Effects of Microplastic on Freshwater Benthic Ecosystems: toxicity and impacts on biogeochemical cycling

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science

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Rochester, NY

July 22nd, 2021

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Table of Contents

Acknowledgements

I would like to firstly extend my deepest thanks to my advisor, Dr. Christy Tyler, whose expertise, patience, and kindness made this project possible. I truly cannot thank her enough for giving me purpose during a time that I couldn't have been more lost, and reminding me why I wanted to pursue environmental science in the first place. Her mentorship has truly made me better person.

I would also like to thank my committee members: Dr. Nathan Eddingsaas, Dr. Matthew Hoffman, and Dr. André Hudson; working on this interdisciplinary project alongside such a diverse group of experts helped me evaluate my project from a variety of perspectives, allowing me to expand my thinking while realizing how much I enjoy working with and learning from others. I would also like to thank the New York Sea Grant College Program and National Oceanic and Atmospheric Administration, as well as the RIT College of Science Dean's Research Initiation Grant for funding all this work.

Additionally, this project would not be possible without all the members of the Aquatic Ecology Lab, especially Ben Hamilton, Evan Squier, Bre Pollard, and Avery Miller; for letting me constantly ask them silly questions and shadow them when running lab protocols or instruments. I also want to thank everyone on the plastics research team, especially Carmella Bangkong, Rocky Diaz, Erika Fernandez, and Ray Malinowski. Their friendship and help throughout this project has meant so much.

Lastly, I would like to thank my family and friends (Mom, Tato, Michael and Cait, Nasha, Sophie, and Ibrahim) who have supported me throughout this journey. Thanks for letting me talk out my research whenever needed, for family dinners watching AEW, and for chaotic hours long FaceTime calls from far away. Thank you all so very much.

List of Tables and Figures

ABSTRACT

The accumulation of plastic debris in waterways is an increasingly complex environmental problem due to the ubiquity and magnitude of plastic debris across freshwater ecosystems, along with the unknown impacts on ecosystems and public health. However, “plastic” is a catchall term for numerous polymers with unique physical and chemical properties. Further complicating the prediction of risk, plastic characteristics may change from environmental exposure, with changes in density, adsorption or leaching of toxins, and accumulation of biofilms that further influence material properties, fate and impact. Recent studies suggest that a substantial proportion of plastic entering freshwater systems is deposited in the benthos, where organisms may be exposed to plastic-associated toxins indirectly or through consumption by invertebrates and higher trophic levels. The presence of toxic materials may hinder crucial ecosystem functions carried out by microbes and invertebrates that are key drivers of benthic ecosystem function and benthic-pelagic coupling. This research addresses the diversity that exists among plastic polymers, and studies how the ecotoxicology of plastic varies both spatially and temporally in the environment. Using toxicity bioassays with the oligochaete *Lumbriculus variegatus* and microcosm experiments, I investigated the eco-toxicity and impacts on biogeochemistry of 6 common consumer plastics in pristine form and after aging in Lake Ontario and a stormwater pond in the watershed, and how the impacts of plastic on benthic organisms extends to impact ecosystem function. While all polymers studied had sublethal impacts on *L. variegatus*, there were unique impacts on nitrogen and phosphorus cycling and ecosystem metabolism among polymers. These effects shifted after environmental exposure and varied between sites, with some materials losing toxicity and others gaining, and unique impacts to biogeochemistry persisting over time. These

results suggest that ecological impacts of plastic pollution are complex, varying among polymers, water bodies and exposure time.

Chapter 1.
Introduction

1.1. Overview of plastic pollution.

Mass production of plastics dates back to the 1950s, providing a low-cost yet durable material to create long-lasting goods. While plastics have demonstrated a practical benefit, cumulative global production has surpassed 6,300 million tonnes (Geyer, et al. 2017). Of this total, it is estimated that only 30% remains in use and only 10% of this total is recycled properly (Geyer, et al. 2017). With the remaining 60% being discarded, immense plastic waste has been left to enter landfills and waterways (Hoellein, et al. 2014; Driedger, et al. 2015), with unclear fate and impacts. Plastics enter freshwater environments from a myriad of sources, including litter deposited at shorelines, and by less visible routes, including atmospheric deposition, wind, wastewater treatment effluent, and stormwater runoff (Baldwin, et al. 2016). Runoff is a significant source of automotive plastic pollution in particular, releasing a variety of materials, including tire-wear particles to aquatic environments (Wik, et al. 2008; Wang, et al. 2017; Lenaker, et al. 2019). Additionally, synthetic textiles have the capacity to escape through both laundry and wastewater treatment processing, directly entering waterways (Baldwin, et al. 2016; Mason, et al. 2016; De Falco, et al. 2018) at an estimated rate of approximately 700,000 microfibers per load of laundry (Napper, et al. 2016) and continue to be released after 10 washes (De Falco, et al. 2019). Corresponding to increased production in recent years, thermoplastic polymers frequently used for single-use items (e.g., polypropylene, polyethylene, and polystyrene) are among the most common observed in both marine and freshwater systems (Browne, et al. 2010), alongside synthetic textiles, such as polyester (Lenaker, et al. 2019).

Plastic has become ubiquitous in the environment and identified in diverse biomes, including freshwater ecosystems. Freshwater ecosystems are often the primary receiving body of watersheds and provide valuable ecosystem services, making contamination to these systems a

concern for pollution extending into connecting waters and public health. Legislation and the popular press often view plastic pollution as a single pollutant, ignoring critical differences in the chemical and physical properties, size, shape, and reactivity (Suhrhoff, et al. 2016; Schonlau, et al. 2019) among common plastic polymers (Table 1). Given these differences, plastic pollution is better considered a diverse suite of contaminants (Rochman, et al. 2019) where these underlying complexities and differential impacts must be considered to fully understand the risks of plastic pollution.

Despite the magnitude of plastic pollution in freshwater, many prior studies in these systems focus solely on surface waters (Eriksen, et al. 2013; Fischer, et al. 2016; Vermaire, et al. 2017), leading to an incomplete picture and perhaps an underestimate of the quantity and impact of plastic in freshwater systems. Moreover, the path plastics take to enter lakes is not always direct, with numerous transport pathways and connecting waterways that plastics can be found before reaching their ultimate destination (Hoellein & Rochman, 2021). These pathways are heavily dependent on level of urbanization, land use, and stormwater management infrastructure (Grbić et al. 2020). Combined sewer systems leading to a wastewater treatment will filter out larger debris, reducing the amount of plastic waste relative to municipal separate storm sewer systems that connect directly to stormwater ponds and other natural waterways. Municipal stormwater infrastructure, like stormwater ponds, often connect to larger waterways and the plastics released to these environments are likely transported to larger systems and exposed to a wide range of environmental conditions that influence the material properties and ultimate fate and impact of plastic pollution in the environment.

As such, it is imperative to better understand the differential fate and impacts of plastic polymers in research and policymaking to identify polymers that may pose the greatest

ecological risks. Environmental behavior and the subsequent impacts are dependent on the differences among plastics, such as chemical composition of the polymer, material density, size, shape, and toxicity. It is particularly important to note that these attributes are subject to change over time in aquatic environments as materials are exposed to environmental conditions, water-borne contaminants, and freshwater organisms ranging from microbes and fungi to fish and mammals.

Table 1.1. Common plastic polymers identified in benthic ecosystems (Ballent, et al. 2016; Lenaker, et al. 2019) and their different physical and chemical properties, including material density, chemical additives, and commercial uses.

Polymer	Abbreviation	Density (g/cm^3)	Common Additives	Common Uses
High density polyethylene	HDPE	0.94-0.97	Irganox 1010 and 1076, Ethanox, BHA, DIOP, DIBP, DEHA, DBP, MEHP, DEHP	Bottle caps, plastic bags, food packaging
Polystyrene	PS	1.03-1.06	Irganox 1076, BHT, Tinuvin 770, Dibromophenol	Solo cups, food packaging, cutlery
Styrene-butadiene rubber	SBR	1.15-1.3	BVUS, carbon black	Tires, playground and athletic turf
Polyethylene terephthalate	PET	1.38-1.41	BVUs, Irganox 1010 and 1076, BHT, DIBP, DEHP, DPP, DOA	Water bottles, fleece fabric
Polyvinyl chloride	PVC	1.3-1.45	DEHP, DHA, HOA, heat stabilizers	Pipe, outdoor furniture, personal hygiene items

BVUs = Benzotriazole based UV stabilizers, BHT = Butylated hydroxytoluene, DIBP = Diisobutyl phthalate, DIOP = Di-octyl isophthalate, DEHP = Bis-(2-ethylhexyl)-phthalate, MEHP = Mono-(2-ethylhexyl)-phthalate, DPP = Dipentyl phthalate, DBP = Dibutyl phthalate, DOA = Dioctyl adipate, DHA = Diheptyl adipate, HOA = Heptyl octyl adipate

1.2. Environmental Impacts of Plastics

Microplastics are divided into two overarching categories, primary and secondary, where primary microplastics are those manufactured to be <5mm in size, and secondary microplastics are the product of larger pieces degrading to this size over time. Microplastics can take on a range of shapes, primarily categorized in the literature as spheres, fragments, fibers, foams, and films. These shapes are often associated with the commercial function of the plastic (e.g., foams for food packaging, fibers for textiles, spheres in cosmetics, etc...). Distribution of plastic pollution in lakes is largely driven by the material density, with higher density particles depositing in the sediment more easily and lower density particles remaining at the surface. In Lake Ontario, estimates suggest that plastic particles began accumulating in the center approximately 18-38 years ago (Corcoran, et al. 2015) and have a presence up to 15 centimeters into the sediment profile (Ballent, et al. 2016).

Recent models and field studies further suggest that shorelines and sediments contain the greatest concentration of plastics (Hoffman & Hittinger, 2017), estimating that 70% of plastic litter sinks and remains preserved in the sediment (Driedger, et al. 2015) and identifying a wide range of polymers in the nearshore benthos at concentrations up to 10,000 particles kg^{-1} (e.g., Zbyszewski & Corcoran 2011; Castaneda, et al. 2014; Corcoran, et al. 2015; Ballent et al. 2016, Dean et al. 2018). When plastics deposit into the sediment profile, photodegradation is slowed from the lack of UV exposure (Andrady, 2011), leading to an even longer residence time in the environment. Though, more recent studies are finding greater spatial variation in the distribution of plastic (Willis, et al. 2017; Lenaker, et al. 2019; Rodrigues, et al. 2019), with particles being found dispersed throughout the water column and sediment, and this may shift over time. Models suggest that plastics originating in surface waters may sink to the bottom over time (Daily &

Hoffman, 2020), where their fate and impact is even less well understood. While resuspension of sunken plastic particles may cause particles to return to the surface, evidence suggests long-term accumulation of particles in the benthos.

There are two main pathways for microplastic particles to sink to the sediment: particles with a greater material density than water at the outset, and particles undergoing biofouling that increases the overall density (Kaiser, et al. 2017; Chen et al. 2019; Semcesen & Wells, 2021). Biofouling is a process under which substrate-limited microscopic organisms utilize plastic surfaces as habitat (Zardus, et al. 2008; Oberbeckmann, et al. 2016). The process is facilitated by the high surface area to volume ratio and hydrophobic nature of plastics that create an ideal novel habitat for microbes that form biofilms. Colonization by these microbes adds mass to plastic particles and may give particles with positive or neutral buoyancy in their pristine form (e.g., HDPE, PS; Table 1) negative buoyancy over time. Biofouling potential is influenced by the surrounding conditions of the ecosystem that may favor certain microbes and algae that will utilize the plastic as habitat, including factors like nutrients, oxygen, and light availability (Smyth, et al. 2021). Differences in biofouling potential may cause particles to sink more rapidly, and lead to different plastic deposition rates across systems.

Moreover, plastics recovered from diverse aquatic samples (freshwater, seawater, and ground water) have been shown to absorb contaminants, including polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (e.g., Mato, et al. 2001; Rios-Mendoza & Jones, 2015; Panno, et al. 2019; Rios-Mendoza & Balcer, 2019; Rodrigues, e al. 2019) and the adsorption of additional hydrophobic contaminants onto plastic surfaces has been shown in lab experiments (Teuten, et al. 2007). This ultimately identifies plastic as both an attractor for toxin accumulation and a vector for toxins to reach the sediment alongside surface

biofilms and may further impact toxicity to organisms. However, it is likely that the extent of contaminant adsorption will vary between systems, where waters with greater levels of contamination may be more likely to adsorb contaminants and may cause differences in plastic toxicity between systems.

Given that plastics are consumable by organisms (Egbeocha, et al. 2018; Franzellitti, et al. 2019), there is concern over toxicity and bioaccumulation of these polymers. At smaller size fractions, plastics in the sediment are consumable by benthic invertebrates, and as key drivers of benthic ecosystem function and coupling between sediments and the water column (Kuntz & Tyler, 2018), the presence of toxic materials may hinder crucial ecosystem functions, and in turn have cascading effects on the delivery of key aquatic ecosystem services (Ponte et al. 2019). These impacts likely differ spatially and temporally and remain understudied. With high concentrations of plastic in the sediment and the risk to benthic organisms, the benthos is likely the most sensitive zone to plastic pollution in freshwater systems, warranting greater study of potential impacts to benthic organisms and ecosystem function.

1.3. Overview of Study

The objectives of this study were to:

1. Evaluate the impact of pristine plastics commonly found in the environment on benthic ecosystem function in Lake Ontario with a focus on nitrogen cycling
2. Evaluate differences in eco-toxicity between multiple microplastic polymers, and how the toxicity of these polymers change over time in different freshwater environments
3. Evaluate how the impacts of plastic pollution to benthic organisms extends to benthic ecosystem function in Lake Ontario

4. Evaluate how the impacts of multiple microplastics on benthic ecosystem function change after environmental exposure

The focus of Chapter 2 is to understand the ecosystem level impact of three pristine microplastics on ecosystem function in Lake Ontario using a microcosm approach, with the prediction that the different polymers will show unique impacts on sediment biogeochemistry. In chapter 3, the changes in eco-toxicity of six microplastic polymers over time in Lake Ontario and a local stormwater pond on the Rochester Institute of Technology (RIT) campus will be investigated using benthic oligochaete, *Lumbriculus variegatus*. Chapter 3 will also discuss how the impacts of these same six polymers on *L. variegatus* extend to the ecosystem level, while also investigating how the ecosystem level impacts of these polymers change after aging in the environment. We believe that toxicity and ecosystem level effects will differ by polymer, and that adverse impacts on organisms will cascade to influence key ecosystem processes such as nitrogen and carbon cycling. It is further predicted that impacts on sediment biogeochemistry will shift over time in the environment as particles age.

