Microplastic fate and behaviour in model freshwater mesocosms

By

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Abstract

Microplastics (plastic particles <5.0 mm in diameter) have been detected in freshwater ecosystems worldwide, yet little is known about the fate and behaviour in Canadian freshwater systems. Knowledge gaps include the influence of ice formation, water quality (i.e., nutrients enhancing both biofilm and filamentous algae growth), and the presence of aquatic plants (cattails) on fate and behaviour of microplastics. I conducted two field-based mesocosm studies at the Prairie Wetland Research Facility at the University of Manitoba. The first mesocosm study was a long-term 622-day study where five different microplastic types (foams, films, fragments, microbeads and fibres) were added to understand long-term microplastic fate and behaviour during distinct seasons and overwintering. The second study was a short-term 251-day study using model constructed freshwater mesocosms where both films and fibres were added to understand whether nutrients, cattails and overwintering affected microplastic fate and behaviour. In both studies, distinct open water seasons (spring, summer, fall), over wintering and subsequent spring melt of ice lead to unique behaviours of different types of microplastics i.e., resuspending, and settling. In the second study, I found that biofilm and filamentous algal growth were enhanced by the nutrient addition were the likely drivers of microplastic settling and resuspension behaviour in the mesocosms through biofouling, defouling (biofilms) and trapping microplastics as enhanced filamentous algal growth occurs. Overall, substrate may not be the ultimate sink for microplastics as previously thought, as microplastic behaviour (e.g., aggregation, biofouling), and characteristics of the aquatic ecosystem (e.g., climate, ice crystal formation, and water temperature-density effects) will add to the complexities of microplastic behaviours and will determine their ultimate fate in freshwater ecosystems. This thesis has begun to advance our understanding of microplastic fate and behaviour in Canadian freshwater systems, their cyclical/seasonal dynamics, biofilm as a driver of microplastic behaviour, resuspension/settling dynamics due to ice formation and subsequent melting, and the potential for floating wetlands using cattails as a bioremediation technique.

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Dedication

Dedicated to my father Barry Warrack.

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Chapter 1. General Introduction

1.1 General Overview

Microplastics (plastic particles <5.0 mm in diameter) have been detected in freshwater ecosystems worldwide. The term “microplastic” is often used as a catch-all for all microplastic particles (different shapes, sizes, colours, polymers), a diverse class of contaminants. Understanding the fate and behaviour of microplastics in aquatic systems is challenging as studies often use a single microplastic type (polymer, specific size, colour), in a specific aquatic system, which does not tell us about fate and behaviour of microplastics in general (Rochman et al. 2019) or provide real world context for aquatic ecosystems. Both the uniqueness of the microplastic (morphology colour, size range, polymer, polarity, polymer additives, eco-toxin, time scale of polymer degradation; Cole et al. 2011; da Costa et al. 2017; Rochman et al. 2019), and characteristics of the aquatic system it has entered (water quality, species composition, climate) will all affect microplastic fate, behaviour, and ultimately where it will end up in the water column.

Research has estimated nearly a 100-fold difference in the estimated densities of microplastics released from point sources compared to the densities actually being detected in the receiving water bodies (Cózar et al. 2014; Warrack et al. 2017); and ultimately, aquatic systems appear to be a sink for these particles. Publications on microplastic fate and behaviour have increased since 2010, to date there are about ten studies focused on fate and behaviour in Canadian freshwater systems (Zbyszewski and Corcoran 2011; Castañeda et al. 2014; Zbyszewski et al. 2014; Corcoran et al. 2015; Anderson et al. 2016; Baldwin et al. 2016; Ballent et al. 2016; Anderson et al. 2017; Campbell et al. 2017; Warrack et al. 2017), and how both our unique sources, Canadian climate, and water quality (nutrient loading) are playing a role. This thesis examines the long-term fate and behaviour of microplastics in shallow model freshwater systems, and how microplastic fate and behaviour respond to the influence of nutrient additions and emergent aquatic vegetation. To investigate this topic, I conducted two

studies at the Prairie Wetland Research Facility (PWRF), at the University of Manitoba, to understand microplastic fate and behaviour in freshwater ecosystems: 1) a long-term mesocosm study, monitoring settling dynamics of five microplastics (foams, fragments, films, microbeads, and fibres), and; 2) a short-term mesocosm study, monitoring two microplastics (films and fibres) to determine whether there are treatment effects (nutrient and plant additions) on microplastic behaviour and settling dynamics through biofouling (biofilm growth as a predictor) and enhanced sedimentation (via cattails). Both studies were sufficient in duration to also allow an evaluation of the potential of ice formation effects on microplastic fate and behaviour.

1.2 Microplastics overview

Macroplastics are organic polymers made from petroleum products (Vert et al. 2012). Types of plastics typically found in aquatic environments are: high and low density polyethylene (HDPE and LDPE), polypropylene (PP), polystyrene (PS), foamed polystyrene, polyvinyl chloride (PVC), cellulose acetate (CA), thermoplastic polyester (PET) and nylon (PA; Appendix A; Table A1). The density of the microplastic will affect its behaviour in aquatic environments as it will determine its buoyancy and fate in the water column (i.e., float at the surface, remain in the water column, orsettle to the sediments). Plastics with low densities (<1 g/cm^3 pure water) are positively buoyant and are likely to float (PE, PP, foamed polystyrene), while plastics with higher densities (PS, PVC, PET, PA, CA) are negatively buoyant and likely to sink (Andrady, 2011; Anderson et al. 2016).

Plastic products used can have a variety of lifespans, ranging from a potential one time use of one day (e.g., consumer packaging) to more than 50 years (e.g., construction materials). Each year Canada discards an estimated 3,268 kilotonnes (kt) of plastic waste, where 87% enters landfills, 9% is recycled, and 4% is incinerated for energy recovery (Environment and Climate Change Canada 2019). Unrecovered plastic waste (i.e., not recycled or incinerated) represents an economic loss of $7.8 billion annually (based on original market value to plastics; Environment and Climate Change Canada 2019). Only 25% of discarded plastic waste is collected for recycling, and after

the sorting process, only 9% was actually recycled mechanically, chemically, or via incineration, with the rest (14%) sent to landfill (Environment and Climate Change Canada 2019). Consumer packaging accounted for the highest percentage (47%) of total plastic waste discarded in Canada compared to other sectors (Appendix A, Figure A1; Environment and Climate Change Canada 2019).

Plastic contamination can either arise from macroplastics (large plastics), or microplastics (microscopic-sized particles; Eerkes-Medrano et al. 2015) entering ecosystems. The National Oceanographic and Atmospheric Association defines microplastics as small plastic particles with their largest dimension <5mm. Plastics can enter aquatic systems via landfill leakages (microplastics leaching into groundwater; Jambeck et al. 2015), littering, aerial deposition (Dris et al. 2015a), municipal tap water (e.g., watering garden; Anderson et al. 2017; Warrack et al. 2017), stormwater (Mason et al. 2016; Liu et al. 2019), and as microplastics leaving wastewater treatment plants (WWTP) via effluent (Mason et al. 2016). Due to the prolific use of plastics by modern society (Romeo et al. 2015), coupled with a very slow degradation time (~100s of years; PlasticsEurope 2017), our understanding of the effects of plastic contamination is still in its infancy (Halden 2015) leading to a growing global concern around possible environmental effects.

Microplastic particles can be categorized into seven morphological categories: fragments, foams, fibres, fibre bundles (20+ fibres), spheres (e.g., microbeads), pellets (3 to 5mm in size, rounded or cylindrical in shape), and films (Table 2.1; Rochman et al. 2019). Microplastics also come in a wide variety of colours (e.g., blue, red, green, tan, gold, off-white; Rochman et al. 2019), which informs their source, and organisms may have preference ingesting microplastics that are similar colours to their prospective prey (de Sá et al. 2015). After microplastics are categorized and enumerated, when retrieved from the environment they should be chemically characterized by Fourier transform infrared (FTIR) or Raman Spectroscopy which use large libraries of plastic particles (e.g., SloPP, SloPP-E), to confirm microplastic polymer identification, and validation of enumeration techniques (Munno et al. 2020). Detailed reporting of microplastic particles using morphology, colour, and polymer helps to trace microplastics back to their source

(Helm 2017), and ultimately enables accurate comparison of microplastics within and between studies.

1.3 Sources and pathways of transport of microplastics in aquatic ecosystems

Microplastic particles are classified as either primary or secondary. “Primary” microplastics are manufactured to be <5mm in size (e.g., microbeads used as abrasives in both industrial and cosmetic products, or preproduction pellets; Table A2). “Secondary” microplastics are the byproduct of larger macroplastics that have fragmented and degraded over time into small, microscopic pieces (e.g., fragments, films, foams, fibres) due to biological, chemical, and physical processes (Napper and Thompson 2016; Hernandez et al. 2017; Falco et al. 2018; Henry et al. 2019; Yang et al. 2019; Table A2). Primary and secondary microplastics predominantly enter aquatic environments through different pathways based on their originating source e.g., microbeads (primary microplastic) enter via effluent discharge (Table A2).

1.3.1 Sources and transport pathways of primary microplastics

Primary microplastics are manufactured at a microscopic size (<5mm), and are found in personal care products as exfoliants, medical applications (Sundt 2014; Lassen et al. 2015), pre-production pellets (Derraik 2002, Moore 2008, Andrady 2011), drilling fluids for oil and gas exploration (Sundt 2014), and in industrial abrasives (Sundt 2014; Table A2). Globally, microbeads are used as abrasive exfoliants and are found in many personal care products (e.g., sunscreen, toothpaste, facewash, shampoos, foundation; Derraik 2002, Cole et al. 2011, Driedger et al. 2015). Microbeads are also still used in medical products as either an abrasive in dentist toothpaste, or a carrier that delivers the pharmaceutical agent in a drug (Sundt 2014; Lassen et al. 2015), despite many national bans. In July 2015, Environment Canada completed a scientific review of microbeads, and listed them as a toxic substance under subsection 64(a) of the Canadian Environmental Protection Act of 1999 (Environment and Climate Change Canada, 2016). In December 2017, Canada prohibited the manufacture and import of

products containing microbeads (Environment and Climate Change Canada, 2016). Many other countries globally (USA, UK, Sweden, Italy, France, India, South Korea, Thailand, Taiwan, and New Zealand) have also banned microbeads from personal care product as an attempt to stop an unnecessary source of microplastics entering the environment. Microbeads predominantly enter the environment via wastewater effluent after they are washed down the drain (Duis and Coors 2016; Table A2). Studies estimated ~8 trillion microbeads were released from WWTPs (prior to microbead ban) in effluent daily across the USA (Magnusson and Norén 2014; Martin and Eizhvertina 2014; Rochman et al. 2015).