Chapter 2.
Impacts of pristine microplastics on benthic ecosystem function

2.1. Introduction

With global plastic production surpassing 300 million tonnes each year (Plastics Europe, 2019), the accumulation of plastic debris in the environment is a pressing issue with largely unknown implications for both ecosystem and public health. Plastic pollution has been identified in diverse aquatic ecosystems (Free, et al. 2014; Brandon, et al. 2019; Hitchcock & Mitrovic, 2019; Jiang, et al. 2019; Firdaus, et al. 2020; Ren, et al. 2021), including the Laurentian Great Lakes (Eriksen et al. 2013, 2014; Baldwin et al. 2016). As one of the world's largest sources of freshwater, plastic pollution in the Great Lakes is noteworthy due to human dependence on this resource for food, drinking water, and tourism. As the terminal lake, Lake Ontario is also the gateway to the Atlantic Ocean through the St. Lawrence Seaway, and may act as an additional source of plastic pollution to the marine environment. Modeling studies (Hoffman & Hittinger 2017; Daily & Hoffman, 2020) and field observations suggest that plastic debris behaves differently in freshwater systems than oceans, where rather than accumulating in a large floating "patch" (Eriksen, et al. 2014), debris distribution in the Great Lakes is determined by source location and transport (Erikson et al. 2013; Driedger et al. 2015; Baldwin et al. 2016; Cable et al. 2017). Plastic ultimately accumulates nearshore in the benthos and on beaches, in concentrations >10,000 particles kg^{-1} (Zbyszewski & Corcoran 2011; Corcoran, et al. 2015; Ballent et al. 2016, Dean et al. 2018). Estimates further suggest that plastic began accumulating in the Lake Ontario benthos 18-38 years ago (Corcoran, et al. 2015), with ecosystem impacts still largely unknown.

Freshwater benthic ecosystems are highly diverse and are critical in regulating trophic dynamics, and recycling and removing carbon and nutrients (Kuntz & Tyler, 2018). These services are commonly carried out by a diverse community of microbes in the sediment, but contamination in the sediment may alter the functional role of these communities and have

cascading effects (Vinebrooke, et al. 2004; Hadley, et al. 2013; Jackson, et al. 2016; Ponte, et al. 2019) with potential to disrupt ecosystem services like water purification and climate regulation. Once in the environment, plastics are subject to conditions that may alter their physical and chemical properties (e.g., microorganisms, UV-exposure, water-borne contaminants), and influence fate and subsequent impacts. The distinct biofilms that microorganisms create on plastic surfaces often differ from the surrounding microbial communities (McCormick et al. 2016; Oberbeckmann, et al. 2016) and may accumulate associated toxins or pathogenic microbes (Rochman et al. 2013; Rios Mendoza & Jones 2015; Harrison et al. 2018; Schönlau et al. 2019; Parthasarathy, et al. 2019; Khalid, et al. 2021; Tu, et al. 2021), and shift ecosystem processes in the water column and sediment.

In riverine systems, increased abundances of nitrifying bacteria have been observed in plastic biofilms (Hoellein, et al. 2014), with the capacity to alter nitrification in sediments and in wastewater effluents, (Muβmann, et al. 2013), and creating nitrification hotspots in affected streams and sediments. Further, different plastic polymers exhibit different impacts on nitrification and denitrification activity (Seeley, et al. 2020), highlighting the need for additional research on the biogeochemical impacts of multiple plastic polymers. Alterations in nitrogen cycling from shifts in sediment microbial communities may impact primary production, with diminished water quality and cascading impacts moving up the food web, making research into the effects on the benthos critical for understanding whole-ecosystem impacts.

Despite often being discussed as a singular pollutant, plastic pollution is in reality a highly complex issue because of the high diversity of materials entering the environment. Polymers vary substantially in their chemical make-up and physical properties, both of which influence the fate and environmental impacts. While plastics have been identified in freshwater

benthos in large quantities, specific polymers are not equally distributed and may have different impacts on biogeochemistry. Polymers with a higher material density, like synthetic microfibers, (e.g., polyester, nylon), polyvinyl chloride (PVC), and tire wear particles comprised of butylated rubber are among the most identified plastics in nearshore sediment samples (Deng, et al. 2019; Lenaker, et al. 2019; Peller, et al. 2019). Fibers can be found in quantities up to 34,000 fibers kg dw^{-1} (Peller et al., 2021) in the Great Lakes basin. Despite being one of the most identified polymers and with substantial recent investigation into microfiber transport pathways (Napper, et al. 2016; Peller, et al. 2019; Kapp, et al. 2020; Liu, et al. 2021), little is known about ecosystem impacts in freshwater environments. PVC is another plastic of concern, due to its high material density, heavy use in construction materials that may release significant amounts of microplastic over time (Geyer et al. 2017), and the potential toxicity associated especially with additives in the polymers (Wagner, 1983). Prior studies have demonstrated negative impacts of PVC on bioturbating organisms (Green, et al. 2016), and reduced denitrification rates (Seeley, et al. 2020) in marine ecosystems. Tire wear particles have been found at densities up to 5,500 particles per kg dw^{-1} (Wang, et al. 2017; Lenaker, et al. 2019). The full effects of tire and road wear particles on aquatic environments are unclear, despite rising concern in the literature (Panko, et al. 2018; Wagner, et al., 2018, Tamis et al. 2021, Chibwe et al. 2021).

To date, many studies explore the impacts of singular plastics, ignoring the diversity that exists among polymers. By evaluating how ecological impacts of plastics differ by polymer, we can better understand the greatest risks and generate more targeted policy. In this study, we use a microcosm approach to evaluate the impacts of three commonly identified consumer microplastics (polyethylene terephthalate (PET); microfibers, styrene-butadiene rubber (SBR);

"crumb rubber", and PVC; particles) on benthic ecosystem function to create a more holistic understanding of the impacts of microplastic pollution in freshwater systems.

2.2. Methods

2.2.1. Microcosm design and set-up

Sediment was collected from Irondequoit Bay to a depth of approximately 10 cm using a 9.5 cm polycarbonate core tube, separated into depth profiles (0-2, 2-5, 5-10 cm) and sieved through a 1-mm mesh to remove rocks, plants, and macroinvertebrates. Microcosms were reconstructed in clean polycarbonate tubing (9.5 x 30 cm) sealed at the bottom with a butyl rubber stopper and wrapped in opaque plastic below the sediment-water interface to prevent light penetration. The headspace of each microcosm was filled with approximately 1L of artificial freshwater (US EPA, 2002) and stored in a 416 L recirculating Living Stream tank (FrigidUnits, Inc., Toledo, OH). The tank was illuminated with full-spectrum lights on a 14:10 hour light:dark cycle to simulate summer conditions and microcosms were individually aerated with room air using airline tubing attached to an aquarium pump. Prior to the start of the experiment, microcosms acclimated for four weeks to restore microbial communities and solute concentrations.

2.2.2. Material Additions

Following the acclimation period, each microcosm received plastic additions at a rate of 0.1% of whole sediment core dry weight. This value was selected using concentrations found in the literature for the Great Lakes (Deng, et al. 2019; Lenaker, et al. 2019; Peller, et al. 2019; Table A1). Plastics were homogeneously mixed into 150 g of surface layer (0-2 cm) sediment with 0.75 g of *Urtica* powder to replenish organic matter and this mixture was added to the top of each core. To prevent particle resuspension into the water column, an additional 45 g of

surface layer sediment was added to the surface for a total end height of 12.5 cm of sediment. Control microcosms received equal additions of untreated surface layer sediment processed as above but without plastic addition. Prepared microcosms were placed back in the tank and incubated for 30 d.

2.2.3. Oxygen and nutrient flux measurements

After 30 d, cores were sampled for sediment-water column fluxes of oxygen, nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^{3-}). Each microcosm was filled completely with artificial freshwater and tightly sealed with a clear polycarbonate lid fitted with rubber o-ring to prevent atmospheric gas exchange during sampling. The rubber stopper inserted into the sampling port in the center of the lid had a magnetic stir bar attached so that the suspended magnet spun freely. Microcosms were tightly wrapped with aluminum foil to create a dark environment for the first half of the flux measurements. During the experiment, microcosms were arranged surrounding a center tube fitted with a large magnetic stir bar spinning at approximately 60 rpm.

Samples were taken every two hr, with the first three in the dark and the last two in the light. Oxygen concentrations were measured using a self-stirring dissolved oxygen probe (Hach LDO-BOD with HQ40D meter) and water samples were taken using a 60-cc syringe. Water removed during sampling was replaced with a known volume of artificial freshwater and this dilution was accounted for in flux calculations. Water samples were immediately filtered through a 0.45 µm PES membrane filter and stored at -20°C until analysis. Nitrate was analyzed using a vanadium-based method (Doane, et al. 2003), ammonium was measured using the phenol-hypochlorite method (Soloranzo, 1969), and phosphate was measured using the ammonium molybdate method (Murphy & Riley, 1962). All flux rates were calculated based on changes in

concentrations in the headspace over time (Tyler, et al. 2001) with daily rates being the summation of light and dark measurements using the 14:10 hr light:dark cycle. Gross primary production (GPP) was calculated using the difference between the oxygen flux in the light and dark, assuming respiration is the only oxygen-consuming process occurring in the dark.

2.2.4. Sediment Properties

The following day, microcosm cores were destructively sampled for oxygen depth, benthic chlorophyll *a*, and potential denitrification. Fecal mounds from tubificid worms were noticed at the surface, likely from juveniles not been removed during sediment collection. Prior to destructive sampling, the number of fecal mounds was recorded and after sediment samples were removed the remaining sediment was sieved through a 1-mm mesh to isolate and quantify tubificids. Oxygen penetration depth into the sediment was assessed based on the visible color change in the sediment (n=3 depths averaged per microcosm). Sediment chlorophyll *a* (Chl *a*) and microbial samples were taken in duplicate using clean 5-cc syringe corers to 1 cm depths and placed in 15 mL centrifuge tubes to be stored at -80°C until further analysis. Chl *a* samples were immediately wrapped in aluminum foil to prevent light exposure, frozen at -80°C, and analyzed within 30 d. Pigments were extracted using sonication in 90% acetone, followed by a 24 hr extraction at -20°C. Samples were then centrifuged, and absorbance of supernatant was measured at 665 nm and 750 nm on a Shimadzu UV-1800 spectrophotometer before and after acidification using 1N HCl (Strickland & Parsons, 1972). Concentrations of Chl *a* and phaeopigment were calculated using the Lorenzen (1967) equations.

Samples for potential denitrification were collected using a 60-cc syringe corer to a depth of 2 cm, immediately placed in 50 mL centrifuge tubes, and refrigerated until the following day. Potential denitrification was measured using lab incubation experiments

following the acetylene inhibition method (Ryden, et al 1987) in 160 mL serum bottles with septa lids. In this method, acetylene inhibits the conversion of nitrous oxide (N_2O) to nitrogen (N_2) gas, with the assumption that all N_2O is converted to N_2 (Groffman, et al. 1999). Nitrous oxide is found in lower concentrations in the atmosphere relative to N_2 gas, making N_2O easier to measure. After adding 20 g of soil, 10 mL of sparged nanopure water and 10 mL of media (nitrate 100 mg^{-1} kg^{-1} + dextrose 40 mg^{-1} kg^{-1}+ chloramphenicol 10 mg^{-1} kg^{-1}), anaerobic conditions were ensured by flushing each serum bottle with N_2 gas for 3 cycles of 2 min each, shaking the bottles in-between flushes. Using a gas tight syringe, 11 mL of acetylene was added to each bottle. Gas samples were taken immediately after the addition of acetylene and injected into an evacuated gas tight vial. Bottles were placed on an orbital shaker (125 RPM) and additional samples taken after 30, 60 and 120 minutes. Gas samples were analyzed using a Shimadzu Gas Auto Analyzer Gas Chromatograph. The ideal gas law was used to calculate N_2O flux in micromoles of nitrogen per gram of soil per day.

2.2.5. Statistical Analysis

Statistical data analysis was completed using JMP 15.0 Pro software. Prior to analysis all data were assessed for normality and heterogeneity of variance to verify assumptions for analysis of variance (ANOVA). One-way ANOVA was used to compare plastic types for all analyses, apart from ammonium and nitrate fluxes, and to compare the presence of worms among the different treatments. When significant effects were found (p-value ≤ 0.05), Dunnett's post-hoc tests were used to identify if treatment groups differed significantly from the control group. Ammonium and nitrate flux data could not be transformed prior to analysis to meet assumptions of ANOVA, and Kruskal-Wallis tests were used. When significant effects were found, Dunn's tests were used to analyze if treatment groups differed significantly from the control group. To

compare hourly nutrient fluxes in the light and dark for each treatment, paired t-tests were used. To further evaluate the whole suite of variables, a principal component analysis (PCA) was run.

2.3. Results

There were significant differences among the plastic polymers in their impact on key ecosystem processes. Presence of worms significantly differed, with microcosms containing PET fibers having a significantly greater abundance of worms (Figure 2.1i, p=0.01). All sediments were net heterotrophic and similar, except for PET fiber, which was significantly more positive than the control (Figure 2.1b, p=0.02). Alongside the observed decrease in NEM, sediments containing PET fiber also appear to have higher sediment oxygen penetration (Figure 2.1c), though insignificant.

The presence of PVC caused unique effects on nutrient cycling relative to other polymers. Both ammonium (Figure 2.1e, p=0.0302) and phosphate (Figure 2.1f, p=0.0143) were released from the sediments to the water column in all treatments except for PVC, where sediment uptake was significant. Ammonium uptake in the PVC treatment occurred only in the light, with release in the dark (p=0.08), suggesting a role of microalgae. No other differences were observed between light and dark fluxes for ammonium or phosphate (p>0.1 for all). The uptake of nitrate in the light was modestly different from the dark (p=0.07) for the control only, however daily nitrate uptake was significantly higher in microcosms containing SBR (Figure 2.1d). Sediments containing PVC had higher benthic Chl *a* content (Figure 2.1g) and decreased potential denitrification (Figure 2.1h), though not significantly so.

The PCA resulted in two main components that together explained 49% of the data variability (28.7% Component 1; 20.3% Component 2). The biplot of the PCA shows distinct

grouping based on the plastic type, with PVC and PET separating from the control and SBR treatments (Figure 2.2).

2.4. Discussion

In this study, we evaluated the impacts of three common microplastic polymers on freshwater benthic ecosystem function, including primary production and nutrient cycling. This is the first study to examine this combination of metrics in freshwater sediments, to our knowledge. Our results illustrate that individual polymers have unique impacts on benthic ecosystem processes.