Pre-production pellets (plastic pellets that are melted down and used in manufacturing of plastic products) are another source of primary microplastics (Duis and Coors 2016) in aquatic ecosystems (Table A2). Pre-production pellets often enter the environment accidently during transport, and have been found in high densities in the environment near plastic manufacturing facilities and enter the environment via run-off (Zbyszewski and Corcoran 2011; Zbyszewski et al. 2014; Lechner and Ramler 2015). Primary microplastics are also found in drilling fluids as both circulation materials and viscosity modification in the oil and gas industry, and often enter the environment accidently through improper disposal as run-off (Sundt 2014). Another important source of primary microplastics are plastic particles used in industrial abrasives i.e., air blasting to remove paint from surfaces and to clean engines (Derraik 2002; Sundt 2014). These industrial abrasives can enter aquatic ecosystems when used outside and not properly disposed of as run-off (Derraik 2002; Sundt 2014; Table A2).

1.3.2 Sources and transport pathways of macroplastics as secondary microplastics

Important sources of secondary microplastics are anthropogenic activities such as littering, dumping of plastic waste, losses during garbage and recycling pickups, losses from poorly managed landfills or recycling plants, agricultural applications, synthetic textiles, hygiene products, and fishing gear (Duis and Coors 2016; Table A2). In marine ecosystems, 75-90% of secondary microplastics have originated from terrestrial anthropogenic sources (e.g., littering), while only 10-25% originate from

ocean/water related sources (e.g., fishing, nets, shipping of cargo; Andrady 2011; Mehlhart and Blepp 2012). The relative contributions to freshwater systems has not been elucidated at this time.

Littering and unintended losses of plastics during garbage and recycling pickups are important sources of secondary microplastics and have the potential to enter aquatic ecosystems via weather phenomena (i.e., wind and stormwater run-off) which blow/wash the plastics into nearby waterways (Pruter 1987; Barnes et al. 2009; Mehlhart 2012; Table A2). Another source of secondary microplastics are losses from poorly managed landfills or recycling plants (Pruter 1987; Barnes et al. 2009; Rillig 2012; Lambert et al. 2014; Jambeck et al. 2015; Table A2). In Canada, it is estimated that 1% of plastic waste (29 kt) from poorly managed landfills enter the environment (Environment and Climate Change Canada 2019) via groundwater seepage (microplastics) and weather phenomena (both macro and microplastics; Table A2).

Plastics used for agricultural applications are another source of secondary microplastics in aquatic ecosystems (Do and Scherer 2012; Rillig 2012; Duis and Coors 2016; Table A2). Plastic mulch is applied to fields to help hold in water, decrease weed growth, and foamed polystyrene is often mixed in soil to improve its quality (Do and Scherer 2012; Rillig 2012; Duis and Coors 2016; Table A2). Plastics used in agriculture are often transported to freshwater systems via weather phenomena, e.g., wind, storm water run-off and smaller particles via seepage into groundwater (Duis and Coors 2016; Rochman et al. 2016; Table A2).

Textiles made of synthetic fibres are a source of secondary microfibres into aquatic ecosystems (Napper and Thompson 2016). As clothing is washed in washing machines, synthetic fibres are shed (Browne et al. 2011). Some of these fibres are not captured by WWTP and are eventually transported, via effluent, into freshwater systems (Browne et al. 2011). Studies have quantified numbers of fibres released in an average load of laundry (5-6 kg of clothes) to be between 1900 to 6,000,000 fibres/load (Browne et al. 2011; Napper and Thompson 2016; Falco et al. 2018). Densities of fibres released during laundering is variable and may be influenced by individual fibre sizes in the synthetic textile, as longer fibres (>1000µm) are often captured at a higher frequency in

drier lint traps compared to shorter fibres (<500 µm; Yang et al. 2019). The age of a particular garment may also affect densities of fibres shed during laundering as less fibres are shed over time, the more a garment is washed (Napper and Thompson 2016). Fibre release from textiles can also be influenced by the use of detergent (an increase is observed; Napper and Thompson 2016; Hernandez et al. 2017; Yang et al. 2019), fabric softener (a decrease is observed; Napper and Thompson 2016; Falco et al. 2018) and temperature of water (higher temperatures release more fibres; Napper and Thompson 2016; Yang et al. 2019). Finally, hygiene products (tampons and sanitary napkins) are another source of secondary microfibres to aquatic ecosystems, as fibres can be released when improperly disposed of via wastewater effluent (Duis and Coors 2016).

1.3.3 Other sources and transport pathways of both primary and secondary microplastics

Biosolids (sewage sludge) are a source of both primary and secondary microplastics in aquatic ecosystems (Zubris and Richards 2005; Browne et al. 2011; Leslie et al. 2013; Nizzetto et al. 2016; Mahon et al. 2017; Kay et al. 2018; Li et al. 2018). Sewage sludge is produced as a byproduct during the WWTP process, and is applied to agricultural fields as a fertilizer (Carr et al. 2016; Mason et al. 2016; Mahon et al. 2017). Microplastics have been found in sludge at densities ranging from ~1600 to 30,000 microplastics per kg of dry sludge (Zubris and Richards 2005; Mahon et al. 2017; Lares et al. 2018; Li et al. 2018). The microplastics' unique characteristics (size, shape, density, polymer type) will affect their ability to settle and become entrained in the sludge (Nizzetto et al. 2016). The majority of microplastics in sludge are fibres (>70%; Magnusson and Norén 2014; Mahon et al. 2017; Lares et al. 2018). After sludge is applied to the agricultural fields, microplastics previously entrained within the sludge can enter aquatic ecosystems via wind, storm water run-off, or other weather phenomena (Nizzetto et al. 2016).

Wind is an important transport pathway of both primary and secondary microplastics into the atmosphere. These airborne microplastics likely originate from

synthetic textiles, household dust, particles created during the construction process, incineration of plastic waste, and landfills (Dris et al. 2016). Once in the atmosphere, wind can transport microplastics globally (lighter weight particles travel further; Horton and Dixon 2017). Microplastics are then deposited into the new ecosystem via atmospheric fallout (Dris et al. 2015; Dris et al. 2016). Microplastics have been detected in unpopulated, isolated aquatic environments e.g., Antarctica, or remote mountain lakes (Free et al. 2014; Obbard et al. 2014; Waller et al. 2017) due to wind transportation and atmospheric deposition.

1.4 Occurrence of microplastics in aquatic ecosystems

Microplastics have been found worldwide in aquatic environments (Ivar Do Sul and Costa 2014; Cole et al. 2014; Eriksen et al. 2014; Waller et al. 2017) including surface waters (Thiel et al. 2003; Eriksen et al. 2013a, Zhao et al. 2014; Cole et al. 2014; Ivar do Sul et al. 2014; Sadri and Thompson 2014; Sutton et al. 2016; Anderson et al. 2017; Cincinelli et al. 2017; Warrack et al. 2017; Zhang et al. 2017), deep sea sediments (Woodall et al. 2014; Koelmans et al. 2017; Van Cauwenberghe et al. 2013), beach sediments (Imhof et al. 2013; Ballent et al. 2016), wastewater effluent (Magnusson and Norén 2014; Carr et al. 2016; Estahbanati and Fahrenfeld 2016; Mason et al. 2016b; Murphy et al. 2016; Ziajahromi et al. 2017; Kay et al. 2018), sea ice in both the Arctic (Obbard et al. 2014; Waller et al. 2017) and Antarctic (Waller et al. 2017), as well as organisms (Depledge et al. 2013; Foekema et al. 2013; Warrack et al. 2017).

1.4.1 Occurrence of microplastics in freshwater

Microplastics have been detected in freshwater systems in Africa, Asia, Europe, South America, and North America (Junk and Nunes De Cunha 2005; Eerkes-Medrano et al. 2015; Anderson et al. 2017; Warrack et al. 2017; Wagner and Lambert 2018). Microplastics have be found in both the surface water and sediments of freshwater systems (lakes, rivers) in Canada (Eriksen et al. 2013; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Campbell et al. 2017; Vermaire et al. 2017; Warrack et al. 2017; Helm 2020; Table A3). Densities of microplastics detected in Canadian surface

waters ranges from 2,779 microplastics/km^2 in Lake Huron (Eriksen et al. 2013) to 1,200,000 microplastics/km^2 in the Assiniboine River, Manitoba (Warrack et al. 2017). Studies have also found that microplastic densities can vary widely at different parts of a water body on the same day; e.g., Lake Huron densities ranged from 0 to 6541 microplastics/km^2 at different sampling sites on a single day (Eriksen et al. 2013). Microplastic morphology, colour, and size differs in Canadian freshwater systems; e.g., >90% fibres detected in surface water of Lake Winnipeg (Anderson et al. 2017) compared to 77% fragments in the surface water of Lake Ontario (Mason et al. 2020).

1.4.2 Occurrence of microplastics in freshwater sediments

Microplastics have been found in both lake and river sediments in Canada (Eriksen et al. 2013; Castaneda et al. 2014; Zbyszewski et al. 2014; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Vermaire et al. 2017; Warrack et al. 2017). Sediment densities are more difficult to compare across studies than surface water densities as researchers employ different sampling techniques when evaluating sediments; for example, the use of corers (Corcoran et al. 2015) sediment trap/grab samplers (Castaneda et al. 2014; Ballent et al. 2016), compared to quadrats (Zbyszewski et al. 2014; Table A3). All microplastic morphologies have been found within Canadian freshwater sediments (Eriksen et al. 2013; Castaneda et al. 2014; Zbyszewski et al. 2014; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Vermaire et al. 2017; Warrack et al. 2017). This study used five main morphologies to reflect the main varieties found in Canadian aquatic ecosystems.

1.5 Fate and behaviour of microplastics in freshwater ecosystems

Fate is often the term used to describe where the microplastic will ultimately end up (i.e., float, or sink to water column/sediments), and behaviour is the term used to describe what microplastics do in the water column/sediments (i.e., do they settle permanently or resuspend). Characteristics of both the microplastic particle and water body, i.e., climate (wind, precipitation, temperature effects, winter, freeze-thaw), water quality (nutrient concentrations, effluent point source), aquatic species (ingestion, egestion, biofouling, trophic transfer, biomagnification) may influence both fate and

behaviour of microplastics in freshwater ecosystems (Rochman et al. 2019). The diversity of microplastic particles (e.g., size, polymer, morphology, polarity) further complicates our ability to predict their exact behaviour as no two particles are identical. Once in freshwater systems, microplastics can undergo a range of abiotic (e.g., degradation, settling, resuspension, aggregation) and biotic (e.g., ingestion, excretion, trophic transfer, bioaccumulation, biofouling) processes and behaviours (Lambert et al. 2014; Anderson et al. 2016; da Costa et al. 2017; Rochman et al. 2019) that ultimately affect their fate.

Climate and seasonality of microplastics

Climate will likely affect microplastic behaviour, creating seasonal patterns in their behaviour. Microplastics entering different climates will be subject to different conditions e.g., precipitation (rain or snow), freeze-thaw cycles, lake turnover, and biofilm species composition, which may affect settling rates, create seasonal patterns in their behaviour in the water column, which will ultimately affect their fate. To date, studies have looked at the pattern between fluctuations in microplastic densities and point sources and have found either no coherent pattern between microplastic density (surface water) and season (Mani and Burkhardt-Holm 2020), or a seasonal pattern, as densities in these studies tended to be higher in spring/early summer compared to fall (Warrack et al. 2017; Wang et al. 2021). These fluctuations in microplastic density are speculated to be due to seasonal increases in point sources such as WWTP input, urban traffic, or precipitation (Anderson et al. 2017; Warrack et al. 2017; Mani and Burkhardt-Holm 2020; Wang et al. 2021). To our knowledge, no work has been conducted to date on understanding how the Canadian climate, and freeze-thaw cycles impact microplastic settling and their behaviour within the water column.

Degradation of microplastics

Once in freshwater ecosystems, abiotic (abrasion, photolysis, thermal, chemical) and biotic processes (ingestion, egestion, microbial colonization) fragment and degrade microplastics (da Costa et al. 2017). The length of time it will take to break it down are unique to the properties of a given particle (e.g., size, morphology, density, polymer)

and the environment they are exposed to (da Costa et al. 2017). The further breakdown of the microplastic in turn results in new microplastic characteristics (size, shape, surface texture) which will affect microplastic particle buoyancy in the water column, giving these entities a highly complex and dynamic range of behaviours.

Biofouling

Many microplastics are initially buoyant in freshwater ecosystems (density <1 g/cm^3) and biofouling can alter their density, often enabling settling (Ye and Andrady 1991; Andrady 2011; Lobelle and Cunliffe 2011; Zettler et al. 2013; Mccormick et al. 2014; Woodall et al. 2014; Napper et al. 2015; Kaiser et al. 2017). As microplastics enter aquatic environments, an instantaneous layer of organic and inorganic substances called a “conditioning film” are adsorbed to its surface (Rummel et al. 2017). This conditioning film may have specific biological properties that influence which species of microorganisms can grow on it (Rummel et al. 2017). The microplastic can then undergo rapid (minutes to hours) biofilm growth, “biofouling” the particle (Cooksey and Wigglesworth-Cooksey 1995). Biofilms are diverse communities of microorganisms: bacteria, algae, fungi, protozoans (Andrady 2011). Biofilm species composition are unique to each aquatic ecosystem as microorganism communities vary spatially, seasonally, and geographically (Andrady 2011; Kaiser et al. 2017; Rummel et al. 2017). Biofilm growth can be influenced by factors such as water chemistry, water column transparency (light preferences), competition, nutrient loading (intensity of growth), and climate (Andrady 2011; Rummel et al. 2017).

The rate of biofilm formation on a microplastic depends on the polymer type, surface area, surface texture (roughness), and water chemistry (nutrients; Ye and Andrady 1991; Rummel et al. 2017). Weathering of the microplastic decreases the physical integrity of the particle, creating a rougher, more heterogenous surface topography which increases surface area for biofilm colonization (Rummel et al. 2017). Biofilms can also biodegrade the microplastic by secreting enzymes which can further break down the particle (da Costa et al. 2016). The growth of a biofilm layer may also change the initial polarity and hydrophobicity of a microplastic (Van Melkebeke et al.

2020). As the biofilm layer reaches a certain thickness, its polarity changes and the microplastic's surface becomes hydrophilic, and can enter the water column and settle (Van Melkebeke et al. 2020).

Microplastics can also defoul as biofilms are sloughed off and removed from the particle (Andrady 2011). Defouling can occur due to low light levels within the water column/sediments which causes the biofilm to decay, or as the biofilm is eaten by a benthic grazer which causes the biofilm to be removed (Andrady 2011). Defouling of the microplastic can alter its buoyancy, enabling it to re-enter the water column and be transported vertically (Wright et al. 2013). Biofouling and defouling of microplastics complicates their ultimate "fate" in aquatic ecosystems (Ye and Andrady 1991), as sediments are believed to be the ultimate sink for microplastics. Densities of microplastics within the water column are currently unaccounted for in most studies (e.g., most studies examine either surface water and/or sediment estimates only), and this behavior of biofouling and defouling may help to understand fate and deposition behaviour of microplastics in aquatic environments.

Settling

Microplastic distributions within Canadian freshwater sediments provides evidence of their ability to settle (Eriksen et al. 2013b; Castaneda et al. 2014; Zbyszewski et al. 2014; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Vermaire et al. 2017). Microplastic settling behaviours are unique to the characteristics of the particle (i.e., density, size, shape, polymer, polarity, surface texture; Rochman et al. 2019), including their ability to form aggregates, biofoul, and characteristics of the aquatic system (e.g., water quality, algal species, water currents).

Microplastic settling velocities have been recently studied in freshwater using modelling approaches (Nguyen et al. 2020), physical experiments (Khatmullina and Isachenko 2017; Waldschläger and Schüttrumpf 2019), and conventional sediment transport equations (e.g., Stokes Law, Dietrich 1982) by calculating theoretical settling velocity of the microplastic in motionless water (Camenen 2007; Zhiyao et al. 2008; Waldschläger and Schüttrumpf 2019). Physical settling rate experiments conducted

under laboratory conditions have found that microplastic settling velocities differed greatly (Camenen 2007; Zhiyao et al. 2008) from the calculated theoretical sediment settling velocities (e.g., Stokes law, Dietrich 1982) and therefore studies are now creating their own equations to describe settling dynamics based on empirical rather than theoretical expectations (Waldschläger and Schüttrumpf 2019). Van Melkebeke et al. (2020), found that theoretical settling velocities of microplastics (films and fibres) were 3 to 4 times faster than measured settling velocity. Both physical settling experiments conducted in a laboratory in motionless water, and sediment transport equations are not truly representative of the complexities of microplastic settling behaviour. Both physical experiments and settling equations do not consider characteristics of the water body (i.e., wind, water quality, weather phenomena creating water turbulence), particle characteristics (surface texture, polarity, polymer), biofouling, defouling, and aggregation within either the experiment or equation. Once microplastics settle to the sediments, they can be further entrained via bioturbation (e.g., burrowing, ingestion, defecation, i.e., all movement of organisms; Kristensen et al. 2012), and sediment deposition (Nakki et al. 2017), decreasing the likelihood of resuspension into the water column. Möhlenkamp et al. (2018), found that microbeads can also be resuspended back into the water column after they have settled which complicates investigations regarding fate.

Resuspension

Resuspension of microplastics occurs as particles that have previously settled re-enter the water column, or microplastics within the water column are vertically transported upward toward the surface water. Movement of water (e.g., wind, storms, turbulence, flooding, lake turnover), or sediment (e.g., benthic feeding), as well as anything that could alter the particles' buoyancy (e.g., defouling of biofilm, degradation), can cause resuspension and redistribution of microplastics throughout the water column (Moore et al. 2002; Cole et al. 2011; Duis and Coors 2016). The unique characteristics of the microplastics polymer (e.g., density), surface area, and texture (which increase both biofouling and defouling) can alter the likelihood of microplastic resuspension (Vaughan et al. 2017). Characteristics of the freshwater system e.g., water column

transparency (darker water leading to the defouling of the microplastic), bathymetry, macrophyte growth (trap microplastics), basin morphology, and trophic species can also affect the likelihood of resuspension (Vaughan et al. 2017).

Aggregation

Microplastic behaviour may also be affected by aggregation, which occurs when abiotic (other microplastics, sediment, rocks), and/or biotic (free floating algal species, or macrophytes) particles associated with microplastics attach together (Long et al. 2015; Lagarde et al. 2016; Long et al. 2017). Microplastics can either form homo-aggregates with the same type of microplastic particles, or hetero-aggregates with different microplastics, sediment, or algal species. As the aggregate forms, its density will change depending on the proportional density of particles within it, which will also affect ultimately where the aggregate will end up in the water column. For example, if buoyant microplastics form a hetero-aggregate with denser particles (filamentous algae, or sediment), the aggregate can become heavy enough to sink (Rummel et al. 2017). Under laboratory conditions, algal species appeared to have polymer preferences when forming aggregates with microplastics (Lagarde et al. 2016), which further adds to the complexities in aquatic systems and the ability to predict microplastic fate and behaviour.

1.6 Mesocosms

Mesocosms are artificial model ecosystem tanks used for experimental studies of similar natural ecosystems (Odum 1984; Kangas and Adey 2008). Mesocosms mimic the structure and function of the natural ecosystems they represent, and may contain aquatic plants, multiple trophic levels of invertebrates, and fish species. Mesocosms bridge the gap between laboratory experimentation and field research (Odum 1984; Caquet et al. 1996; Kangas and Adey 2008), as they allow for controlled testing of the contaminant that can often not be released in field experiments (Kangas and Adey 2008). Advantages of using mesocosms are that they provide realistic conditions that exist in field studies compared to labs (Solomon, 1996) and they also provide control over variability that can confound results in field experiments (Kangas and Adey 2008).

Another advantage is that mesocosms allow for replication due to their size that is often not possible in field studies.

1.7 Research Objectives

No studies have been conducted to date that evaluate the long-term behaviour and settling dynamics of microplastics, nor of the role of either nutrients or emergent vegetation (e.g. cattails) on microplastic behaviour (settling/resuspension). The purpose of my thesis was to examine the behaviour of microplastics in freshwater systems, and the potential impact of nutrients and cattails on both settling and resuspension. To investigate these attributes of microplastics, I developed four research objectives:

Long-term study

1. To investigate and evaluate trends, if any, between five different microplastic types and their settling dynamics in aquatic mesocosm conditions over a 622-day period at the Prairie Wetland Research Facility at the University of Manitoba.
2. To investigate ice formation effects, if any, on microplastic densities before versus after ice formation in aquatic mesocosm conditions over a 622-day period at the Prairie Wetland Research Facility at the University of Manitoba.