Overall, sediments containing PVC exhibited the strongest differences from both the control treatment and other plastic treatments, particularly in ammonium and phosphate flux (Figure 2.1e, Figure 2.1f). The significant enhancement of ammonium uptake in the presence of microplastic is consistent with findings in intertidal systems (Cluzard, et al. 2015), though inconsistent with a different mesocosm studies evaluating marine sediments (Green, et al. 2016; Li, et al. 2020). The presence of PVC may have increased nitrification activity in freshwater sediment (Muβmann, et al. 2013), accounting for the increased uptake of ammonium and slight positive release of nitrate to the water column, and the increase in benthic microalgal abundance in surface sediments. Results of the PCA suggest that PVC separates somewhat on the second component, where GPP and chlorophyll *a* are positively loaded and phosphate and ammonium flux are negatively loaded (Figure 2.2). This suggests that the link in variance between GPP and benthic microalgae may have increased sediment nutrient retention, further corroborated by hourly ammonium flux showing significant uptake in the light (due to photosynthetic uptake) and release in the dark (Table 2.2). With the two variables together, it is possible that primary production in the presence of PVC facilitates the growth of benthic microalgae, while the two

together may create aerobic conditions that favor nitrifying bacteria and the enhanced uptake of ammonium providing nutrients to microalgae to continue this loop. Moreover, the presence of PVC could have also promoted annamox bacteria in the anoxic layer, offering an additional pathway for ammonium uptake and nitrogen removal from sediments alongside enhanced microalgal activity.

While this study did not measure chemical effects, PVC is known to leach several chemicals to the water column (Flournoy, et al. 1999; Lithner, et al. 2011), which may also contribute to the differences observed. The presence of chemical additives may have also adversely impacted denitrifying bacteria while promoting nitrifying bacteria and growth of benthic microalgae in response. Titanium dioxide is a common UV stabilizer used in plastic manufacturing, and is a known emerging contaminant (Shah, et al. 2017). Titanium dioxide has demonstrated negative impacts on nitrification and denitrification activity in soils (Simonin, et al. 2016) and may have contributed to suppressing denitrifying activity in the sediment in our study.

The PCA results also suggest that PET fibers, which is separated on the first principal component demonstrates shifted metabolism and increasing oxygenation of surface sediments, likely driven by enhanced worm colonization (Figure 2.2). Microcosms containing PET had a significantly higher tubificid worm abundance (Figure 2.1i), which may contribute to the increased oxygen penetration and reduced NEM at the conclusion of the experiment because of consumption of organic matter in the top few mm of sediment over the course of the 30 days, causing the observed decrease in ecosystem metabolism because OM reserves were exhausted. However, upon termination of the experiment, the tubificid worms quantified were entangled in the fiber particles with many identified as deceased, suggesting a possible interaction with the plastic particles.

Toxicity of plastic on organisms was not directly measured here, making it difficult to determine to what extent plastic toxicity may have had on ecosystem function. With worms being identified as a major driver of the effects observed in microcosms containing PET fibers (Figure 2.2.) and the organisms having observed interaction with these fibers, it begs the question if the interactions and effects observed with this plastic type are due to the morphology being attractive to these organisms, or if there is a chemical or physical toxicity component that impacts organisms and extends to impact ecosystem processes. Given the role of benthic organisms as ecosystem engineers (Kuntz & Tyler, 2018), further work is required to link microplastic toxicity on benthic invertebrates to ecosystem functions. Additional work investigating the drivers of the toxicity of different polymers (i.e., morphology, chemical composition, ingestion, entanglement) would also be needed to better tease out what influences the effects of plastic pollution in waterways. With unique ecological impacts identified among polymers, this work provides a first glimpse into the potential cascading environmental effects of plastic pollution in freshwater ecosystems and highlights the complexity and need to investigate impacts of plastic pollution as a multi-faceted issue.

Figures

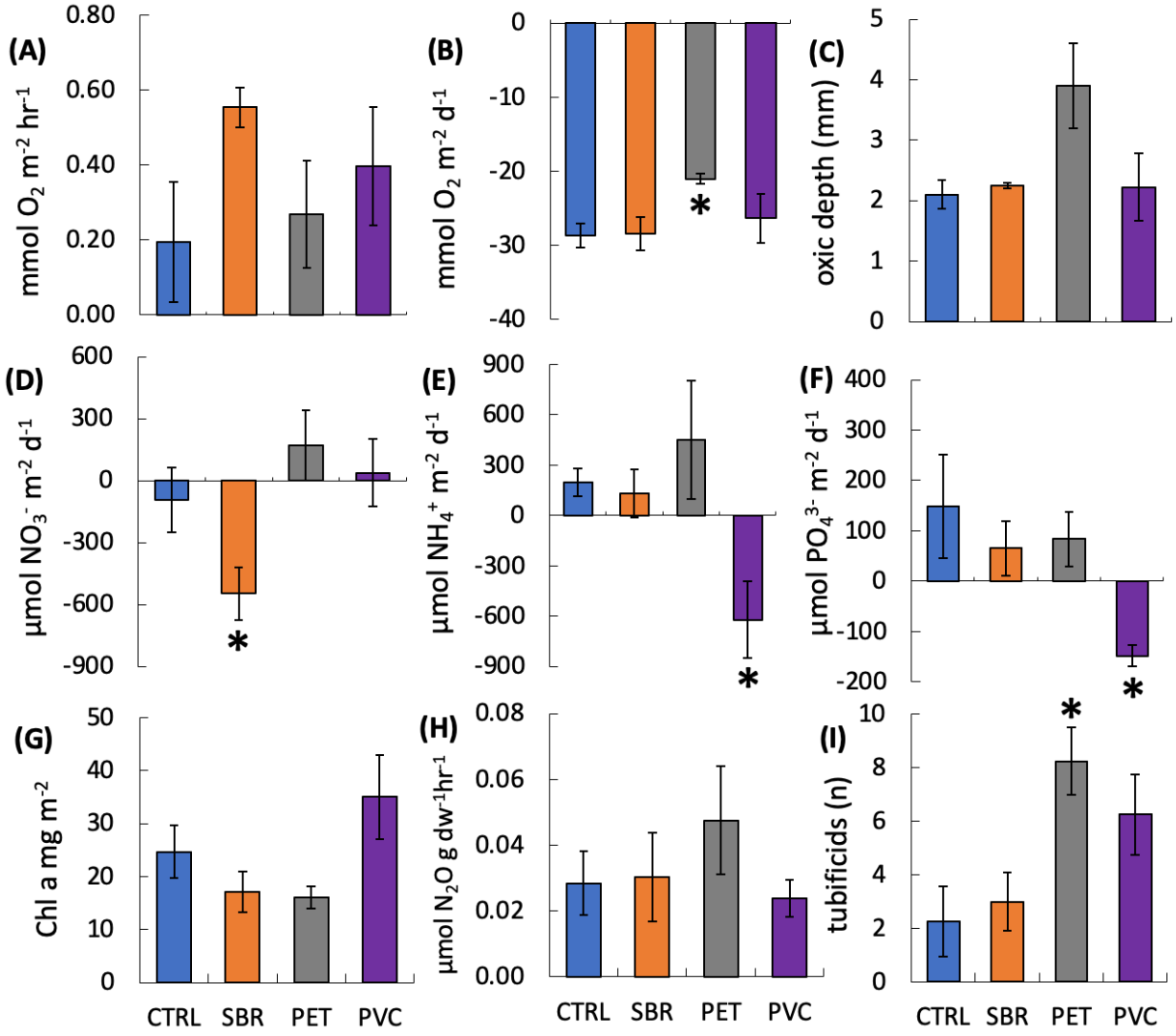
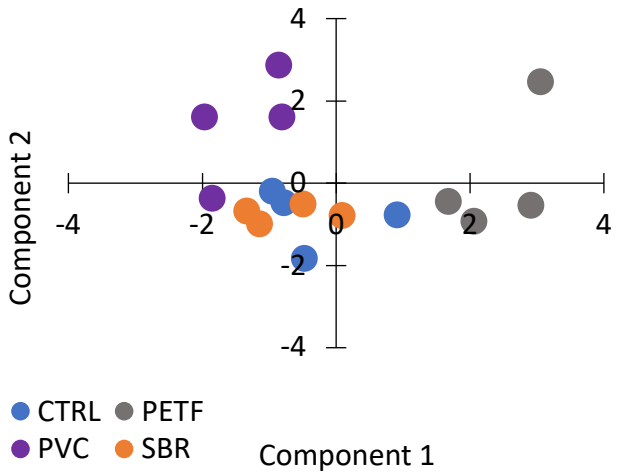


Fig 2.1. Gross primary production (GPP; A); net ecosystem metabolism (NEM; B); sediment oxygen penetration (C); daily sediment-water column fluxes for NO_3^- (D); NH_4^+ (E); and PO_4^{3-} (F); benthic microalgal Chl *a* (G), and potential denitrification (H) and organism abundance (I) measured after 30 d. Values are mean ± SE, n=4. Asterisks represent significant differences compared to control.



	Component 1	Component 2
NEM	0.42	0.28
GPP	-0.07	0.44
NO_3^- Flux	0.23	0.30
NH_4^+ Flux	0.38	-0.37
PO_4^{3-} Flux	0.23	-0.38
Pot DNF	0.18	-0.20
Chl *a*	-0.18	0.45
Oxic depth	0.45	0.33
Worms	0.54	0.07

Fig 2.2. Principal components analysis showing biplot and factor loading for each component. Component 1 explains 28.7% of variability in the data. Component 2 explains 20.3% of variability in the data.

Tables

Table 2.1. Results of one-way ANOVA or Kruskall-Wallis tests on the effects of plastic type for colonization of organisms, NEM, GPP, sediment oxygen penetration, daily nitrate flux, daily ammonium flux, daily phosphate flux, chlorophyll *a* content (Chl *a*) and potential denitrification (PDNF). Significant effects ($p<0.05$) are bolded. Values with asterisks indicate a Chi-square value from Kruskall-Wallis tests.

Factor	**Plastic Type**		
	df	F/X^2	*p*
NEM	3	4.97	**0.02**
GPP	3	1.32	0.31
Oxic Depth	3	3.38	0.05
Daily NO_3^-	3	7.91*	**0.04**
Daily NH_4^+	3	8.93	**0.03**
Daily PO_4^{3-}	3	3.93*	**0.03**
Worm count	3	5.59	**0.01**
Chl *a*	3	2.84	0.08
PDNF	3	2.84	0.08

Table 2.2. Hourly fluxes of nitrate (µmol m^{-2} h^{-1}), ammonium (µmol m^{-2} h^{-1}), phosphate (µmol m^{-2} h^{-1}) in the light and dark (mean ± SE, n=4). Values in bold are significantly different between light and dark at $p<0.1$.

			CTRL	SBR	PET	PVC
NO_3^-	Day 30	Light	**-43.8±18.3**	-24.6±14.0	26.4±9.7	-23.6±12.5
		Dark	**52.1±17.5**	-20.2±27.7	-19.9±23.1	36.8±24.4
NH_4^+	Day 30	Light	-7.1±12.6	21.3±11.6	9.1±20.0	**-68.0±31.7**
		Dark	29.9±13.7	-16.5±8.7	30.7±10.2	**33.2±24.3**
PO_4^{3-}	Day 30	Light	11.6±9.1	6.1±5.4	9.7±4.6	-2.9±1.3
		Dark	3.9±3.3	1.7±4.5	0.5±4.6	-7.0±1.3

Chapter 3.
Microplastic toxicity and cascading impacts to ecosystem function over time and space

3.1. Introduction

The accumulation of plastic debris in marine and freshwater ecosystems has received increased attention in both scientific studies and the popular press due to the observed magnitude of plastic debris (Castaneda, et al. 2014; Cable, et al. 2017; Hendrickson, et al. 2018), the ubiquity of plastic across all studied ecosystems (Evangeliou, et al. 2020; González-Pleiter, et al. 2020), and the unknown impacts on both ecosystem and public health. As one of the world's largest freshwater systems, the Laurentian Great Lakes are a critical resource for food, drinking water and tourism. Thus, understanding the potential impact of plastics on ecosystem functions and services is critical. This system is also a gateway to the Atlantic Ocean through the St. Lawrence Seaway and may act as a significant source of plastic pollution to the marine environment. However, "plastic" is a catchall term for numerous polymers used for different purposes, with unique physical and chemical properties (Rochman, 2019). This complicates our ability to draw comprehensive conclusions about fate and impact. Further, with environmental exposure, the physical and chemical characteristics may change and further influence the physical and chemical properties.

Although microplastics may be consumed by benthic organisms (Browne, et al. 2013; Scherer, et al. 2017; Botterell, et al 2019), interacting factors contributing to bioavailability, including particle size, color, shape, and abundance that may increase impact for certain groups of species. Locations with higher concentrations of plastic have greater bioavailability (Messinetti, et al. 2017) because of the greater potential of interaction between organisms and plastic particles. Material density and how it changes over time plays a significant role in local microplastic abundance, with negatively buoyant and biofouled polymers being common in the sediment. Smaller plastic particles (Desforges, et al. 2015) and those without jagged edges (e.g.,

fibers and beads) are more likely to be consumed (Cole & Galloway, 2015; Steer, et al. 2017; Sun, et al. 2017) by accommodating a wider range of organism sizes and feeding types (Covich, et al. 1999; Scherer, et al. 2017). Thus, heterogeneous consumption of plastic particles leads to different potential routes of toxicity to organisms, depending on whether the exposure is external to the organism, or internal following consumption.

There are additional challenges in understanding the eco-toxicity of microplastics in addition to the existing diversity among polymers, with difficulties in determining whether plastic particles themselves or leaching of associated additives (dyes, flame retardants, plasticizers, UV stabilizers, etc.) and/or chemical adsorption from the environment are most consequential (Gandara e Silva, et al. 2016; Hahladakis, et al. 2018; Capolupo, et al. 2020). Prior studies have shown that ingestion of a wide range of plastic polymers has led to reduced feeding, weight, and fertility in a variety of benthic species (Browne, et al. 2013; Jemec, et al. 2016; Hurley, et al. 2017; Scherer, et al. 2017; Scherer, et al. 2020), where it is suggested that feeding type also holds impact on ingestion of particles (Browne, et al. 2013; Scherer, et al. 2017; Bour, et al. 2018), though results are inconsistent between studies. A study examining the microplastic effect threshold of polystyrene on a suite of freshwater benthic macroinvertebrates found no significant effects on survival, reproduction, or growth in concentrations up to 40% sediment dry weight (Redondo-Hasselerharm, et al., 2018) with similar findings in another study examining the chronic toxicity of tire wear particles (Redondo-Hasselerharm, et al. 2018). However, many microplastic bioassays utilize materials solely in their pristine state, and do not include post-consumer items or materials after they have been in the environment for a period of time. This may not be environmentally realistic as most inputs are from consumer plastics that persist in the

environment for many years, and the physical and chemical characteristics change as they age (Bejgarn, et al. 2015; Oberbeckmann, et al. 2018; Ding, et al. 2020; Dudek, et al. 2020).