Short-term study

3. To investigate and evaluate effects, if any, between replicated treatments (control; nutrient and cattail additions) on microplastic behaviour (settling/resuspension rate) of two microplastic types using a modelling approach in aquatic mesocosm conditions over a 72-day period at the Prairie Wetland Research Facility at the University of Manitoba. My study was part of a larger study not discussed within the thesis.
4. To investigate ice formation and treatment effects, if any, on microplastic densities before versus after ice melt in aquatic mesocosm conditions over a 179-day overwinter period at the Prairie Wetland Research Facility at the University of Manitoba.

1.8 Research hypotheses

My hypotheses for my objectives are as follows:

Long-term study

1. Since microplastics have unique sizes, polymers, shapes, and densities which will affect their buoyancy, behaviour and fate in aquatic ecosystems, I hypothesize that the different microplastic polymers used within the study (foams (PS), films (PE), fragments (PE), microbeads (PE) and fibres (PET)), will have different settling and resuspension dynamics. Foams, and fragments will not settle due to the buoyancy of the plastic polymer. Fibres, microbeads and films will settle due to their buoyancy, size, shape and polymer. Since biofilm growth increases microplastic settling rates, biofilm development will be correlated to microplastic settling rates, and the higher the biofilm densities are within the tanks, the more microplastics that will be deposited into the sediments.
2. Since the water and sediments in the mesocosms will freeze during winter, ice formation will likely affect microplastic behaviour in both the surface water and sediments, altering their densities after ice melts in the spring.

Short-term study

3. Since nutrients stimulate aquatic plant growth, I hypothesize that an initial experimental nutrient addition (Nutrient and Nutrient+Plant treatments) will stimulate periphyton growth in the short-term within the mesocosm, leading to increased biofouling and settling rates of the microplastics (films and fibres), compared with control treatment. Since cattails enhance sedimentation of suspended particles, I hypothesize that the addition of cattails in the Nutrient+Plant treatment will enhance microplastic settling rates and reduce resuspension.
4. Since films settled after ice melted in the spring in the long-term study, I hypothesize that ice formation will also enhance the settling of films. Since fibres

resuspended after ice melted in the spring in the long-term study, I hypothesize that ice formation will also enhance the resuspension of fibres.

1.9 References

Anderson, J.C., Park, B.J., and Palace, V.P. 2016. Microplastics in aquatic environments: Implications for Canadian ecosystems. Environ. Pollut. **218**: 269–280.

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. **225**: 223–231.

Andrady, A.L. 2011. Microplastics in the marine environment. Mar. Pollut. Bull. **62**(8): 1596–1605.

Baldwin, A.K., Corsi, S.R., and Mason, S.A. 2016. Plastic debris in 29 Great Lakes tributaries: relations to watershed attributes and hydrology. Environ. Sci. Technol. **50**(19): 10377–10385.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., and Longstaffe, F.J. 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. **110**(1): 383-395.

Barnes, D.K.A., Galgani, F., Thompson, R.C., and Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. **364**(1526): 1985–1998.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E.L., Tonkin, A., Galloway, T., and Thompson, R.C. 2011. Accumulations of microplastic on shorelines worldwide: sources and sinks. Environ. Sci. Technol. (September): 9175–9179.

Camenen, B. 2007. Simple and general formula for the settling velocity of particles. J. Hydraul. Eng. **133**(2): 229–233.

Campbell, S.H., Williamson, P.R., and Hall, B.D. 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. Facets **2**(1): 395–409.

Caquet, T., Lagadic, L., Jonot, O., Baturo, W., Kilanda, M., Simon, P., Le Bras, S., Echaubard, M., and Ramade, F. 1996. Outdoor experimental ponds (mesocosms) designed for long-term ecotoxicological studies in aquatic environment. Ecotoxicol. Environ. Saf. **34**(2): 125–133.

Carr, S.A., Liu, J., and Tesoro, A.G. 2016. Transport and fate of microplastic particles in wastewater treatment plants. Water Res. **91**: 174–182.

Castañeda, R.A., Avlijas, S., Anouk Simard, M., and Ricciardi, A. 2014. Microplastic pollution in St. Lawrence river sediments. Can. J. Fish. Aquat. Sci. **71**(12): 1767–1771.

Cauwenberghe, L. Van, Vanreusel, A., Mees, J., and Janssen, C.R. 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. **182**: 495–499.

Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Cristina, M., Corsolini, S., and Sea, R. 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): occurrence, distribution and characterization by FTIR. Chemosphere **175**: 391–400.

Cole, M., Lindeque, P., Halsband, C., and Galloway, T.S. 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. **62**(12): 2588–2597.

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., and Galloway, T.S. 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. **4**: 1–8.

Cooksey, K.E., and Wigglesworth-Cooksey, B. 1995. Adhesion of bacteria and diatoms to surfaces in the sea: A review. Aquat. Microb. Ecol. **9**(1): 87–96.

Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., and Marvin, C.H. 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environ. Pollut. **204**: 17–25.

da Costa, J.P., Duarte, A.C., and Rocha-Santos, T.A.P. 2017. Microplastics –

occurrence, fate and behaviour in the environment. Compr. Anal. Chem. **75**: 1–24.

da Costa, J.P., Santos, P.S.M., Duarte, A.C., and Rocha-Santos, T. 2016. (Nano)plastics in the environment - sources, fates and effects. Sci. Total Environ. **566**–**567**: 15–26.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., and Duarte, C.M. 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. U. S. A. **111**(28): 10239–10244.

Depledge, M.H., Galgani, F., Panti, C., Caliani, I., Casini, S., and Fossi, M.C. 2013. Plastic litter in the sea. Mar. Environ. Res. **92**: 279–281.

Derraik, J.G.B. 2002a. The pollution of the marine environment by plastic debris : a review. Mar. Pollut. Bull. **44**: 842–852.

Dietrich, W.E. 1982. Settling velocity of natural particles. Water Resour. Res. **18**(6): 1615–1626.

Do, T.C.V., and Scherer, H.W. 2012. Compost and biogas residues as basic materials for

potting substrates. Plant, Soil Environ. **58**(10): 459–464.

Driedger, A.G.J., Dürr, H.H., Mitchell, K., and Van Cappellen, P. 2015. Plastic debris in the Laurentian Great Lakes: A review. J. Great Lakes Res. **41**(1): 9–19. International Association for Great Lakes Research.

Dris, R., Gasperi, J., Rocher, V., Saad, M., Renault, N., and Tassin, B. 2015a. Microplastic contamination in an urban area: a case study in Greater Paris. Environ. Chem. **12**(5): 592–599.

Dris, R., Gasperi, J., Saad, M., Mirande, C., and Tassin, B. 2016. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar. Pollut. Bull. **104**(1–2): 290–293.

Duis, K., and Coors, A. 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. **28**(1): 1–25. Springer Berlin Heidelberg.

Eerkes-Medrano, D., Thompson, R.C., and Aldridge, D.C. 2015. Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res. **75**: 63–82.

Environment and Climate Change Canada. 2019. Economic study of the Canadian plastic industry, markets and waste : summary report to Environment and Climate Change Canada, pp. 1-43.

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F.,

Ryan, P.G., and Reisser, J. 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One **9**(12): 1–15.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., and Amato, S. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. **77**(1–2): 177–182.

Estahbanati, S., and Fahrenfeld, N.L. 2016. Influence of wastewater treatment plant discharges on microplastic concentrations in surface water. Chemosphere **162**: 277–284.

Falco, F. De, Pia, M., Gentile, G., Di, E., Escudero, R., Villalba, R., Mossotti, R., Montarsolo, A., Gavignano, S., Tonin, C., and Avella, M. 2018. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. Environ. Pollut. **236**: 916–925.

Foekema, E.M., Gruijter, C. De, Mergia, M.T., Franeker, J.A. Van, Murk, A.J., and Koelmans, A.A. 2013. Plastic in North Sea fish. Environ. Sci. Technol. **47**: 8818–8824.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., and Boldgiv, B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. **85**(1): 156–163.

Georg Mehlhart, M.B. 2012. Study on land-sourced litter(LSL) in marine environments: review of sources and literature in the context of initiative of Declaration of the Global Plastics Associations for Solutions on marine Litter. Öko-Institut eV, Darmstadt/freibg. **49**(0): 30–40.

Halden, R.U. 2015. Epistemology of contaminants of emerging concern and literature meta-analysis. J. Hazard. Mater. **282**: 2–9.

Helm, P.A. 2017. Improving microplastics source apportionment: A role for microplastic morphology and taxonomy? Anal. Methods **9**(9): 1328–1331. Royal Society of Chemistry.

Helm, P.A. 2020. Occurrence, Sources, Transport, and fate of microplastics in the Great Lakes--St. Lawrence river basin. *In* Contaminants of the Great Lakes. *Edited by* J. Crossman and C. Weisener. Springer International Publishing, Cham. pp. 15–47.

Henry, B., Laitala, K., and Klepp, I.G. 2019. Microfibres from apparel and home textiles: prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. **652**: 483–494.

Hernandez, E., Nowack, B., and Mitrano, D.M. 2017. Polyester textiles as a source of microplastics from households: a mechanistic study to understand microfiber release during washing. Environ. Sci. Technol. **51**(12): 7036–7046.

Horton, A.A., and Dixon, S.J. 2017. Microplastics: An introduction to environmental transport processes. Wiley Interdiscip. Rev. Water (September 2017): e1268.

Imhof, H.K., Ivleva, N.P., Schmid, J., Niessner, R., and Laforsch, C. 2013. Contamination of beach sediments of a subalpine lake with microplastic particles. Curr. Biol. **23**(19): R867–R868.

Ivar Do Sul, J.A., and Costa, M.F. 2014. The present and future of microplastic pollution in the marine environment. **185**: 352-364

Ivar do Sul, J.A., Costa, M.F., Silva-Cavalcanti, J.S., and Araújo, M.C.B. 2014. Plastic debris retention and exportation by a mangrove forest patch. Mar. Pollut. Bull. **78**(1–2): 252–257.

Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., and Law, K.L. 2015. The ocean : **347**(6223): 3–6.

Junk, W.J., and Nunes De Cunha, C. 2005. Pantanal: A large South American wetland at a crossroads. Ecol. Eng. **24**(4 SPEC. ISS.): 391–401.

Kaiser, D., Kowalski, N., and Waniek, J.J. 2017. Effects of biofouling on the sinking behavior of microplastics. Environ. Res. Lett. **12**(12).

Kay, P., Hiscoe, R., Moberley, I., Bajic, L., and McKenna, N. 2018. Wastewater treatment plants as a source of microplastics in river catchments. Environ. Sci. Pollut. Res. **25**(20): 20264–20267.

Khatmullina, L., and Isachenko, I. 2017. Settling velocity of microplastic particles of regular shapes. Mar. Pollut. Bull. **114**(2): 871–880.

Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Ossendorp, B.C., Redondo-Hasselerharm, P.E., Verschoor, A., Van Wezel, A.P., and Scheffer, M. 2017. Risks of plastic debris: unravelling fact, opinion, perception, and belief. Environ. Sci. Technol. **51**(20): 11513–11519.

Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C.O., and Banta, G.T. 2012. What is bioturbation? the need for a precise definition for fauna in aquatic sciences. Mar. Ecol. Prog. Ser. **446**: 285–302.

Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., and Caruso, A. 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. **215**: 331–339.

Lambert, S., Sinclair, C., and Boxall, A. 2014. Occurrence, degradation and effect of polymer-based materials in the environment. Rev. Environ. Contam. Toxicol. **227**: 1–53.

Lares, M., Ncibi, M.C., Sillanpää, M., and Sillanpää, M. 2018. Occurrence, identification and removal of microplastic particles and fibers in conventional activated sludge process and advanced MBR technology. Water Res. **133**: 236–246.

Lassen, C., Hansen, S.F., Magnusson, K., Hartmann, N.B., Rehne Jensen, P., Nielsen, T.G., and Brinch, A. 2015. Microplastics occurrence, effects and sources of releases. *In* Danish Environmental Protection Agency. pp. 1-6.

Lechner, A., and Ramler, D. 2015. The discharge of certain amounts of industrial microplastic from a production plant into the River Danube is permitted by the Austrian legislation. Environ. Pollut. **200**: 159–160.

Leslie, H.A., van Velzen, M.J.M., and Vethaak, A.D. 2013. Microplastic survey of the Dutch environment: novel data set of microplastics in North Sea sediments, treated wastewater effluents and marine biota. IVM Inst. Environ. Stud. **476**(September): 1–30.

Li, X., Chen, L., Mei, Q., Dong, B., Dai, X., Ding, G., and Zeng, E.Y. 2018. Microplastics in sewage sludge from the wastewater treatment plants in China. Water Res. **142**: 75–85.

Liu, F., Vianello, A., and Vollertsen, J. 2019. Retention of microplastics in sediments of urban and highway stormwater retention ponds. Environ. Pollut. **255**: 113335.

Lobelle, D., and Cunliffe, M. 2011. Early microbial biofilm formation on marine plastic debris. Mar. Pollut. Bull. **62**(1): 197–200.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., and Soudant, P. 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. **175**: 39–46.

Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., and

Soudant, P. 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. **228**: 454–463.

Magnusson, K., and Norén, F. 2014. Screening of microplastic particles in and down-stream a wastewater treatment plant. IVL Swedish Environ. Res. Inst. **C 55**(C): 22.

Mahon, A.M., Connell, B.O., Healy, M.G., Connor, I.O., O, R., Nash, R., and Morrison, L. 2017. Microplastics in sewage sludge: effects of treatment. Environ. Sci. Technol. **51**(2): 810–818.

Mani, T., and Burkhardt-Holm, P. 2020. Seasonal microplastics variation in nival and pluvial stretches of the Rhine River – from the Swiss catchment towards the North Sea. Sci. Total Environ. **707**: 135579.

Martin, C.; Eizhvertina, O. Quantitative analysis of microplastics in WWTP effluent in the Niagara region, technical report published for Niagara College Environmental Technician Field and Lab (co-op): Final Term Project, 2014; Niagara College Canada: Niagara-on-the- Lake, Canada, 2014.

Mason, S.A., Daily, J., Aleid, G., Ricotta, R., Smith, M., Donnelly, K., Knauff, R., Edwards, W., and Hoffman, M.J. 2020. High levels of pelagic plastic pollution within the surface waters of Lakes Erie and Ontario. J. Great Lakes Res. **46**(2): 277–288.

Mason, S.A., Garneau, D., Sutton, R., Chu, Y., Ehmann, K., Barnes, J., Fink, P., Papazissimos, D., and Rogers, D.L. 2016. Microplastic pollution is widely detected in US municipal wastewater treatment plant effluent. Environ. Pollut. pp. 1-10.

McCormick, A., Hoellein, T.J., Mason, S.A., Schluep, J., and Kelly, J.J. 2014. Microplastic is an abundant and distinct microbial habitat in an urban river. Environ. Sci. Technol. **48**(20): 11863–11871.

Van Melkebeke, M., Janssen, C., and De Meester, S. 2020. Characteristics and

sinking behavior of typical microplastics including the potential effect of biofouling: Implications for Remediation. Environ. Sci. Technol. **54**(14): 8668–8680.

Möhlenkamp, P., Purser, A., and Thomsen, L. 2018. Plastic microbeads from cosmetic products: an experimental study of their hydrodynamic behaviour, vertical transport and resuspension in phytoplankton and sediment aggregates. Elementa. **6:** 1-16.

Moore, C.J. 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. **108**(2): 131–139.

Moore, C.J., Moore, S.L., Weisberg, S.B., Lattin, G.L., and Zellers, A.F. 2002. A comparison of neustonic plastic and zooplankton abundance in southern California's coastal waters. Mar. Pollut. Bull. **44**(10): 1035–1038.

Munno, K., De Frond, H., O'donnell, B., and Rochman, C.M. 2020. Increasing the accessibility for characterizing microplastics: introducing new application-based and spectral libraries of plastic particles (SLoPP and SLoPP-E). Anal. Chem. **92**(3): 2443–2451.

Murphy, F., Ewins, C., Carbonnier, F., and Quinn, B. 2016. Wastewater Treatment Works (WwTW) as a Source of Microplastics in the aquatic environment. Environ. Pollut. **234**: 487-494.

Nakki, P., Setala, O., and Lehtiniemi, M. 2017. Bioturbation transports secondary microplastics to depper layers in soft marine sedinets of the northern Baltic sea. Mar. Pollut. Bull. **119**(1): 255–261.

Napper, I.E., Bakir, A., Rowland, S.J., and Thompson, R.C. 2015. Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar. Pollut. Bull. **99**(1–2): 178–185.

Napper, I.E., and Thompson, R.C. 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. **112**(1–2): 39–45.

Nguyen, T.H., Tang, F.H.M., and Maggi, F. 2020. Sinking of microbial-associated microplastics in natural waters. PLoS One **15**(2): 1–20.

Nizzetto, L., Futter, M., and Langaas, S. 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. **50**(20): 10777–10779.

Obbard, R.W., Sadri, S., Wong, Y.Q., Khitun, A.A., Baker, I., and Richard, C. 2014. Global warming releases microplastic legacy frozen in Arctic Sea. Earth's Futur. **2**: 315–320.

Odum, E.P. 1984. The Mesocosm. Bioscience **34**(9): 558–562.

PlasticsEurope Market Research Group (PEMRG) / Consultic Marketing & Industrieberatung GmbH. 2017. Plastics – the facts 2017. https://www.plasticseurope.org/application/files/5715/1717/4180/Plastics_the_facts_2017_FINAL_for_website_one_page.pdf. pp. 1-41.

Pruter, A.T. 1987. Sources, quantities and distribution of persistent plastics in the marine environment. Mar. Pollut. Bull. **18**(6 SUPPL. B): 305–310.

Rillig, M.C. 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. **46**(12): 6453–6454.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H. De, Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., and Hung, C. 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. **38**(4): 703–711.

Rochman, C.M., Cook, A., and Koelmans, A.A. 2016. Plastic debris and policy: using current scientific understanding to invoke positive change. Environ. Toxicol. Chem. **35**(7): 1617–1626.

Rochman, C.M., Kross, S.M., Armstrong, J.B., Bogan, M.T., Darling, E.S., Green, S.J., Smyth, A.R., and Veríssimo, D. 2015. Scientific evidence supports a ban on

microbeads. Environ. Sci. Technol. **49**(18): 10759–10761.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., and Schmitt-Jansen, M. 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. **4**(7): 258–267.

de Sá, L.C., Luís, L.G., and Guilhermino, L. 2015. Effects of microplastics on juveniles of the common goby (Pomatoschistus microps): confusion with prey, reduction of the predatory performance and efficiency, and possible influence of developmental conditions. Environ. Pollut. **196**: 359–362.

Sadri, S.S., and Thompson, R.C. 2014. On the quantity and composition of floating plastic debris entering and leaving the Tamar Estuary, Southwest England. Mar. Pollut. Bull. **81**(1): 55–60.

Sundt, P. et al. 2014. Sundt, Schultze, Syversen - 2014 - Sources of microplastic-pollution to the marine environment Project report. https://www.miljodirektoratet.no/globalassets/publikasjoner/M321/M321.pdf. pp.1-86.

Sutton, R., Mason, S.A., Stanek, S.K., Willis-norton, E., Wren, I.F., and Box, C. 2016. Microplastic contamination in the San Francisco Bay, California , USA. MPB **109**(1): 230–235.

Thiel, M., Hinojosa, I., and Macaya, E. 2003. Floating marine debris in coastal waters of the SE-Pacific (Chile). **46**: 224–231.

Vaughan, R., Turner, S.D., and Rose, N.L. 2017. Microplastics in the sediments of a UK urban lake. Environ. Pollut. **229**: 10–18.

Vermaire, J.C., Pomeroy, C., Herczegh, S.M., and Haggart, O. 2017. Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. Facets **2**: 301–314.

Wagner, M., and Lambert, S. 2018. Freshwater microplastics: emerging environmental contaminants? pp. 1-293.

Waldschläger, K., and Schüttrumpf, H. 2019. Effects of particle properties on the settling and rise velocities of microplastics in freshwater under laboratory conditions. Environ. Sci. Technol. **53**: 1958–1966.

Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., and Hughes, K.A. 2017. Microplastics in the Antarctic marine system: An emerging area of research. Sci. Total Environ. **598**: 220–227.

Wang, G., Lu, J., Li, W., Ning, J., Zhou, L., Tong, Y., Liu, Z., Zhou, H., and Xiayihazi, N. 2021. Seasonal variation and risk assessment of microplastics in surface water of the Manas River Basin, China. Ecotoxicol. Environ. Saf. **208**: 111477.

Warrack, S., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastics flowing into Lake Winnipeg: densities, sources, flux, and fish exposures. Proc. Manitoba's Undergrad. Sci. Eng. Res. **3**: 5–15. University of Manitoba Libraries.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., and Thompson, R.C. 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. **1**(4): 140317–140317.

Wright, S.L., Thompson, R.C., and Galloway, T.S. 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. **178**: 483–492.

Yang, L., Qiao, F., Lei, K., Li, H., Kang, Y., Cui, S., and An, L. 2019. Microfiber release from different fabrics during washing. Environ. Pollut. **249**: 136–143.

Ye, S., and Andrady, A.L. 1991. Fouling of floating plastic debris under Biscayne Bay exposure conditions. **22**(12): 608-613.