Moreover, there are numerous routes plastics follow in the environment before reaching their destination. The different pathways are heavily driven by urbanization, land use, and stormwater management infrastructure (Hoellein & Rochman, 2021). Municipal stormwater infrastructure, like stormwater ponds, often connect to larger waterways and plastics released directly to these environments are likely transported to larger systems while being exposed to a wide range of environmental conditions (i.e., light availability, currents), microorganisms, and water-borne contaminants (Smyth, et al., 2021). Environmental conditions induce shifts in the physical and chemical properties of plastics, altering the material density, chemistry, toxicity, and biology. Material density may be shifted positively or negatively over time, where colonization of unique microorganisms onto the plastic surface contributes to a higher material density (Kaiser, et al. 2017; Chen et al. 2019; Semcesen & Wells, 2021), but photooxidation leads to reduced density (Weinstein, et al. 2016) and possible leaching of chemical additives to the surrounding environment (Hongwei Luo, et al. 2020). Shifts in material density alter the vertical distribution of plastics within the ecosystem, causing plastics that were previously negatively buoyant to sink, and vice-versa.

Consequently, the biofilms that travel alongside plastics over time may have effects on toxicity and ecosystem function. Microbes in plastic biofilms are often unique compared to the surrounding communities and may foster pathogenic bacteria and associated toxins (Mato, et al. 2001; Rios-Mendoza & Jones, 2015; Panno, et al. 2019), thus shifting ecosystem processes in the water column and sediment. Plastic biofilms may harbor nitrifying bacteria, creating nitrification hotspots in streams and microcosms (Muβmann, et al. 2013) which may have adverse effects in

freshwater ecosystems over time, shifting nitrate flux and nitrogen removal within sediments. Recent models and field studies have identified the benthos as most polluted by plastic (Zbyszewski & Corcoran 2011; Castaneda, et al. 2014; Dean et al. 2018; Lenaker, et al. 2019; Rodrigues, et al. 2019), due to the high material density of many plastics even in their pristine form, and from biofilm accumulation causing sinking over time (Kaiser, et al. 2017; Chen et al. 2019; Semescen, et al. 2021). These changes in the physical, chemical and biological properties with environmental exposure have unknown implications for the impact on ecosystems, and accumulation in the benthos suggests that these habitats are among the most impacted.

Benthic ecosystems are highly diverse areas that are critical in regulating trophic dynamics, and recycling and removing carbon and nutrients (Covich, et al. 2004; Kuntz & Tyler, 2018). These services are commonly carried out by diverse communities of microbes and invertebrates in the sediment, but contamination may alter the functional role of these communities and have cascading effects on ecosystem function (Vinebrooke, et al. 2004; Hadley, et al. 2013; Jackson, et al. 2016; Ponte, et al. 2019), with potential to disrupt ecosystem services like water purification and climate regulation. The interactions between benthic invertebrates, microbes, and the sediment play an important role in the biogeochemistry of aquatic ecosystems. Benthic invertebrates impact biogeochemistry directly through respiration and excretion, and indirectly through behaviors like bioturbation. Bioturbation refers to the activities of organisms that alters the physical environment, including the creation of burrows (Kristensen, et al. 2012). The creation of these burrows reworks surficial sediment, providing space that facilitates sediment-water column exchanges of nutrients and oxygen (Vanni, 2002; Lohrer, et al. 2004; Kuntz, 2015) and enhances organic matter decomposition (Chauvet & Gessner, 1993). In turn, the greater influx of inorganic nutrients and increased surface area from

these burrows further support the microbial communities that are key in transforming nutrients and organic matter (Lohrer, 2004).

Key bioturbators in freshwater systems include tubificid worms and aquatic lumbriculidae, *Lumbriculus variegatus*. By feeding head-down in the sediment, *L. variegatus* consumes detritus found among sediment particles, assimilating nutrients, and excreting sediment and leaving burrows behind. The ubiquitous presence of *L. variegatus* across freshwater ecosystems, alongside their ability to be easily cultured in a laboratory setting makes them a key model organism for both acute and chronic toxicity assays (Beckingham & Gosh, 2010; Vought & Wang, 2018) and ecosystem level studies. Even sublethal effects of toxins may have a negative impact on ecosystem function and biogeochemical processes by influencing the organisms that serve as key drivers of ecosystem function and benthic-pelagic coupling (Cesar, et al. 2012; Pigneret, et al. 2016; Blankson, et al. 2017; Ponte, et al. 2019), though this has not been studied in response to plastic exposure in freshwater sediments.

Furthermore, little is known about how impacts vary across polymer type and with environmental exposure. As plastics continue to accumulate in the benthos, understanding the interactions between organisms and microplastic particles and how they shift over time is critical to mapping the long-term fate and impact of microplastic pollution in freshwater ecosystems. To better understand the impacts of plastic pollution throughout different stages of the life cycle in the environment, we investigated the ecological impacts of six common consumer plastics. Traditional toxicity bioassays were conducted on *L. variegatus* using microplastics in their pristine form and after aging in Lake Ontario and a stormwater pond in Rochester, NY. The cascading effects of these same six polymers in pristine and aged form on sediment biogeochemistry, benthic-pelagic coupling, and microalgal abundance, along with the ability of

L. variegatus to influence these key ecosystem functions was also measured. This is the first study to examine the cascading impact of plastic pollution on ecosystem services in freshwater ecosystems over time.

3.2. Methods

3.2.1. Site Descriptions.

For this study, the field incubations took place in Lake Ontario and in the RIT J-Lot Stormwater Pond (Figure 3.1). Incubations in Lake Ontario took place offshore of private residence in Sodus, New York. This site features a shallow and rocky coast with high wave action during storm events. The RIT J-Lot Stormwater Pond is a 1.0-acre retention pond characterized by fine silts and clays (Burkett, 2014). The pond is 14 years old and adjacent to campus roads. The stormwater pond is also linked to Lake Ontario, connecting to the Genesee River to flow into Lake Ontario. Samples were collected from the surface and benthic frames at both sites after 1 and 4 mo for further use in toxicity assays, and after 4 mo in Lake Ontario for use in ecosystem level experiments.

Fig 3.1. Aerial images of site locations for incubation experiments: Lake Ontario (A) and J-Lot Stormwater Pond (B). Location of the surface water frame for the stormwater pond is indicated in blue marking and location for the benthic frame is indicated in yellow marking.

3.2.2. Field Aging Experiments

Post-consumer products of six plastic polymers in particulate form (polyethylene terephthalate (PET) bottle and microfiber, styrene-butadiene (SBR) crumb rubber, high-density polyethylene (HDPE) bottle cap, polystyrene (PS) solo cup, and poly-vinyl chloride (PVC) particles) were aged in field incubation experiments measure how the characteristics and impacts of plastics change over time in different freshwater settings. Plastics were sourced as described in Table 2, and ground to size in a household blender or coffee mill when necessary. Parallel incubations occurred in the water column and sediment at each site in consideration that many plastics may be exposed to both water and sediment throughout the microplastic life cycle.

Table 3.1. List and properties of polymers used in field aging experiments.

Material	Source	Size	Color
PET-F	Polyester fleece fabric	ND	Orange
PET-B	Water bottles - Poland Spring (<1L bottle, #1)	14-mm	Clear
SBR	Commercial crumb rubber	4-mm	Black
PVC	Sigma Aldrich	ND	White
HDPE	Water bottle cap - Poland Spring #2	10-mm	Clear
PS	Clear PS solo cup - Wegmans brand	10-mm	Clear

Water column incubations were conducted using 0.75 x 1.5 m x 0.23 m box frames constructed out of 2 cm or 2.5 cm PVC pipe (schedule 40). Benthic frames were constructed from 0.23 m x 0.45 m 1.2 cm PVC pipe. Corners were glued using PVC adhesive. Each frame was fitted with 6 - 1/16th" stainless steel cables running the length of the frame. Hollow bamboo "spacers" were strung onto the cables to separate the samples. Each water column frame was outfitted with an Onset HOBO temperature and light logger (MX2202) set to log every 10 min. Particles for the water column incubations were housed in 6 cm length x 2.54 cm ID vinyl tubing. Tubing containers were capped on either end with 80 µm Nitex mesh (Wildco Co). Particles for benthic incubations are housed in 8 x 8 cm 80 µm

Nitex mesh bags. Empty containers, both tubing and mesh bags, were also included to serve as blanks. Containers were attached to steel cables with hollow bamboo separators on the frames in a block design (Figure 2; n=3 per collection time; n=9 per frame). The water depth varied over time at each site, but was roughly 0.75-1.5 m at both sites. Frames in Lake Ontario incubations were located so that the water column and benthic frames were roughly on top of one another, however, incubations taking place in the RIT J-Lot Stormwater Pond were separated to ensure that the benthic frame would not be disturbed by fishing. Each field location was sampled every 3-4 months for light penetration, and water column dissolved oxygen, conductivity, pH, nitrate, phosphate, and ammonium (Table A.2.).

Figure 3.2. Completed surface water raft used in field aging experiments

3.2.3. Aging Experiment Sample Processing

At each sampling time point, the cable wires holding the incubation containers were cut and placed into a plastic container with site water and covered during transport. In the lab, individual containers were cut from the wire and subdivided. Particles for toxicity and ecosystem level experiments were rinsed with nanopure water on a 64-µm sieve and placed into nano-rinsed glass vials. Samples were dried at room temperature for 48 hr. Once dry, samples were stored at -20°C when not in use. Subsamples were weighed out for distribution in toxicity bioassays and ecosystem level experiments.

3.2.4. Chronic Toxicity to Lumbriculus variegatus

L. variegatus were obtained in September 2018 and cultured in the lab at room temperature in an 11 L aquarium with a water depth of 3–5 cm of continuously aerated artificial freshwater. Pre-soaked unbleached paper towel was used as a substrate and worms were fed with commercial goldfish flakes twice a week with tank water changed biweekly (Pakarinen et al., 2011; Wang et al., 2014).

The experimental setup included 250 mL jars filled with artificial sediment and artificial freshwater. Artificial sediment was prepared according to OECD guidelines (2007) and mixed using a rotating mixer for 4-hr. Following a resting period of 24-hr, 60 g of sediment (wet weight) was added to each jar, with plastic mixed in using a glass rod to homogenize the sediment-plastic mixture and prevent resuspension of lower density materials. Toxicity bioassays were conducted using microplastics in their pristine form, as well as after 1 and 4 months of aging in Lake Ontario and the stormwater pond. Microplastic additions were determined using mass estimates found in the literature (Castelvetro, et al. 2019; Deng, et al. 2019; Lenaker, et al. 2019; Peller, et al. 2019), alongside properties of the artificial sediment. Materials were added at a value of 0.5% sediment dry weight to represent a higher particle concentration while remaining environmentally realistic (n=6 per treatment). Following plastic addition, 100 mL of artificial freshwater (EPA protocol described by Weber, 1991) was added to each jar and the headspace was gently aerated using Pasteur glass pipettes and airline tubing. Following an acclimation period of 24-hr where sediment settling occurred, 15 individuals were added to each jar. Six parallel replicates of 15 randomly selected individuals are weighed all together in a pre-tared aluminum pan to obtain initial total wet weight, and then dried in a 65ºC drying oven for 24-hr to obtain initial total dry weight. Individual wet weight and dry weight was obtained by dividing the

total mass by the number of individuals. Each jar was covered with parafilm to prevent outside plastic contamination while also minimizing water evaporation. Throughout the duration of the 30-d exposure period, deionized water was added as needed to compensate for evaporation without raising salt content. Organisms were not fed during the exposure period, aside from organic matter provided by the artificial sediment mixture.

After the exposure period, each jar was carefully poured over a 35-µm mesh sieve and individuals from the water column are collected and counted. Worms remaining in the sediment were carefully extracted, counted, and rinsed with deionized water to remove sediment and other residue. The number of total individuals was noted, recording the number alive and deceased. Organisms were patted dry with a Kim-wipe and added to small pre-tared glass vials to measure total and individual wet weight following recovery. The vials were placed in a drying oven at 60^{o} C for 24 hours and reassessed for total and individual dry weight.

3.2.5. Acute Toxicity to L. variegatus

Alongside chronic toxicity experiments, a series of acute toxicity studies designed to evaluate the toxicity of plastic leachate relative to impacts of the particles were conducted to better understand the potential role of chemical additives in toxicological response. The transparent body wall and closed circulatory system of *L. variegatus* makes pulsation rate an easily detectable and quantifiable acute response, demonstrated in prior studies (US EPA, 1990). For these experiments, leachate of each polymer was made by exposing the respective polymer to sunlight on a rotoshaker in vials of artificial freshwater for 1 week. A control of clean AFW absent of plastic was exposed to the same aging conditions to control for any possible effects of exposure to sunlight and motion.

As a toxin control, the known effect that caffeine has on pulsation rate in these organisms was also measured (Drewes, 1995). In addition to the control, treatments consisted of leachate with and without the corresponding polymer, where the particle was either left or removed after the week of leaching. After the week, 4 random *L. variegatus* individuals were added to each treatment vial (n=4 vials per treatment) and exposed for 48-hr. Baseline pulsation rates were measured prior to exposure. These assays were conducted on all six plastics in their pristine form, and on samples of HDPE bottle cap, PET microfiber and bottle, and SBR after aging in the surface waters of the stormwater pond and Lake Ontario. Due to losses, only PET microfiber and bottle samples aged in the surface waters of the stormwater pond were tested at the 4-mo sampling time point.

Pulsation rate was measured using a plastic pipette dropper to take up *L. variegatus* individuals and carefully place them into a capillary tube open on both ends. The capillary tube was placed under a standard light microscope and individual pulsations were counted on the same selected area for three separate 15 second increments. Measurements were taken on the same segment to avoid miscounting. When organism movement was heavy due to light stress, time was given to wait until movement subsided.

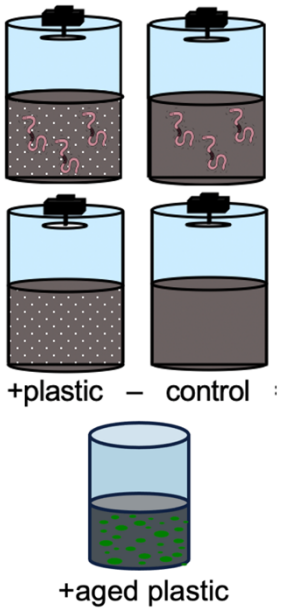


Figure 3.3. Outline of treatments used in microcosm experiments

3.2.6. Microcosm Design

Sediment for microcosms was collected from Irondequoit Bay in Rochester, NY to a depth of 5 cm using 9.5 x 30 cm clear polycarbonate tubes, sieved in the field through a 1-mm mesh to

remove rocks, macroinvertebrates, and plants and sorted to depths of 0-2 cm and 2-5 cm. Cores were reconstructed in the lab in 1-L wide-mouth glass mason jars. Material additions were 0.22% of whole sediment core dry weight (0.5 g), determined by estimated masses and volumes of benthic plastic found in the literature (Deng, et al. 2019; Lenaker, et al. 2019; Peller, et al. 2019; Table A.1.), alongside experimental concentrations used by studies with similar goals (Green, et al. 2016; Seeley, et al. 2020). Treatments are shown in Figure 3.3, and included a sediment control absent of plastic, an organism control absent of plastic, aged plastic, and pristine plastic in the presence and absence of *L. variegatus*. For SBR and PVC, only pristine plastics were evaluated in the presence and absence of organisms. Material additions were mixed into the 0-2 cm layer to prevent resuspension to the headspace. Each microcosm was wrapped with opaque plastic below the sediment-water interface to prevent light penetration and placed in a 416 L Living Stream Tank (FrigidUnits, Inc. Toledo, OH) with AFW for 4 weeks. The tank was set to a 14:10 hour light:dark cycle under full spectrum lights and cores were individually aerated by air stone bubblers attached to an aquarium air pump. Following a 24-hr acclimation period, treatments incorporating *L. variegatus* had 50 randomly selected individuals added gently to each core.

3.2.7. Microcosm Oxygen and Nutrient Sampling

Cores were acclimated in the tank for one week to restore microbial communities and solute concentrations. Once a week for three weeks, water samples were drawn to measure sediment-water column fluxes of nitrate, ammonium, and phosphate. Artificial freshwater of known concentration replaced the water withdrawn for these samples and this replacement was considered in flux calculations. Nitrate was measured using a vanadium-based method and measuring absorbance on a Shimadzu UV-1900 spectrophotometer (Doane, et al. 2003),

ammonium was measured using the phenol hypochlorite method (Solórzano, 1969), and phosphate was analyzed using the ammonium molybdate method (Murphy & Riley, 1962). After 4 weeks, oxygen flux was measured in each core in the light and the dark using a gastight chamber top with two drilled holes to allow 3/16" tubing fitted in a rubber grommet to pass through. A recirculating water pump was attached to the tubing to mix the water column throughout the sampling period. At each sampling point, water was collected in an anaerobic chamber with tubing capped with a three-way stopcock, taking one measurement approximately every hour. The chamber was built to fit a dissolved oxygen probe (Hach LDO101 Field Sensor) securely at the top to measure oxygen concentration at each sampling point. Four measurements were taken in the dark and another four were taken in the light. The 14:10 hour light:dark diel cycle was used to calculate daily net ecosystem metabolism (NEM) from the hourly light and dark measurements. Gross primary production (GPP) was calculated using the difference between the oxygen flux in the light and dark, assuming respiration is the only oxygen consuming process occurring in the dark. All nutrient flux rates were calculated based on changes in concentrations in the headspace over time (Tyler, et al. 2001).

3.2.8. Analysis of Sediment Properties

After flux sampling, each microcosm was sampled for sediment oxygen penetration depth (assessed visually) using methods described by Solan et al. 2004, organic matter content and benthic chlorophyll *a* (Chl *a*). Organic matter samples were extracted using a 60-cc syringe corer marked to 2 cm to extract surface layer sediment. Organic matter content was measured using the loss on combustion (LOC) (Heiri, et al. 2001). Chl *a* samples were extracted using a 5 mL syringe corer to extract a 1 cm sample, placed in 15 mL centrifuge tubes, and stored at -80°C. To avoid light exposure, chlorophyll samples were immediately wrapped in aluminum foil and

analyzed within 30 days. At time of analysis, Chl *a* samples were extracted from cells using sonication in 90% acetone followed by a 24-hr extraction period. The following day, samples were centrifuged, and absorbance of supernatant was measured absorbance at 665 nm and 750 nm on a Shimadzu UV-1800 spectrophotometer before and after addition of 1N HCl (Strickland & Parsons, 1972). Concentrations of Chl *a* and phaeopigment were calculated using the Lorenzen (1967) equations.

3.2.9. Statistical Analysis

Statistical data analysis was completed using JMP 15.0 Pro software. Prior to analysis, all data were assessed for normality and heterogeneity of variance to verify assumptions for analysis of variance (ANOVA). Changes in population and individual dry mass of *L. variegatus* after 30 d exposure to pristine plastics, and changes in the pulsation rate of *L. variegatus* after 2 d exposure to pristine materials were compared using one-way ANOVA. When significant effects were found ($p<0.05$), a Dunnett's test was used to identify differences from the control. To analyze how metrics of chronic and acute toxicity of all the plastics change across ecosystems and with environmental exposure, full-factorial mixed models were used to identify significant drivers of shifts in toxicity using time, site, and aging location and their interactions as variables. Due to substantial loss of samples, data on the chronic effects of PVC in the sediment and water column were collapsed and pooled, and changes in toxicity over time and space were analyzed using full-factorial mixed-models with site and time and the interaction were used. When significant effects were found, Tukey's HSD post-hoc analysis was used to identify significant differences.

A full-factorial two-way ANOVA was used to compare pristine plastics in the presence and absence of worms on oxygen and nutrient fluxes, and sediment properties, for each polymer. When significant interactions between plastic and worms were found, a Tukey's HSD post-hoc

test was used to identify significant differences. An additional one-way ANOVA to compare the control with pristine and aged polymers was also conducted using the same control and pristine polymer values as in the previous analysis. When significant effects were found, a Tukey's HSD analysis was used to identify significant differences.

3.3. Results

3.3.1. Chronic toxicity of plastic to L. variegatus

There were significant lethal and sublethal impacts of all six plastic types on *L. variegatus* after 30-d exposures (Figure 3.8), and these effects shifted with time and across ecosystems. In the pristine form, all plastics showed significant reductions in organism abundance, all being 2-3 times lower than the control (Figure 3.4); however, these effects change with time and site. While worm abundances remained lower than their corresponding control at each site and time point tested, the difference changes over time and varies by site. In the stormwater pond, the difference in abundance compared to the control becomes more positive over time (Figure 3.5), indicating plastics in this system may become less lethal as they age. In Lake Ontario, however, the difference in abundance becomes generally more negative over time, indicating plastics may become more lethal.

The mixed model identified time as a significant factor on shifts in worm abundance after exposure to PET microfiber and SBR crumb rubber (Table 3.2), suggesting that lethal effects change over time for these polymers in a similar way across sites. The model further identified both site and the interaction between site and time as significant factors on shifts in worm abundance for HDPE (Table 3.2), showing that abundance increases in the stormwater pond but decreases in Lake Ontario. Sublethal impacts to *L. variegatus* also shifted over time, with

relative individual mass generally increasing (Figure 3.6). The mixed model identified time as a significant factor of shifts in individual mass for all polymers except PET microfiber, but there was a significant interaction between site and time for all polymers (Table 3.3) indicating that changes in individual mass over time are dependent on site, with significant increases in individual mass in after exposure to plastics aged in Lake Ontario compared to plastics aged in the stormwater pond (Figure 3.6). Alongside increases in individual mass, there were unique differences in total biomass (Figure 3.7), corroborating potential reproductive stress. In pristine form, total biomass decreased in every polymer by up to 220 mg and continued to show decreases even after both 1 and 4 months in response to plastic isolated from the stormwater pond. On the other hand, total biomass after exposure to plastics aged in the benthos of Lake Ontario for 1-month show an increase up to 275 mg, though there is no data for the 4-month time point at this location for any polymer. This increase was also observed after exposure to plastics aged at the surface of Lake Ontario, apart from PET microfiber and bottle where there was a significant decrease. The mixed model (Table 3.4) identified that both time alone and the interaction between site and time was significant for all polymers. For HDPE, all variables and their interactions were significant, suggesting that changes in total biomass over time are dependent both on the overall site, as well as whether plastics were aged in the sediment or water column. This was also shown for both PET microfiber and bottle, where all variables except for site alone were significant.

3.3.2. Acute toxicity of plastic to L. variegatus

There were unique effects of treatments containing leachate both with and without the corresponding particle on the pulsation rate of *L. variegatus* in pristine form and after aging in the environment. In pristine form with only plastic leachate, all treatments apart from those using

HDPE and PS led to significantly reduced pulsation rate compared to the control (Figure 3.9a), though, in the presence of both plastic leachate and the particle, PS showed significant reductions in pulsation rate, while treatments with PETB did not (Figure 3.9b). These unique effects continued to change over time and varied by site (Figure 3.10). In mixed model analyses, time was identified as a significant driver of change in pulsation rate for treatments with HDPE leachate, and for both treatment types using SBR. Though it appears that the difference in pulsation rate in response to treatments using aged SBR lessens over time, suggesting leachate from these materials may become less hazardous (Table 3.5). On the other hand, there was no significant difference in pulsation rate in either treatment using pristine HDPE, but it appears that leachate treatments made from aged HDPE from both sites had a significant decrease in pulsation rate, indicating that leachate from these plastics may become more hazardous over time. (Table 3.5). For PET bottle, there were also more significant effects on pulsation rate over time in the presence of just the leachate (Figure 3.10). For PET fibers, the interaction between site and time was a significant factor in the changes identified in both leachate and particle with leachate treatments, suggesting differences in acute toxicity over time are site-dependent (Table 3.6). Moreover, pulsation rate in response to both treatment types using PET microfiber and bottle were most reduced after 4 months in the stormwater pond (Figure 3.10), though, there is no 4-month time point in Lake Ontario to compare these data against.

3.3.3. Ecosystem level impacts of plastic pollution

There were additional significant and unique differences among the plastic types on key ecosystem processes, and on the functional role of *L. variegatus* as an ecosystem engineer. The presence of *L. variegatus* significantly enhanced net ecosystem metabolism (NEM) and the uptake of nitrate to sediments in all polymer groups. However, NEM was more negative and

nitrate uptake was reduced in sediments with *L. variegatus* and PET bottle (Figure 3.11a, Figure 3.12a), suggesting the functional role of the organism may have been negatively impacted by the presence of the plastic. Significant interaction between worms and plastic was further observed in daily ammonium flux in sediments containing HDPE, PS, and PVC (Figure 3.12b), where there was reduced sediment uptake. There were additional impacts on ammonium flux over time in sediments containing HDPE and PS, with a significantly reduced release to the water column in pristine form and after 4 months of aging when compared to the sediment control. There were additional effects on nutrient cycling in sediments containing PS and PVC with significant release of phosphate to the water column in the presence of plastic (Figure 3.12c), and highest releases from sediments containing pristine plastic. Sediments containing HDPE, PS, and PVC continued to show effects on sediment properties, demonstrating enhanced organic matter content (Figure 3.13a). For sediments containing HDPE, this increase was seen in pristine plastics, but sediments containing PS continued to have a higher organic matter content even after aging for 4 months. The presence of worms further showed significant enhancement of benthic microalgal content in sediments containing PS (Figure 3.13b).

3.4. Discussion

3.4.1. Toxicity of plastics over time and space

In this study, the ecological impacts of six different plastic polymers over time in two different freshwater environments were studied to create a better understanding of the complexities of plastic pollution. In examining the impacts over time, the net effect of both the particle and biofilm were evaluated. While other studies have investigated toxicity of plastic in the pristine form or with plastic leachate, this is the first study to evaluate chronic toxicity and the ecosystem impacts of plastic particles over time in freshwater sediments, to our knowledge.

Overall, we observed negative effects on both abundances and mass of *L. variegatus* after exposure to all plastics in their pristine form and saw changes in these effects over time and between the two systems. Worm abundance relative to the control remained negative, suggesting plastics remained toxic to *L. variegatus* after 4 months. In Lake Ontario, relative abundances became more negative, suggesting increased toxicity over time at this site, while relative abundances become more positive after time in the stormwater pond. The observed increases in individual mass alongside reductions in total biomass indicate potential reproductive stress on *L. variegatus*. Lumbriculids reproduce asexually by fragmentation and increases in individual mass may be indicative of ecological stress on the organism (Martinez, et al. 2006). In pristine form, plastics have a range of chemical additives that may be toxic to organisms, however, the chemistry of the plastic may be altered by UV-exposure (Song, et al. 2017; Cai, et al. 2018;), or leach additives into the environment as they age. Plastics may also adsorb outside contaminants over time, leading to increased toxicity (Antunes, et al. 2013; O'Donovan, et al. 2018; Yu, et al. 2019). Plastics aged in Lake Ontario may have adsorbed outside toxins that made these materials more toxic, while materials aged in the stormwater pond could have lost toxic additives. Environmental conditions at these sites differ substantially with different chemistry that may play a role in toxin adsorption or release. As a major lake located among urbanized areas, Lake Ontario has been shown to have a variety of toxins (i.e., PCBs, PAHs) in low amounts, but accumulating over time (Rios & Evans, 2013; McDonough, et al. 2014; 201Rios et al. 2016; Rios & Balcer, 2020). These same toxins have been demonstrated to be adsorbed onto the surfaces of various microplastics in freshwater environments (Rios, et al. 2007; Faure et al. 2015) with potential to be transferred up the food web upon ingestion (Lee, et al. 2019). On the other hand, stormwater ponds are primarily impacted by road runoff, with road salt and nutrients

being among the most common (Sonderup, et al. 2015; Lusk, et al. 2020). The stormwater pond used in this study is a young pond located in a relatively underdeveloped area, making heavy contamination to this system unlikely. The differences in the chemistry of the two sites likely influence the differences observed in toxicity, where simultaneous occurrence of organic matter or surface biofilms formed on plastics isolated from the stormwater pond having a shielding effect and minimizing toxicity of the material (Cerrillo, et al. 2016) while toxin adsorption from plastics in Lake Ontario increasing toxicity is plausible.

Results from acute toxicity bioassays suggest that chemical composition is an important factor in plastic toxicity while shifting over time. Contrary to results from chronic toxicity bioassays, pulsation rate was most reduced by plastics aged in the stormwater pond, making it plausible that chronic effects of materials from the stormwater pond were minimized from surface biofilms or organic matter, though, acute bioassays were only run on a handful of materials sampled only from the surface and there is no 4-month data for samples from Lake Ontario. Additionally, the polymers we evaluated differed in morphology, and this may contribute to differences in toxicity (Vroom, et al. 2017; Choi et al. 2018). It is possible that the shape of the fibers compared to the small spheres or jagged fragments used may add a physical component to the toxicity of materials, where organisms can become entangled in the fibers. Further studies comparing both natural (i.e., cotton, wool) and synthetic materials as fibers may help clarify different components of plastic toxicity.

3.4.2. Impacts of plastic on freshwater biogeochemistry

This study also investigated how the impacts of plastics on *L. variegatus* impacts their role as an ecosystem engineer, alongside how plastics impact key ecosystem processes, including sediment biogeochemistry, benthic-pelagic coupling, and benthic microalgae over time. The

results suggest that pristine plastics have unique impacts on key ecosystem processes by themselves, as well as impacts the functional role of *L. variegatus*.