Zbyszewski, M., and Corcoran, P.L. 2011. Distribution and degradation of fresh water plastic particles along the beaches of Lake Huron, Canada. **220**: 365–372.

Zbyszewski, M., Corcoran, P.L., and Hockin, A. 2014. Comparison of the distribution and degradation of plastic debris along shorelines of the Great Lakes, North America. J. Great Lakes Res. **40**(2): 288–299.

Zettler, E.R., Mincer, T.J., and Amaral-Zettler, L.A. 2013. Life in the plastisphere: microbial communities on plastic marine debris. Environ. Sci. Technol. **47**: 7137.

Zhang, W., Zhang, S., Wang, J., Wang, Y., Mu, J., Wang, P., Lin, X., and Ma, D. 2017. Microplastic pollution in the surface waters of the Bohai Sea, China. Environ. Pollut. **231**: 541–548.

Zhang, Y., Liang, J., Zeng, G., Tang, W., Lu, Y., Luo, Y., Xing, W., Tang, N., Ye, S., Li, X., and Huang, W. 2020. How climate change and eutrophication interact with microplastic pollution and sediment resuspension in shallow lakes: A review. Sci. Total Environ. **705**: 135979.

Zhao, S., Zhu, L., Wang, T., and Li, D. 2014. Suspended microplastics in the surface water of the Yangtze estuary system, China: first observations on occurrence, distribution. Mar. Pollut. Bull. **86**(1–2): 562–568.

Zhiyao, S., Tingting, W., Fumin, X., and Ruijie, L. 2008. A simple formula for predicting settling velocity of sediment particles. Water Sci. Eng. **1**(1): 37–43.

Ziajahromi, S., Neale, P.A., Rintoul, L., and Leusch, F.D.L. 2017. Wastewater treatment plants as a pathway for microplastics: development of a new approach to sample wastewater-based microplastics. Water Res. **112**: 93–99.

Zubris, K.A. V., and Richards, B.K. 2005. Synthetic fibers as an indicator of land application of sludge. Environ. Pollut. **138**(2): 201–211.

Chapter 2. Long-term Settling dynamics of microplastics: a field-based mesocosm study

2.1 Abstract

Microplastics have been detected in freshwater ecosystems worldwide. Despite this, relatively few studies have focused on long-term fate and behaviour in freshwater ecosystems. Knowledge gaps include understanding their fate and behaviour in the Canadian climate, including how ice formation might affect microplastic behaviour. To address this, outdoor freshwater mesocosms (n=9) were dosed with five types of microplastics (foams, films, fragments, microbeads and fibres) to understand their long-term fate and behaviour, and how ice formation may affect behaviour over a 622-day period. Not all microplastics (foams and fragments) settled (reached the sediments) over the course of the study. All microplastics displayed seasonal setting trends during the open water season in the surface water, as microplastic densities tended to decrease from spring to fall. Both films and fibres displayed seasonal trends during the open water season in the sediments, as microplastic deposition increased from spring to early fall. My results suggest that biofilm development was the most likely driver of microplastic deposition. Foams, microbeads and fibres formed aggregates within the mesocosms, yet aggregation alone did not appear sufficient to alter the density of foams enabling them to settle. Aggregation likely enhanced deposition rates of microbeads and fibres. Ice formation effects were specific to each microplastic type e.g., enhancing the settling of films, compared to the resuspension of fibres after the ice melted. Sediments may not be the ultimate sink for microplastics, as microplastic behaviour (e.g., aggregation, biofouling), and characteristics of the aquatic ecosystem (e.g., climate, ice crystal formation, and water temperature-density effects) may add to the complexities of microplastic behaviours that will determine their ultimate fate in aquatic ecosystems.

2.2 Introduction

Microplastics (plastic particles <5.0 mm in diameter) have been detected in freshwater ecosystems (lakes, rivers and streams) worldwide. Documented sources of microplastics into freshwater ecosystems include landfill seepage to groundwater (Environment and Climate Change Canada 2019), sludge applied to agricultural fields (Free et al. 2014; Magnusson et al. 2016), fishing gear (Pruter 1987), and synthetic textiles (Browne et al. 2011; Rillig 2012). Microplastic particles are classified as either primary or secondary. “Primary” microplastics are manufactured to be <5mm in size (e.g., microbeads used as abrasives in both industrial and cosmetic products). “Secondary” microplastics are a by-product of larger macroplastics that have fragmented and degraded over time into small, microscopic pieces (e.g., fragments, films, foams, fibres) due to biological, chemical, and physical processes (Napper and Thompson 2016; Hernandez et al. 2017; Falco et al. 2018; Henry et al. 2019; Yang et al. 2019). Both primary and secondary microplastics can enter freshwater ecosystems through pathways based on their originating source, e.g., microbeads (primary microplastic) enter via effluent discharge (Browne et al. 2011), whereas fibres (secondary microplastic) appear to enter primarily via aerial deposition (Dris et al. 2016). Microplastics have be found in surface water and sediments of freshwater systems (lakes, rivers, streams) including those in Canada (Eriksen et al. 2013; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Campbell et al. 2017; Vermaire et al. 2017; Warrack et al. 2017; Helm 2020). Once microplastics enter freshwater systems, they can undergo a range of abiotic (abrasion, photolysis, thermal, chemical) and biotic processes (ingestion, egestion, microbial colonization; Lambert et al. 2014; Anderson et al. 2016; da Costa et al. 2017; Rochman et al. 2019) which can affect their behavior and ultimate fate in aquatic ecosystems.

Microplastic fate (i.e., where the microplastic will ultimately end up, e.g., floating on the surface water, or settling to the sediments), and behaviour (i.e., what microplastics do in the water column/sediments, e.g., settle, resuspend, aggregate) is complex. Microplastic settling behaviour is currently studied in freshwater using modelling approaches (Nguyen et al. 2020), physical experiments (Khatmullina and

Isachenko 2017; Waldschläger and Schüttrumpf 2019), and conventional sediment transport equations (e.g., Stokes Law, Dietrich 1982) by calculating theoretical settling velocity of the microplastic in motionless water (Camenen 2007; Zhiyao et al. 2008; Waldschläger and Schüttrumpf 2019). Resuspension can also affect microplastic fate and behaviour in the water column, as microplastics are transported vertically from the bottom of the water body upward. Another microplastic behaviour, aggregation, occurs when abiotic (other microplastics, sediment, rocks), and/or biotic (free floating algal species, or macrophytes) factors act to facilitate particles to attach together (Long et al. 2015; Lagarde et al. 2016; Long et al. 2017). As aggregates form, their density will change depending on the particles within it, which will also affect where the aggregate will end up in the water column.

Both microplastic fate and behaviour are affected by the characteristics of both the microplastic particle (polymer, shape, size, surface texture, density) and the water body it enters (Rochman et al. 2019). The waterbody characteristics such as climate, biofilm species composition, and water quality (Rochman et al. 2019) will affect microplastic behaviour. Climate has the potential to affect microplastic behaviour through wind, precipitation, temperature effects, winter, ice formation, and freeze-thaw cycles. As water cools ice crystals begin to form around particles in the water column (including microplastics). Microplastics trapped within the ice crystals can be resuspended to the surface water as ice crystals are less dense than liquid water (Wetzel, 1975). Microplastics floating on the surface of the water body may also get trapped as surface ice forms. In spring, as the ice begins to thaw, microplastics trapped within ice are rereleased back into the water column, and the microplastics' fate will depend on particle density, polymer, and how long it takes for the microplastic to be released from the ice. To our knowledge, no studies have compared effects of colder climate freeze-thaw cycles on microplastic fate, behaviour and their subsequent settling dynamics.

Biofilm specie composition within the freshwater system may also affect fate and behaviour of microplastics (Rochman et al. 2019). Microorganisms that make up the biofilm community (bacteria, algae, fungi, protozoans) vary spatially, seasonally, and

geographically and are typically unique to each aquatic ecosystem (Andrady 2011; Kaiser et al. 2017; Rummel et al. 2017). The rate of biofilm formation on a microplastic depends on polymer type, surface area, surface texture (roughness), and water chemistry (nutrients; Ye and Andrady 1991; Rummel et al. 2017). As the biofilm layer reaches a certain thickness, its polarity changes and the microplastics' surface can become hydrophilic, thus entering the water column and settling (Van Melkebeke et al. 2020). Microplastics can also de-foul as biofilms are removed from the (Andrady 2011), altering the particles buoyancy and enabling it to re-enter the water column (resuspend) and be transported vertically (Wright et al. 2013). Water quality parameters (i.e., nutrients, DO, pH, conductivity, water clarity) affect the biofilm species composition of freshwater ecosystems (Villeneuve et al. 2013) and potentially also the fate and behaviour of microplastics via biofouling rates given the water quality in an environment.

No studies to date have evaluated the long-term behaviour and settling dynamics of microplastics in freshwater ecosystems. Microplastic settling experiments conducted under laboratory settings have lasted only seconds to minutes (Khatmullina and Isachenko 2017; Waldschläger and Schüttrumpf 2019). The purpose of this chapter was to examine the long-term (622 days) fate and behaviour of microplastics in freshwater systems and the influence of ice formation. My objectives were: (1) To investigate and evaluate trends, if any, between five different microplastic types and their settling dynamics in aquatic mesocosm conditions over several years, and (2) To take advantage of this multi-year investigation to investigate ice formation effects, if any, on microplastic densities before versus after ice formation in aquatic mesocosm conditions. I hypothesized that (1) different microplastic polymers used within the study (e.g., foams (PS), films (PE), fragments (PE), microbeads (PE) and fibres (PET)), would have different settling and resuspension dynamics, based on their buoyancy in water: specifically, that foams, and fragments would not settle, but that fibres, microbeads and films would settle due to their buoyancy, size, shape and polymer, and (2) the role of biofilm development will enhance settling dynamics. Further, I hypothesized that (3) ice formation would alter microplastic buoyancy characteristics, and therefore densities in both the surface and sediments before and after ice formation.

2.3 Materials and methods

2.3.1 Long-term mesocosm study

Test facility and mesocosm preparation

The study was conducted at the Prairie Wetland Research Facility (PWRF), at the University of Manitoba. The PWRF has eighteen circular, above ground, flat-bottomed, low-density polyethylene, 3500 L (2.7 m diameter x 0.72 m height; 17.56 m^2 total area) tanks. In 2011, each tank was filled with ~0.23m of soil (Anseeuw Brothers Ltd., Winnipeg, MB), which mimicked freshwater sediments (see Cardinal et al. 2014 for more details). The soil used for the sediments was clay-dominated, and contained 50.9% clay, 35.4% silt, and 13.7% sand (Cardinal et al. 2014). Soil was used as the sediment layer for the study since it has been used previously in other mesocosm based studies at the PWRF, and the soil was established (>5 years). Biota in the mesocosms included macrophytes (*Typha* spp. (cattails), *Myriophyllum sibiricum* (short spiked water milfoil), Utricularia vulgaris (bladderwort) and *Potamogeton* spp. (pond weed), algae, zooplankton, and benthos, which were previously attained from Oak Hammock Marsh, Stonewall Manitoba. Insect colonization occurred naturally due to the mesocosms being open to their surrounding environment.

A total of six mesocosms were used for this study. Tanks were randomly assigned as control or microplastic treatment (Appendix B; Figure B1). The three microplastic treatment tanks received the five microplastic types (foams, films, fragments, microbeads, and fibres) and controls received no additions of any materials. These particular microplastics were chosen because they are made of different plastic types (foams were foamed polystyrene; films, fragments and microbeads were polyethylene; and fibres were polyester), and were five different but commonly found morphologies in Manitoban lakes and rivers, which are also ubiquitous in other freshwater aquatic environments. The study commenced on August 4, 2017, with 59 days pre-treatment monitoring and regular monitoring until April 18, 2019. Monitoring of mesocosms consisted of measuring water quality parameters, taking weekly photos,

collecting surface and sediment samples for microplastics, and the collection of biofilm tiles.

Each tank was topped up with dechlorinated water as needed (water level was below the lip of the tank after sampling was conducted), depending on the evaporation rates throughout the summer season (about 2.5% of the water evaporated/day, ~70 litres) to maintain water levels. Water was added in 2017 on June 12 (day -53), June 22 (day -43), July 6 (day -29), July 27 (day -8), August 2 (day -2), August 11 (day 7), August 31 (day 20) and in 2018 on May 9 (day 271), May 26 (day 288), June 7 (day 300), July 4 (day 327), July 27 (day 350), August 21 (day 375), and September 13 (day 398). Water was added very slowly and carefully with the hose nozzle directed at a piece of wood to diffuse flow and decrease disturbance).

Preparation of microplastics

A total of five microplastic types (foams, films, fragments, microbeads, and fibres) were used in the study. The densities added to the mesocosms were not environmentally relevant densities, as I wanted to understand the long-term behaviours of microplastics. I therefore needed to dose the tanks with enough microplastics that I could detect them readily throughout our sampling efforts.

Foams (foamed polystyrene) were beanbag chair beads, spherical in shape, white in colour, <5mm, purchased from a multinational retailer (Table 2.1). The average weight of one foam was ~0.001 grams, which was calculated by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 foams on an analytical balance, and creating a density curve (Appendix B; Figure B2). The initial amount of foams added to each mesocosm was ~2200. This was estimated by weight (using our density curve) and divided into four equal (550 foams) amounts (using weight) and put into four separate plastic bags for dosing. The four equal amounts of microplastics/mesocosm, was a dosing technique, as the mesocosms were divided into 4 quadrants to help with initial even distribution of microplastics within the tank during the initial dosing.

Films (polyester) were plastic post-it flags purchased from a multinational retailer (Table 2.1). The films were pink, orange, yellow, green, and blue in colour. The post-it

flags were sliced into squares (<5 mm) by hand using a rotary cutter. The films were then weighed on an analytical balance using a range to create a density curve (Appendix B; Figure B3). The average weight of one film was ~0.0017 grams. The initial amount of films added to each mesocosm was ~2200. This was estimated by weight (using our density curve) and divided into four equal (550 film) amounts (using weight) for each mesocosm (to dose each of the four quadrants in each mesocosm) and put into four separate glass vials.

Fragments (low density polyethylene) were “perler beads” (crafting beads arranged in pegboards to form patterns and fused together using a hot iron) and were purchased at a multinational retailer (Table 2.1). The fragments were multicoloured (clear, white, red, pink, yellow, brown, black, orange, green and blue) and were hollow and cylindrical in shape. The fragments were quartered by hand lengthwise using scissors to be <5mm in diameter and were irregular, rectangular in shape. The fragments were then weighed on an analytical balance using a range (see above in foams; Appendix B; Figure B4), and the average weight of one fragment was ~0.012 grams. The initial amount of fragments added to each mesocosm ~2200. This was estimated by weight (using our density curve) and divided into four equal (550 fragment) amounts (using weight) and put into plastic bags for dosing.

Microbeads (polyethylene) were from facewash, purchased from the beauty department of multinational retailer (Table 2.1). The microbeads were white and blue in colour, spherical in shape, and were ~0.33mm in size. The microbeads were squeezed out of the facewash tubes, into a 220 μm sieve and the soap from the facewash was washed away using DI water. The microbeads were then placed in paper envelopes and into a drying oven (70°C) for 24 hours. Then the microbeads were weighed on an analytical balance using a range (Appendix B; Figure B5), and the average weight of one microbead was ~0.000083 grams. The initial amount of microbeads added to each mesocosm was ~81492. This was estimated by weight (using our density curve) and divided into four equal (20373 microbead) amounts (using weight) and put into four separate glass vials.

Fibres (polyester) fleece fabric purchased from a multinational sewing store (Table 2.1). The colour of the fibres used in this study was orange, and it was used as a QA/QC measure. During previous analyses of samples in Lake Winnipeg and the Red, Assiniboine, and Nelson Rivers (Anderson et al. 2017b; Warrack et al. 2017), orange fibres were never encountered. Therefore, orange fibres were more likely to be differentiated from other colours of fibres (blue, clear, black) that may be introduced unintentionally from aerial deposition, experimenters clothing, and sample processing. Fibres were sliced off the main fabric by hand using razors and scissors. The fibres were then measured to make sure they were <5mm in diameter. The fibres were weighed on an analytical balance using a range (Appendix B; Figure B6), and the average weight of one fibre was ~0.000071 grams. The initial number of fibres added to each mesocosm ~8800. This was estimated by weight (using our density curve) and divided into four equal (2200 fibre) amounts (using weight) and put into plastic bags for dosing.

Microplastic additions

To dose the tanks, cotton string was used to create four equal quadrants and duct tape was used to tact the string down. The quadrants were temporarily used to ensure microplastics were evenly distributed throughout the tanks. Each quadrant received equal amounts of each of the pre-weighed microplastic type (see above). The microplastics were added by four individuals to each quadrant simultaneously (Figure 2.1 a). The microplastics were distributed on the surface of the water in a zigzag pattern to try to evenly distribute the microplastics throughout the respective quadrants. The string quadrants were then removed. Fragments were added to each mesocosm using a Scotts® handheld Handy Green® spreader typically used to apply fertilizer and lawn seed (Figure 2.1 b). The fragments were added to the top of the spreader, and as the individual slowly circled the quadrant, the spreader randomly displaced the fragments (Figure 2.1 b). This method was used to help ensure fragments were randomly distributed throughout the tank and decrease human error.

A total of ~12,100 individual microplastics were added to each mesocosm on August 4, 2017 (exposure day 0). The three microplastic treatment mesocosms were

dosed again with fibres and microbeads on September 1, 2017 (day 28), using the same approach as above, because after the initial dosing (exposure day 0), visual inspection of the tanks raised concern that fibres and microbeads were too sparse to facilitate ongoing sampling requirements. Another ~4,400 fibres/mesocosm were added (day 28; total fibres: ~8800 fibres), and ~80,392 microbeads (a full vial) were added (total microbeads: ~81,492 microbeads; Table 2.1). Each tank then contained a total of ~96,892 individual microplastics after these subsequent additions.

2.3.2 Water quality parameters

YSI measurements

YSI 6600 V2 Sonde (Yellow Springs, OH), temperature (˚C), specific conductivity (mS/cm), pH, chlorophyll content (µg/L), and dissolved oxygen (mg/L) were monitored each weekday between 8-9:30am, and once a week between 1:30-3:30pm in each tank at a depth of ~0.20 m to characterize fluctuations of water quality throughout the study. Pre-exposure YSI monitoring occurred from June 12, 2017 (day -54) to August 3, 2017 (day -1), post exposure monitoring occurred from August 4, 2017 (exposure day 0) to October 18, 2018 (day 440). Measurements were not taken in the winter (October 26, 2017 (day 83) to May 9, 2018 (day 279)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued daily until October 10, 2018.

Photosynthetically active radiation (PAR)

Photosynthetically active radiation (PAR) was measured with an Apogee MQ-200 quantum sensor (in µmol*m^2/s) with AL-100 sensor levelling plate (Hoskin Scientific, Burlington, ON). Measurements were taken weekly around noon (between 11:45 am to 1:00 pm) at sediment level (same spot used each time, marked with a flag) in each mesocosm. Pre-exposure monitoring occurred from June 12, 2017 (day -54) to July 27, 2017 (day -8). Post-exposure monitoring occurred after dosing on August 4, 2017 (exposure day 0) to September 18, 2018 (day 410), on clear days. September 18, 2018 was the last day PAR was measured as it was cloudy every other week when trying to

monitor. Measurements were not taken in the winter (October 26, 2017 (day 83) to May 25, 2018 (day 295)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued weekly until September 18, 2018.

Filamentous algae

Qualitative filamentous algae assessments were conducted weekly by the investigator, and other trained individuals. Each tank was assessed using a scale of 1 to 3 (1= no algae present, 2= distinct algal masses visible, 3= full algal colonization), to approximate algal growth or productivity (Baxter et al. 2013). Pre-exposure monitoring occurred June 12, 2017 (day -54) to August 3, 2017 (day -1). Post exposure monitoring occurred after August 4, 2017 (exposure day 0) to April 18, 2019 (day 622). Measurements were not taken in the winter 2017-2018 (October 14, 2017 (day 72) to May 13, 2018 (day 282)), and winter 2018-2019 (October 10 (day 433) to April 17 (day 621)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued weekly until October 10, 2018.

Depth

A total of six depth measurements were taken in each tank weekly, in random spots, where average depth was then determined. Depths were taken pre- and post-fill of the tanks when topped off with water three times (August 2, 2017 day -2, August 11, 2017 (day 7), August 31, 2017 (day 27)) to calculate evaporation rates. Depths were used to calculate water volume for the study. Pre-exposure monitoring occurred from June 12, 2017 (day -54) to August 2, 2017 (day -2). Weekly post exposure monitoring occurred from August 4, 2017 (exposure day 0) to October 8, 2018 (day 431). Measurements were not taken in the winter (October 14, 2017 (day 72) to May 13, 2018 (day 238)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued weekly until October 6, 2018.