There were unique effects of plastics without organisms on ecosystem processes, with both sediments containing PS, SBR, and PVC showing greater release of phosphate to the water column (Figure 3.11c). Furthermore, PS in both pristine form and after aging showed enhanced organic matter content (Figure 3.13a). Microplastic may be a novel source of carbon for microorganisms and benthic invertebrates (Hu, et al. 2019; Shiu, et al. 2020), enhancing organic matter over time. Microplastics have also been shown to increase sediment porosity (Cluzard, et al. 2015), which may explain higher phosphate fluxes in sediments containing PS, SBR, and PVC. However, both sediments containing PS and HDPE also showed significantly reduced fluxes of ammonium compared to the sediment control (Figure 3.12b), where it is also possible that microplastics may enhance microbial nitrification activity (Muβmann, et al. 2013), while creating small anaerobic habitats (Li et al. 2020) that may promote annamox or denitrifying bacteria alongside. These conditions may cause the reduced release of ammonium in sediments containing microplastic while offering a nitrogen removal pathway, though this was not directly measured here. Many of these unique effects in sediments containing PS and HDPE continued to be seen after 4-mo of aging, suggesting that effects of plastic on ecosystem processes may have long-term implications, particularly related to nitrogen cycling.

Moreover, the presence of worms and the subsequent interactions with plastic showed unique effects on ecosystem processes. As a bioturbating organism, the burrows created by *L. variegatus* significantly contribute to benthic-pelagic coupling, and the effects of worms were significant across all treatment groupings in enhancing NEM and daily nitrate flux, consistent with prior studies (Kuntz & Tyler, 2018; Ponte, et al. 2019). However, this study identified

polymers in the pristine form to have negative impacts to *L. variegatus* and results additionally show that these negative effects extend to impact ecosystem function, though effects are unique by polymer and on different aspects. PET bottle appears to reduce the role of *L. variegatus* in both oxygen and nitrate flux (Figure 3.10a, Figure 3.11a), while PS, HDPE and PVC appear to reduce the role in ammonium flux (3.12b). These findings are consistent with prior studies identifying the negative impacts of different microplastics to bioturbating organisms (Green et al. 2016; Huang, et al. 2021) and their ability to rework sediment with impacts to nutrient cycling. While behavior of lumbriculids may differ in the natural environment compared to microcosms, it is possible that interaction with the plastic had impact on the ability for these organisms to burrow. Alongside the unique impacts plastics have by themselves on nitrogen cycling, reducing the role of *L. variegatus* may have additional long-term effects. The burrows created by these organisms provide aeration to the sediment and facilitate aerobic processes such as nitrification, while additionally supporting microbial communities (Lohrer, 2004). Though certain plastics alone may enhance nitrification, there may still be a disruption to the sediment profile and benthic microbial communities that may impact ecosystem function moving up the food web. With sediments containing PET-B, PS, HDPE, and PVC showing significant impacts to key biogeochemical cycles in the presence and absence of organisms, this identifies these polymers as some with the largest ecological risk, particularly as these polymers are some of the most identified in the environment and are used in common consumer items. Overall, these results show that plastic pollution shows both unique impacts to organisms and freshwater biogeochemistry, and that the two intersect. These impacts also shift over time, making plastic pollution a multi-faceted issue that must be treated with the diversity that exists among polymers, and monitored closely over time at different sites to understand and minimize risk to ecosystems.

Figures

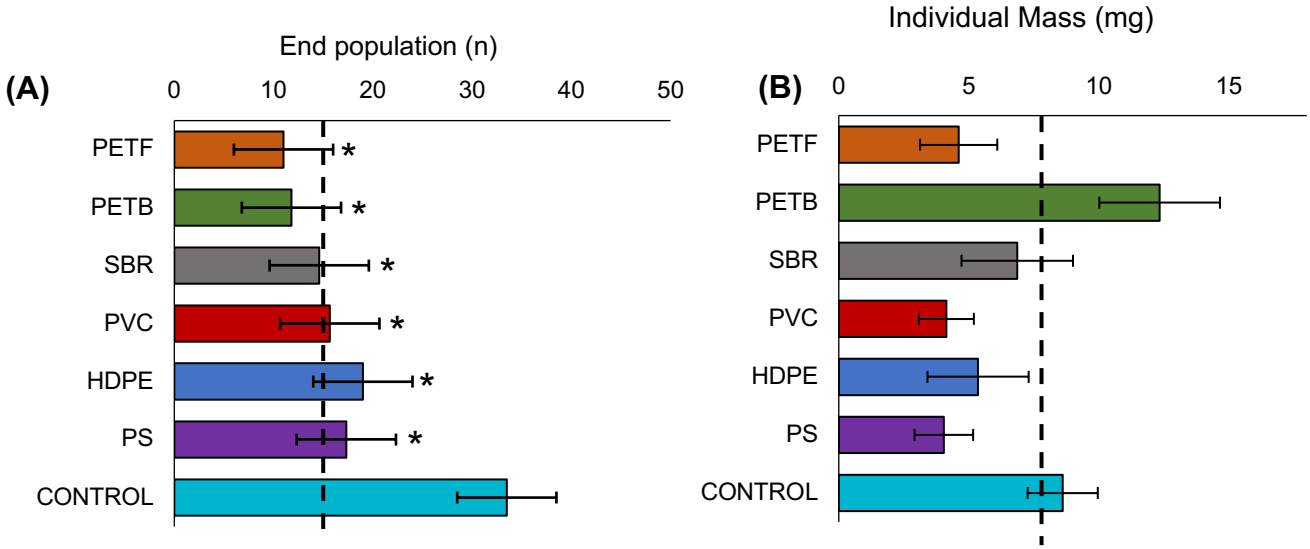
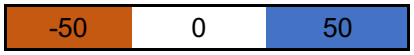


Figure 3.4. Toxicity of plastics for *L. variegatus* in their pristine form, measuring changes in population (A), and individual dry mass (B) after 30 d. Values are mean ± SE. Dashed lines indicate initial conditions. Asterisks indicate significant differences from control ($p<0.05$).

			HDPE	PET-B	PET-F	PS	PVC	SBR
Pristine		T0	**-15**	**-22**	**-20**	**-16**	**-18**	**-20**
SW Pond	Surface	T1	**-13**	**-11**	**-18**	**-14**	-9	-1
		T4	**-11**	-7	-1	-2	7	-1
	Benthic	T1	-14	-11	-12	**-14**	**-11**	-8
		T4	**-6**	-6	**-9**	-8	-3	**-10**
Lake Ontario	Surface	T1	**-20**	**-22**	**-21**	**-21**	**-18**	**-21**
		T4	**-28**	ND	**-26**	ND	ND	ND
	Benthic	T1	**-19**	**-17**	-11	-16	ND	-8



difference in abundance relative to control

Figure 3.5. Heat map showing differences in abundance of *L. variegatus* recovered from chronic toxicity bioassays on pristine materials and after aging in the surface and benthos of both sites relative to control data. Positive values indicate greater abundances relative to control. Negative values indicate lower abundances relative to control. Bolded values were identified as significantly different from control ($p<0.05$) in paired t-tests.

			HDPE	PET-B	PET-F	PS	PVC	SBR
Pristine		T0	-3	1	-4	**-4**	-2	1
SW Pond	Surface	T1	3	**16**	17	**13**	5	7
		T4	0	1	3	3	-1	-1
	Benthic	T1	**9**	5	2	0	13	2
		T4	-3	-1	-3	1	-2	-2
Lake Ontario	Surface	T1	**16**	15	2	**16**	**29**	13
		T4	-5	ND	0	ND	ND	ND
	Benthic	T1	**20**	**29**	7	21	ND	16

-50 0 50

difference in individual mass (mg) relative to control

Figure 3.6. Heat map showing differences in individual mass (mg) of *L. variegatus* recovered from chronic toxicity bioassays on pristine materials and after aging in the surface and benthos of both sites relative to control data. Positive values indicate greater individual mass relative to control. Negative values indicate lower individual dry mass relative to control. Bolded values were identified as significantly different from control ($p<0.05$) in paired t-tests.

			HDPE	PET-B	PET-F	PS	PVC	SBR
Pristine		T0	**-197**	**-184**	**-221**	**-212**	**-188**	**-172**
SW Pond	Surface	T1	-34	**279**	61	129	51	**115**
		T4	**-121**	-20	-43	**-104**	-47	14
	Benthic	T1	-5	15	8	111	95	-65
		T4	**-137**	**-104**	**-137**	-104	-90	**-99**
Lake Ontario	Surface	T1	74	**-124**	**-157**	12	302	13
		T4	ND	-185	-77	ND	ND	ND
	Benthic	T1	81	155	39	207	ND	273

-100 0 100

difference in biomass (mg) relative to control

Figure 3.7. Heat map showing differences in total biomass (mg) of *L. variegatus* recovered from chronic toxicity bioassays on pristine materials and after aging in the surface and benthos of both sites relative to control data. Positive values indicate greater individual mass relative to control. Negative values indicate lower individual dry mass relative to control. Bolded values were identified as significantly different from control ($p<0.05$) in paired t-tests.

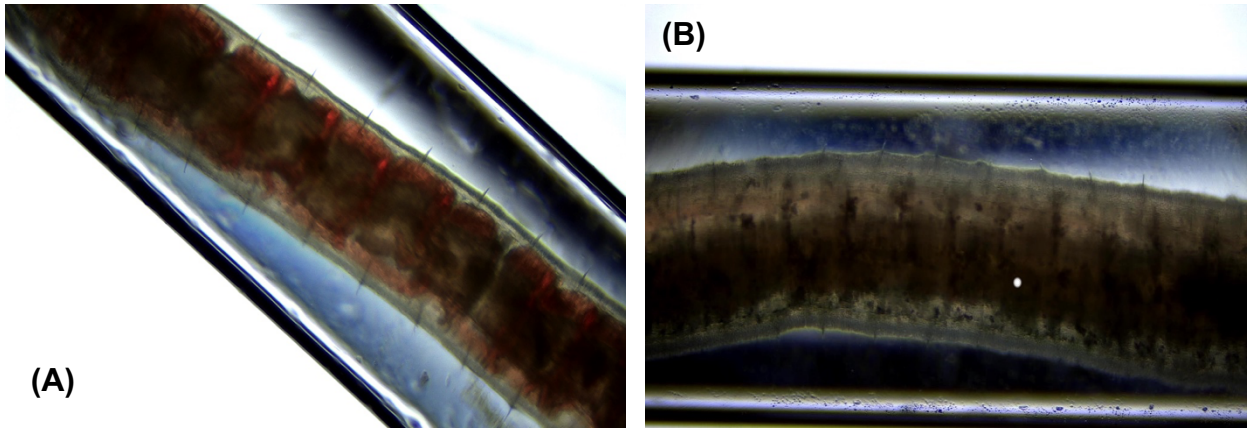


Figure 3.8. Microscope images of body condition of *L. variegatus* before chronic toxicity bioassays (A) and after a 30-d exposure to HDPE aged in the surface of Lake Ontario for 1 month (B).

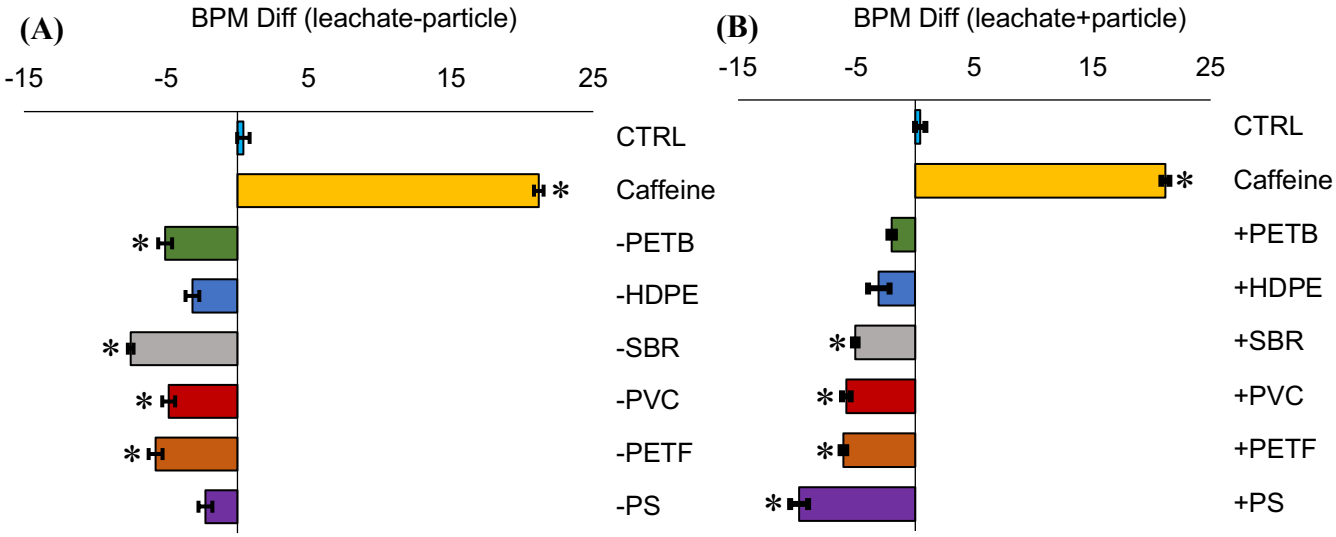
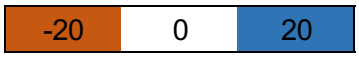


Figure 3.9. Acute toxicity of plastics for *L. variegatus* in their pristine form, measuring changes in pulsation rate after exposure to plastic leachate with and without the corresponding particle, and individual dry mass (B) after 30 d. Values are mean ± SE. Dashed lines indicate initial conditions. Asterisks represent treatments significantly different from the control ($p<0.05$) in a one-way ANOVA.

			HDPE		SBR		PET-B		PET-F	
			particle+ leachate	leachate only	particle+ leachate	leachate only	particle+ leachate	leachate only	particle+ leachate	leachate only
	Pristine	T0	-3	-3	**-5**	**-7**	-2	**-5**	**-6**	**-6**
SW Pond	Surface	T1	-4	**-8**	-1	-2	2	**-6**	-2	-2
	Surface	T4	ND	ND	ND	ND	**2**	**-12**	**-9**	**-8**
L Ontario	Surface	T1	-2	**-6**	-1	-2	0	**-6**	**-2**	-3



difference in pulsation rate relative to control

Figure 3.10. Heat map showing differences in pulsation rate of *L. variegatus* after exposure to plastic leachate with and without particle from pristine materials and after aging in the surface of Lake Ontario and the Stormwater Pond relative to control data. Positive values indicate greater individual mass relative to control. Negative values indicate lower individual dry mass relative to control. Bolded values were identified as significantly different from control ($p<0.05$) in paired t-tests.

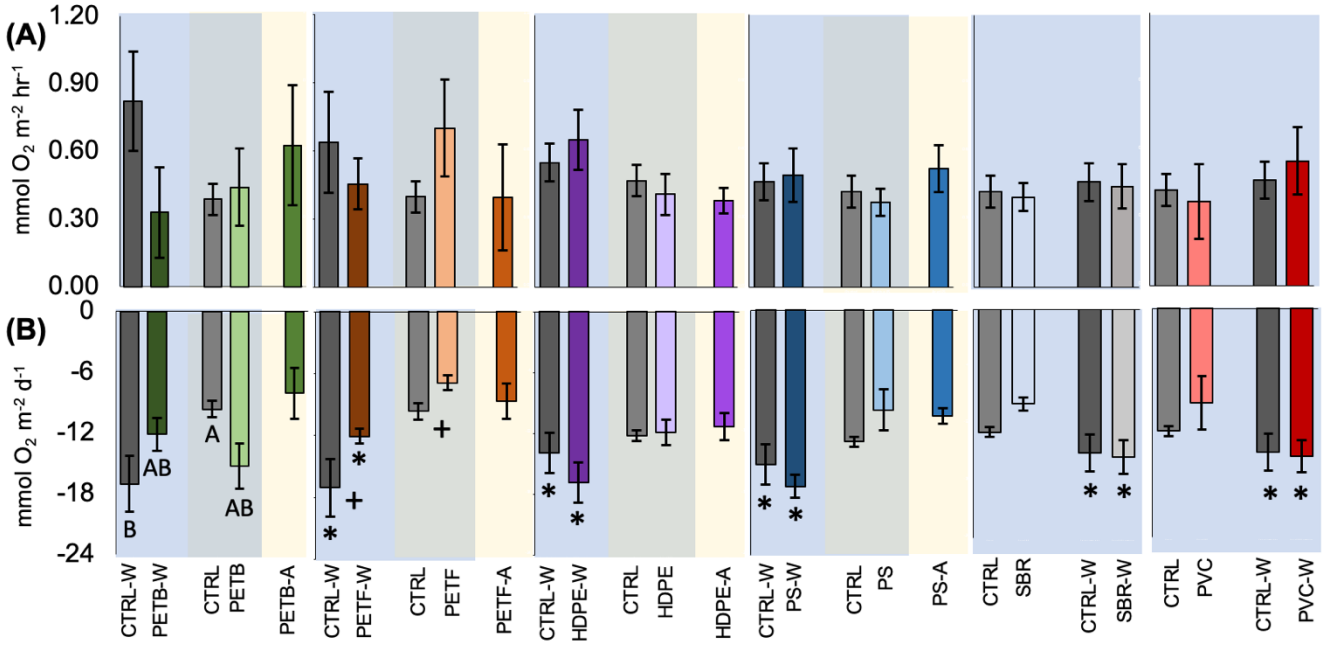


Figure 3.11. Gross primary production (GPP; A) and net ecosystem metabolism (NEM; B) after 30 d, in response to six plastic polymers in their pristine form in the presence and absence of *L. variegatus*, and after aging for 4 months in Lake Ontario benthos. Values are mean ± SE. Asterisks indicate significant differences between treatments with and without worms, plus signs indicate significant differences between treatments with and without plastics. Significant effects due to the interaction of worms and plastic are represented by different ABC lettering.

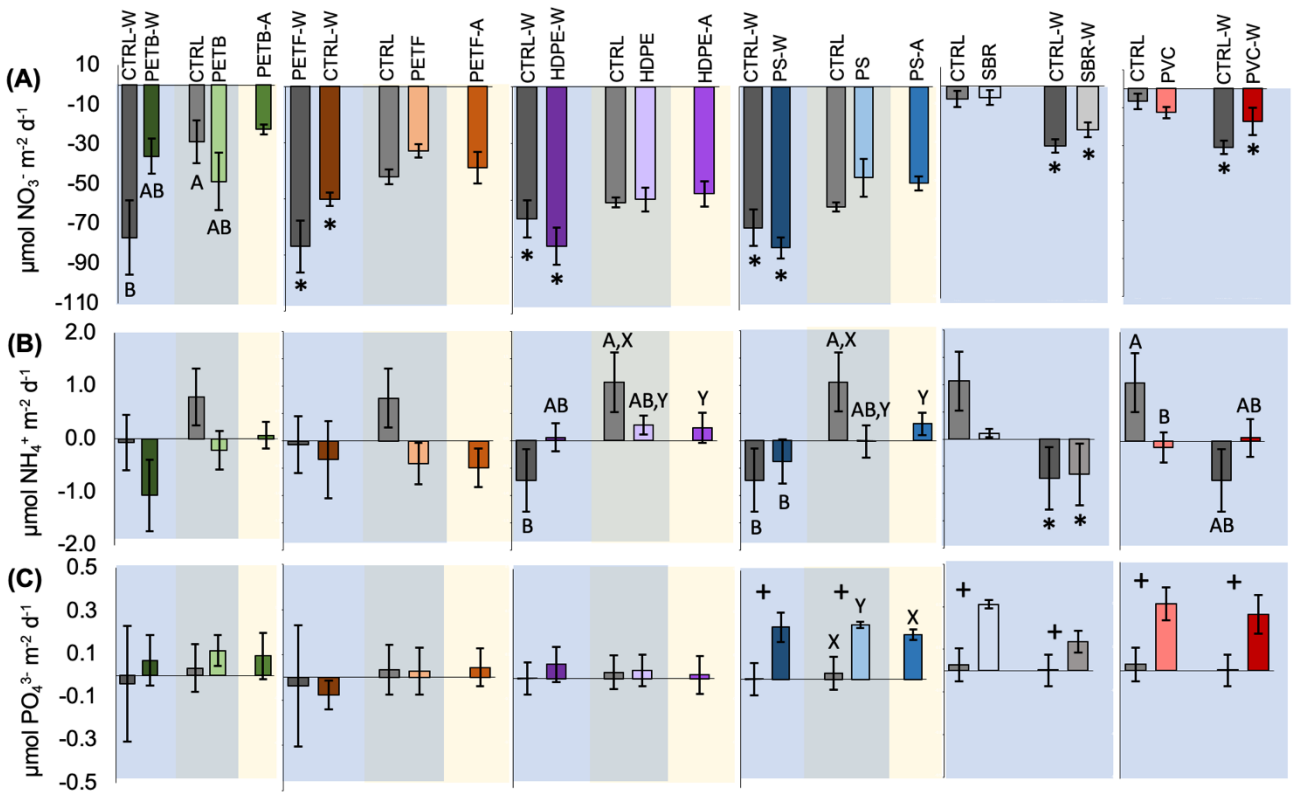


Figure 3.12. Daily fluxes of nitrate (NO_3^-; A), ammonium (NH_4^+; B), and phosphate (PO_4^{3-}; C) in response to six plastic polymers in their pristine form in the presence and absence of *L. variegatus*, and after aging for 4 months in Lake Ontario benthos. Values are mean ± SE. Asterisks indicate significant differences between treatments with and without worms, plus signs indicate significant differences between treatments with and without plastics. Significant effects due to the interaction of worms and plastic are represented by differing ABC lettering. Significant effects of time are indicated by differing XYZ lettering.

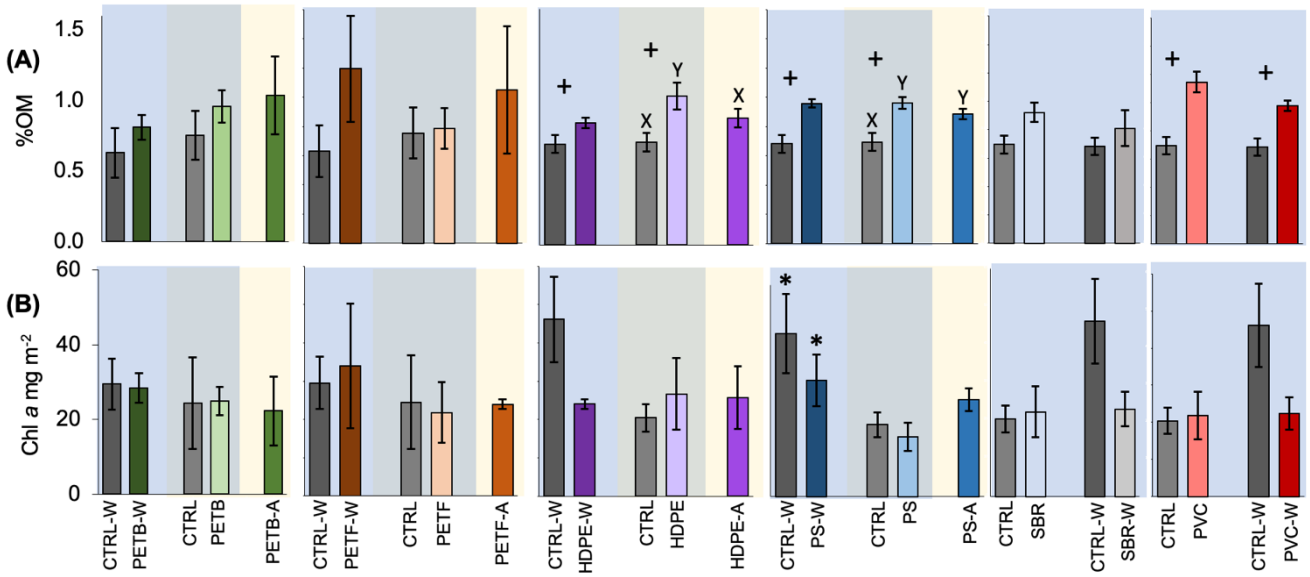


Figure 3.13. Benthic chlorophyll content (A) and organic matter content (B) after 30 d in response to six plastic polymers in their pristine form in the presence and absence of *L. variegatus*, and after aging for 4 months in Lake Ontario benthos. Values are mean ± SE. Asterisks indicate significant differences between treatments with and without worms, plus signs indicate significant differences between treatments with and without plastics. Significant effects due to the interaction of worms and plastic are represented by differing ABC lettering. Significant effects of time are indicated by differing XYZ lettering.

Tables

Table 3.2. Output of mixed model on relative abundance of *L. variegatus* recovered from chronic toxicity bioassays. Significant effects and interactions *(p*<0.05) are bolded.

Factor		HDPE	PET-B	PET-F	SBR	PS	PVC
Site	F	9.44	1.14	0.20	0.00	3.39	3.04
	p	**<0.0036**	0.29	0.65	0.93	0.07	0.08
Time	F	0.04	2.02	10.7	4.80	0.05	1.66
	p	0.85	0.16	**0.0019**	**0.0343**	0.81	0.20
Location	F	0.71	0.82	2.64	0.93	0.55	
	p	0.40	0.37	0.11	0.33	0.46	
Site x Loc	F	1.6	0.84	3.05	3.33	1.36	
	p	0.20	0.36	0.08	0.07	0.25	
Site x Time	F	4.2	0.07	0.49	0.90	2.65	1.91
	p	**0.04**	0.79	0.49	0.35	0.11	0.17
Loc x Time	F	2.6	0.57	0.60	0.83	0.30	
	p	0.11	0.46	0.44	0.36	0.59	
Site x Loc x Time	F	1.5	0.47	2.66	1.76	1.14	
	p	0.22	0.49	0.10	0.19	0.29	

Table 3.3. Output of mixed model on relative individual mass of *L. variegatus* recovered from chronic toxicity bioassays. Significant effects and interactions (*p*<0.05) are bolded.

Factor		HDPE	PET-B	PET-F	SBR	PS	PVC
Site	F	27.6	19.6	1.5	25.1	29.3	69.7
	p	**<0.0001**	**<0.0001**	0.22	**<0.0001**	**<0.0001**	**<0.0001**
Time	F	15.3	13.9	3.18	16.2	33.1	63.2
	p	**0.0003**	**0.0006**	0.08	**0.0002**	**0.0001**	**<0.0001**
Location	F	12.9	1.3	2.5	0.49	0.71	
	p	**0.0008**	0.24	0.12	0.48	0.40	
Site x Loc	F	14.0	4.1	5.3	0.05	0.48	
	p	**0.0005**	**0.04**	**0.02**	0.82	0.49	
Site x Time	F	16.5	16.3	5.2	18.5	32.8	65.3
	p	**0.0002**	**0.0002**	**0.02**	**0.0001**	**0.0001**	**<0.0001**
Loc x Time	F	19.4	1.5	3.24	0.14	0.57	
	p	**<0.0001**	0.22	0.07	0.70	0.45	
Site x Loc x Time	F	19.2	1.7	3.4	0.13	0.29	
	p	**0.0001**	0.19	0.06	0.71	0.58	

Table 3.4. Output of mixed model on total biomass of *L. variegatus* recovered from chronic toxicity bioassays. Significant effects and interactions (p<0.05) are bolded.

Factor		HDPE	PET-B	PET-F	SBR	PS	PVC
Site	F	7.18	2.22	4.02	18.70	13.8	29.6
	p	**0.0103**	0.1434	0.05	**<0.0001**	**0.0006**	**<0.0001**
Time	F	7.19	6.82	12.8	23.5	21.3	36.0
	p	**0.0102**	**0.0126**	**0.0008**	**<0.0001**	**<0.0001**	**<0.0001**
Location	F	4.52	2.40	7.07	3.12	2.49	
	p	**0.0391**	0.12	**0.0105**	0.08	0.1219	
Site x Loc	F	4.90	8.05	11.02	8.4	2.69	
	p	**0.0318**	**0.0071**	**0.0017**	**0.006**	0.1078	
Site x Time	F	5.40	5.58	8.22	18.0	19.02	30.6
	p	**0.0246**	**0.023**	**0.006**	**0.0001**	**<0.0001**	**<0.0001**
Loc x Time	F	7.220	2.89	4.91	2.99	1.87	
	p	**0.0101**	0.096	**0.0313**	0.091	0.117	
Site x Loc x Time	F	7.62	3.14	7.64	4.05	1.83	
	p	**0.0083**	**0.083**	**0.008**	0.05	0.1826	

Table 3.5. Output of mixed model on shift in *L. variegatus* pulsation rate after exposure to different acute toxicity bioassay treatments. Significant interactions (p<0.05) are bolded.

Factor		HDPE		PET-B		PET-F		SBR	
		Particle +leachate	Leachate	Particle +leachate	Leachate	Particle +leachate	Leachate	Particle +leachate	Leachate
Site	F	0.29	1.46	1.35	0.54	5.36	2.74	0.06	0.09
	p	0.60	0.25	0.26	0.47	**0.03**	0.12	0.81	0.76
Time	F	0.10	13.2	0.05	0.81	4.14	2.17	69.1	31.5
	p	0.75	**0.003**	0.82	0.38	0.6	0.16	**<0.0001**	**0.0001**
Site x Time	F	0.29	1.46	4.14	0.63	7.96	4.61	0.06	0.09
	p	0.60	0.25	0.06	0.44	**0.01**	**0.048**	0.81	0.76

Table 3.6. Results of two-way ANOVA on the effects of plastic type, worm presence, and the interaction between plastics and worms (P*W) on all metrics evaluated. Degrees of freedom were 3 for all. Significant effects (p<0.05) are bolded.

		%OM		Chl *a*		PO_4^{3-}		NH_4^+		NO_3^-		NEM		GPP		
		t	*p*	t	*p*	t	*p*	t	*p*	t	*p*	t	*p*	t	*p*	
PET-B	Plastic	-1.33	0.21	0.96	0.36	-0.65	0.53	1.95	0.07	-2.01	0.07	0.23	0.82	1.4	0.19	
	Worm	0.95	0.36	-2.05	0.07	0.41	0.69	1.65	0.13	2.82	**0.01**	1.35	0.21	-1.02	0.33	
	P*W	-0.09	0.93	-1.12	0.29	0.09	0.93	0.01	0.99	2.66	**0.02**	3.33	**0.006**	-1.74	0.11	
PET-F	Plastic	-0.73	0.48	-0.14	0.89	0.15	0.89	0.45	0.66	-2.09	0.06	-4.98	**0.0004**	0.18	0.86	
	Worm	0.12	0.91	-1.48	0.16	0.58	0.57	-0.72	0.49	4.48	**0.0009**	8.07	**<0.0001**	-0.64	0.54	
	P*W	0.56	0.59	0.60	0.56	-0.11	0.92	1.9	0.09	1.22	0.25	1.44	0.18	-2.31	**0.04**	
HDPE	Plastic	-2.93	**0.02**	0.97	0.35	-0.48	0.64	-0.78	0.45	0.23	0.83	0.30	0.77	-0.22	0.83	
	Worm	1.26	0.24	-1.42	0.18	0	0.99	3.63	**0.004**	3.84	**0.003**	3.53	**0.005**	-1.75	0.11	
	P*W	-1.10	0.30	-1.73	0.11	0.37	0.72	2.98	**0.01**	0.60	0.56	-0.57	0.58	0.88	0.40	
PS	Plastic	-5.39	**0.0002**	1.14	0.28	-3.61	**0.004**	0.24	0.81	-0.26	0.80	-0.37	0.72	0.90	0.38	
	Worm	0.16	0.87	-2.86	**0.01**	0.31	0.76	3.25	**0.008**	5.55	**0.0002**	4.26	**0.0017**	-1.16	0.27	
	P*W	0.08	0.94	-0.67	0.52	0.13	0.91	2.37	**0.03**	-0.41	0.63	-1.51	0.16	0.22	0.83	
SBR	Plastic	-2.11	0.06	1.49	0.16	-3.51	**0.004**	0.91	0.38	-1.22	0.25	-0.24	0.81	1.12	0.28	
	Worm	0.75	0.47	-1.86	0.08	1.7	0.12	2.63	**0.02**	5.42	**0.0002**	2.62	**0.022**	-0.80	0.44	
	P*W	-0.60	0.56	-1.76	0.10	-1.23	0.24	1.07	0.31	1.02	0.33	-1.39	0.19	-0.20	0.84	
PVC	Plastic	-2.83	**0.02**	1.59	0.14	-3.40	**0.005**	-0.18	0.86	-0.83	0.42	-0.34	0.74	0.41	0.69	
	Worm	1.11	0.29	-1.87	0.09	0.50	0.63	2.68	**0.02**	3.07	**0.009**	2.90	**0.014**	-1.05	0.32	
	P*W	-0.16	0.88	-1.79	0.10	-0.14	0.89	3.12	**0.01**	2.09	0.05	-0.09	0.93	0.4	0.69	

Table 3.7. Results of one-way ANOVA comparing effects of plastic over time on each metric. Degrees of freedom were 2 for all. Significant effects are bolded (p<0.05).

	PET-B		PET-F		HDPE		PS	
	F	*p*	F	*p*	F	*p*	F	*p*
GPP	0.45	0.65	0.91	0.44	0.38	0.69	0.88	0.45
NEM	3.62	0.07	1.47	0.28	0.18	0.84	1.51	0.28
NO_3^-	0.63	0.56	0.37	0.70	0.44	0.66	1.12	0.37
NH_4^+	0.66	0.54	1.30	0.32	9.51	**0.007**	10.01	**0.006**
PO_4^{3-}	0.18	0.84	0.00	0.99	0.01	0.98	6.22	**0.02**
Chl a	0.06	0.94	0.18	0.85	0.30	0.75	2.20	0.17
%OM	0.63	0.56	0.30	0.75	4.52	**0.04**	8.33	**0.009**

Chapter 4.
Conclusions

Our study showcases that plastic pollution is a highly complex issue, finding that toxicological and ecological impacts varying by polymer, and further identifying that these same impacts shift across time and space. With the ubiquity of plastic in the environment, these results show that plastic cannot be treated as one universal pollutant that is equal across all systems and requires thorough examination into differences between polymers and in specific ecosystems to better understand the biggest risks from plastic accumulation in freshwater environments.

We studied six common consumer items, evaluating both chronic and acute toxicity on *L. variegatus*, and the impacts on biogeochemical cycles. We investigated these impacts in their pristine form and after and 1 and 4-months of aging at two differing locations: Lake Ontario, and RIT J-Lot Stormwater Pond. Our results determined that all 6 plastics show lethal and sublethal effects on *L. variegatus*. Results also suggest that plastic leachate across polymers is acutely toxic to *L. variegatus*, showing significant decreases in pulsation rate after 2-day exposures. Though we are left with many questions and the multiple drivers of plastic ecotoxicity remains complicated, these results identify that chemical composition of polymers may be an important component to ecotoxicity, and that it changes over time. With the strongest toxicological effects being seen from pristine plastics, the differences we observed over time and site may be driven by the leaching and adsorption of contaminants at the two locations, or from differences in biofilms formed on plastic that may minimize or enhance toxicological effects. Both factors are influenced by outside environmental conditions, like light and nutrient availability and pre-existing contaminants in the water, making plastic ecotoxicity site-specific. Moreover, with many of these polymers differing in morphology, this may be an additional driver of ecotoxicity, particularly with morphologies like fibers that may cause physical entanglement of organisms and reduce mobility alongside any chemical effects.

We further identified that there are unique impacts on biogeochemical cycling by polymer, consistent with our original predictions. In the first microcosm experiment, we identified that PET microfibers and PVC particles had unique impacts on different processes, with reduced ecosystem metabolism and increased sediment oxygen penetration in sediments containing PET microfibers. This remained consistent in the second microcosm experiment and did not change over time. PVC stimulated significant uptake of ammonium and phosphate to the sediment and had greater benthic microalgal content, likely related to primary production in these sediments, showing that alterations to one ecosystem process will cause others to shift alongside. In the second microcosm experiment, sediments containing PVC continued to show unique effects on nitrogen and sediment properties, though this was not evaluated over time for this polymer. Impacts to nitrogen cycling were further shown in sediments containing PS and HDPE, showing reductions in ammonium flux in pristine form and after aging, suggesting plastic pollution with these polymers may have long-term effects on nitrogen cycling.

This study also sought to link microplastic toxicity to *L. variegatus* to ecosystem function. The results show that negative impacts on *L. variegatus* extend to ecosystem function in unique ways. Results show that while worms enhanced oxygen and nitrate flux across the treatment groupings, these effects were diminished in the presence of PET bottle, PS, HDPE, and PVC. With these plastics showing negative effects to *L. variegatus* with cascading effects to ecosystem function, alongside unique impacts to biogeochemistry on their own, this may place these polymers among the greatest risk regarding plastic pollution in freshwater benthic environments. Though there are still many questions to be answered, this study provides the first glimpse into both the toxicological and ecological impacts of different plastics across time and

space in freshwater environments, providing a better holistic understanding of plastic pollution that may be used to inform plastic manufacturing and policy.

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Appendices

Table A.1. Table of sources used to determine plastic additions for toxicity bioassays and microcosm experiments.

Polymer	Location	System	Plastic Estimate	Reference
SBR	Menomonee River, Wisconsin	River - downstream	5500 p/kg dw^{-1}	Lenaker, et al. 2019
SBR	Menomonee River, Wisconsin	River – upstream	200 p/k dw^{-1}	Lenaker, et al. 2019
PET	Milwaukee River, Wisconsin	River - downstream	800 p/kg dw^{-1}	Lenaker, et al. 2019
PET	Lake Michigan, WI	Lake – offshore	35 p/kg dw^{-1}	Lenaker, et al. 2019
PET	Lake Michigan, IN	Lake	75 p/kg dw^{-1}	Peller, et al. 2019
PET	Shaoxing City, China	River	300 p/kg dw^{-1}	Deng, et al. 2019
PET	Lamberts Channel and Baynes Sound	Marine	140 p/kg dw^{-1}	Kazmiruk, et al. 2018
PVC	Lake Ontario, Toronto	Lake	725 p/kg dw^{-1}	Ballent, et al. 2016
PVC	Lake Ontario, Toronto	Lake	24 p/kg dw^{-1}	Ballent, et al. 2016
PVC	Hong Kong	River - estuary	9 p/kg dw^{-1}	Cheang, et al. 2018
PVC	Germany	River	287 p/m^{-2}	Klein, et al. 2017

Table A.2. Water quality data collected during field aging experiment in Lake Ontario and the stormwater pond. Variables include surface and benthic values of light availability, water column dissolved oxygen, temperature, conductivity, and salinity, and mid-water column pH and concentrations of nitrate, ammonium, and phosphate.

	Lake Ontario			Stormwater Pond		
	July 2020	Oct 2020	June 2021	July 2020	Oct 2020	June 2021
Surface Light (µmol photons/m^{-2}/sec)	316.2 ± 0.8	1090 ± 21	1394 ± 37	756.7 ± 54.6	791.5 ± 12	1394.7 ± 37
Bottom Light (µmol photons/m^{-2}/sec)	211.3 ± 8.1	862 ± 11	501 ± 139	78.5 ± 63.2	318.2 ± 78	501.3 ± 139
Surface DO (mg/L)	9.3 ± 0.1	11.3 ± 0.1	11.9 ± 0.2	12.7 ± 0.2	12.5 ± 0.2	4.8 ± 0.1
Bottom DO (mg/L)	9.5 ± 0.1	11.5 ± 0.1	11.7 ± 0.2	11 ± 1	12.4 ± 0.5	4.9 ± 0.3
Surface Temp (C)	24 ± 0.1	20.4 ± 0.5	18	29.1 ± 0.2	22.0 ± 0.5	21.3 ± 0.1
Bottom Temp (C)	23.8 ± 0.1	18.6 ± 0.3	18 ± 0.1	28 ± 0.3	19.9 ± 0.5	21.2 ± 0.1
Surface Conductivity (µS/cm)	294.3 ± 0.3	221.3 ± 0.9	252	881.3 ± 2.7	967.3 ± 2.4	2048.7 ± 1.3
Bottom Conductivity (µS/cm)	293.3 ± 0.3	207.3 ± 0.2	252.3 ± 0.3	868.7 ± 4.7	920.0 ± 5.9	2040.3 ± 1.5
Salinity (ppt)	0.1	0.1	0.1	0.4	0.5	1.1
pH	6.5	7.0	0.1	8.8	7.6	ND
NO_3^- (mg N/L)	0.34 ± 0.01	0.30 ± 0.00	0.17 ± 0.02	0.21 ± 0.00	0.21 ± 0.0	0.059 ± 0.01
NH_4^+ (µM)	0.12 ± 0.15	0.34 ± 0.16	ND	1.05 ± 0.24	1.06 ± 0.25	ND
PO_4^{3-} (µM)	0.13 ± 0.02	0.10 ± 0.03	0.18 ± 0.02	0.14 ± 0.03	0.13 ± 0.05	0.21 ± 0.02

Table A.3. Final population size of *L. variegatus* recovered from chronic toxicity bioassays. Values are mean ± SE.

			HDPE	PET-B	PET-F	PS	PVC	SBR
Pristine	Pristine	T0	19.0 ± 2.4	11.8 ± 1.9	11.0 ± 3.3	17.3 ± 3.3	15.7 ± 1.5	14.6 ± 1.9
SW Pond	Surface	T1	20.3 ± 3.0	2.3 ± 3.4	16.0 ± 2.7	19.3 ± 0.6	24.5 ± 1.5	32.8 ± 6.1
		T4	28.6 ± 1.0	25.6 ± 3.8	31.6 ± 2.1	30.8 ± 3.2	40.0 ± 2.4	31.6 ± 2.7
	Benthic	T1	19.8 ± 6.7	22.3 ± 6.5	21.6 ± 4.5	19.5 ± 1.8	17.3 ± 5.9	25.2 ± 6.8
		T4	26.8 ± 1.9	26.5 ± 3.7	24.2 ± 2.0	24.8 ± 5.4	30.2 ± 3.2	23.2 ± 2.6
Lake Ontario	Surface	T1	13.6 ± 2.4	11.3 ± 2.4	12.8 ± 5.9	12.8 ± 3.4	15.5 ± 1.3	12.2 ± 2.6
		T4	30.0 ± 1.5	ND	29.2 ± 3.6	ND	ND	ND
	Benthic	T1	11.4 ± 4.6	16.8 ± 5.1	22.3 ± 6.3	18.2 ± 6.9	ND	16.8 ± 6.5

Table A.4. Individual dry mass (mg) of *L. variegatus* recovered from chronic toxicity bioassays. Values are mean ± SE.

			HDPE	PET-B	PET-F	PS	PVC	SBR
Pristine	Pristine	T0	5.4 ± 1.9	10.3 ± 2.8	4.6 ± 1.5	4.0 ± 1.1	6.1 ± 2.2	9.5 ± 3.2
SW Pond	Surface	T1	11.6 ± 3.1	24.7 ± 1.0	25.1 ± 7.5	21.6 ± 3.8	13.5 ± 6.1	15.4 ± 8.0
		T4	5.8 ± 1.5	11.8 ± 2.9	7.6 ± 1.4	6.6 ± 1.5	6.1 ± 1.3	9.1 ± 1.6
	Benthic	T1	8.3 ± 3.3	13.4 ± 2.3	17.1 ± 5.7	21.2 ± 4.1	12.9 ± 5.4	10.1 ± 2.2
		T4	5.8 ± 1.1	7.5 ± 1.4	5.8 ± 1.5	9.7 ± 2.4	7.0 ± 1.8	7.7 ± 0.9
Lake Ontario	Surface	T1	25.0 ± 4.9	24.0 ± 10.9	10.3 ± 3.4	24.7 ± 3.9	37.5 ± 6.1	22.1 ± 9.8
		T4	5.3 ± 1.3	ND	5.0 ± 1.3	ND	ND	ND
	Benthic	T1	22.6 ± 6.2	37.0 ± 9.7	15.0 ± 3.2	19.7 ± 9.1	ND	30.0 ± 6.7

Figure A.5. Infrared spectra of PETF (A), HDPE (B), PET-B (C), PS (D) and PVC (E) used in field aging experiments. Spectra were taken on a Shimadzu IRTracer-100.