General Hardness and Alkalinity

General hardness and alkalinity were measured weekly using integrative sampling (for more details refer to Solomon et al. 1982). Zooplankton mesh (200 μm) was placed on the end of the hose, so no microplastics were lost while sampling. To ensure QA/QC, each treatment used its own integrative sampler, which was rinsed with DI water between each use. Integrative sampling consisted of six grab samples from different depths and places within each mesocosm. Each grab sample (~0.9 L) was poured into a clean plastic bucket (~5.4 L in total). A dropper deposited 5 ml into a glass vial and taken back into the lab for analysis. Both general hardness mg/L ($CaCo_3$) and alkalinity mg/L ($CaCo_3$) were measured using Nutrafin aquarium test kits (Rolf C. Hagen Inc., Montreal, QC). The water samples were measured within two hours of taking the sample. Pre-exposure monitoring occurred from July 20, 2017 (day -16) to August 3, 2017 (day -1). Weekly post exposure monitoring occurred in 2017: August 17 (day 14) to October 13 (day 68), 2018: May 13 (day 283) to October 10 (day 432), and 2019: April 18 (day 622). Measurements were not taken in either winter 2017-2018 (October 14, 2017 (day 72) to May 13, 2018 (day 238)), or winter 2018-2019 (October 11, 2017 (day 433) to April 17, 2019 (day 621)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued biweekly until October 10, 2018.

2.3.3 Surface water microplastic sampling and analysis

To detect microplastics in the surface waters, I developed our own surface water sampler. The circular sampler was built using piping (PVC-FGV coupling 0.051 m by 0.076 m) as the mouth (0.085 m diameter), and a 200 μm mesh was placed on the end of the piping to trap microplastics. The sampler was then duct tapped onto a piece of wood (width: 0.051 m by height: 0.102 m) to maneuver it throughout the water (Figure 2.2 a). Quadrants of equal size in the mesocosm were made using cotton string and tacked down using duct tape. The sampler was submerged 0.0425 m (radius of the sampler, marked with a permanent marker) and was towed across the diameter of the mesocosm using the string quadrants as guidance, making an “X”. The total surface

area sampled was 0.7 m^2. The 200 μm mesh of the circular sampler was sprayed with Milli-Q water into a glass litre mason jar with 70% ethanol for later processing. The rope quadrants were rotated by 0.25 m each sampling date to ensure random sampling of the mesocosms surface waters. The quadrants were measured prior to the study and marked with tape on the side of the mesocosm. Pre-exposure monitoring occurred on July 28, 2017 (day -8) and was conducted in triplicate for all mesocosms. Samples were collected biweekly post exposure from August 18, 2017 (day 14) to April 18, 2018 (day 622). Measurements were not taken in winter 2017-2018 (October 14, 2017 (day 71) to May 8, 2018 (day 277)), and winter 2018-2019 (October 9, 2018 (day 421) to April 17, 2019 (day 621)), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued. Microplastics have not been found to affect ice growth rates, and the densities used in our study were too low to affect albedo, and therefore I can assume microplastics did not alter ice formation within our study (Geilfus et al., 2019).

In the lab, surface water samples were emptied into a 250 μm sieve after collection and rinsed using DI water for two minutes to ensure all the ethanol was removed. The samples were then reconstituted to 1250 ml with DI water, and stirred on a stirring hot plate. A subsample of 250 ml was collected and processed using a wet peroxide oxidation (WPO) treatment (Masura et al. 2015), as samples contained aquatic plants and algae. In order to remove organic material, 20 ml of a 0.05 M Fe (II) solution and 20 ml of 30% H_2O_2 was added while the solution was heated to 75˚C on a stirring hotplate. The Fe (II) solution was prepared by adding 7.5 g of $FeSO_4 \cdot H_2O$ to 500 ml of Milli-Q water and 3 ml of concentrated sulfuric acid. Subsamples were left covered for 24 hours. The digested samples were filtered again and viewed under a dissecting microscope. The number and type of microplastic (foam, film, fragment, microbead or fibre) was recorded. As microplastic particles were enumerated, they were transferred to ethanol in a glass vial with a rubber stopper for long-term storage.

2.3.4 Sediment sampling and analysis

To detect microplastics in the sediments, each mesocosm received five plastic boxes without lids (Fisherbrand SureOne filter tip boxes), with a surface area of 0.0096 m^2 (length 0.12 m, width 0.08 m, depth 0.05 m) were placed in random locations in the mesocosms by pushing the box 0.02 m into the sediment. Beside each box was a numbered flag that made sampling easier, as the box could easily be located. A random number generator was used to select sampling order of boxes. Boxes were placed in the mesocosms on June 5, 2017 (day -60). To sample the sediments, the lid was closed on the box while the box was submerged and the box and contents were removed from the tank. The lid retained all "settled" microplastics within the box while it was being retrieved (Figure 2.2 b). Contents of the box were rinsed into a Ziploc bag using 500 ml Milli-Q water. Samples were frozen (-20°C) for later processing. To process, sediment samples were thawed and contents were washed out of the Ziploc bag using DI water into a 250 μm sieve. The sample was then rinsed in the sieve, and the fine sediment was washed away. The remaining sample (aquatic plants, organic material and microplastics) was then reconstituted to 1250 ml with DI water, and stirred on a stirring hot plate. A subsample of 250 ml was collected and processed using a wet peroxide oxidation (WPO) treatment (see surface water sampling section 2.3.3 Masura et al. 2015). The number and type of microplastic (foam, film, fragment, microbead or fibre) was recorded. As microplastic particles were enumerated, they were transferred to ethanol in a glass vial with a rubber stopper for long-term storage.

Pre-exposure sampling occurred on July 15, 2017 (day -20) and July 30, 2017 (day -5). Biweekly sampling occurred in 2017 after dosing (August 4, 2017; exposure day 0) on August 12, 2017 (day 8) to October 13, 2017 (day 70). For the pre-exposure monitoring we took triplicate 125 ml glass jars were used to take a grab sample from three random locations in the mesocosms. Sediment sampling did not occur in the winter (Oct 14, 2017 to June 16, 2018), as the mesocosms had ice cover on the surface. The initial sampling after winter was a sediment sampled occurred in triplicate on May 11, 2018, and used the method of three 125 ml glass jars to take grab samples. As soon as the mesocosms were thawed (0% ice cover), on May 11, 2018 (day 284) six

new sediment boxes were placed in each mesocosm with flags. Sampling occurred monthly in 2018 (Year two), June 16, 2018 (day 316) to October 16, 2018 (day 438). No samples were taken over winter again. As soon as the mesocosms thawed, a final triplicate sediment sample from each mesocosm was taken on April 18, 2019 (day 622). To collect the final sediment sample, three 125 ml glass jars were used to take a grab sample of the sediments. The jar was pressed into the sediments, carefully to not disturb any microplastics, the sediments were scooped, and the lid was placed on the jar before it was pulled out of the tank. These locations had not been sampled before, so there was no disturbance from previous sampling events. The contents of each container was washed into a Ziploc bag, and frozen at -20°C.

2.3.5 Quality assurance and quality control (QA/QC)

QA/QC involved aerial deposition, surface water, DI water, and sediment blanks. I also ensured that no one wore orange fleece during the study, to try to eliminate possible contamination sources. All blanks were conducted in duplicate or triplicate. Blanks were only conducted at the beginning of the study to give a sense whether more blanks should have been employed. I used specific microplastics that were specific in colour and shape to make sure I could identify the microplastics added to the study, compared to microplastics introduced through aerial deposition, surface water, DI water, or sediment sampling. Therefore I was able to do minimal QA/QC as all microplastics introduced through sampling and processing were completely different from those initially spiked.

Aerial deposition blanks

Two air blanks were employed to understand whether microplastics (same colour and shape as our experiment) were being introduced into our samples while I was processing and enumerating under the dissecting microscope. Two aerial deposition blanks were deployed in the lab by leaving one litre glass mason jars of Milli-Q out on the lab counter for 24 hours. After 24 hours, lids were placed on the jars, and the blank was processed in the same way that the other samples were using the WPO method (Masura et al. 2015). Within the two blanks, a total of eight and seven fibres (clear and

blue in colour) were introduced over the 24-hour time-period, or 0.3 fibres/hour. Since the average time for sorting of samples under the dissection microscope was four hours, I estimate that on average 1.25 microplastic particles were introduced from the lab air. However, because I only enumerated orange-coloured fibres (the colour added to mesocosms), I can assume fibres from aerial deposition did not influence our microplastic counts.

Surface water blank

Preliminary surface water samples were taken to determine what types of microplastics were already within the system. Three surface water blanks were employed for each tank where I used the same sampling method for surface water sampling. Each sampler was rinsed using one litre of Milli-Q water, and visually inspected before the next sample was taken. Each tank had its own sampler, which helped avoid cross contamination between tanks. Samples were then processed using WPO method (Masura et al. 2015) and enumerated under the dissecting microscope. Clear fibres, and purple fibres were the only microplastics found within the surface water. Since I used orange-coloured fibres, I can assume fibres within our tanks did not influence our microplastic counts.

Deionized water blanks

Lab blanks were used to determine whether microplastics were being introduced into our samples from DI water during processing in the lab. Four DI blanks were conducted. I ran the DI water tap at a rate of 8 L/minute (480 L total) at the University of Manitoba for 60 minutes on a clean 355 µm brass sieve. Any contents within the sieve were then rinsed into a petri dish, and viewed under a dissecting microscope. Within the four blanks: 13, 5, 16 and 9 fibres (clear and blue in colour) were found. This suggests that on average one microplastic particle (fibre) was introduced for every 48 L of DI water used when processing the samples. The average rinse time of a sample is five minutes with DI water (at 8 L/minute), with reconstitution to 1.25 L prior to subsampling, I can estimate that on average, 0.85 fibres were introduced to our samples, from the DI

water alone. Again, only orange-coloured fibres were used in the experiment, so I can assume that these fibres did not influence our microplastic counts.

Sediment blank

Preliminary sediment samples were taken to determine what types of microplastics were already within the system. Three sediment blanks were employed for each tank by using clean 480 mL glass jars to take a grab sample. The jar was pressed into the sediments, carefully to not disturb any microplastics, the sediments were scooped, and the lid was placed on the jar before it was pulled out of the tank. The contents of each container was washed into a Ziploc bag, and frozen at -20°C. Sediment samples were processed using WPO treatment (Masura et al. 2015), and enumerated under the dissecting microscope. Clear, red, and black fibres were found in the sediments, and therefore I could assume that there were no microplastics similar to those chosen for the study (shape, and colour) were detected in the sediments before the initial dosing, and therefore we did not need to do any more sediment blanks throughout the study.

2.3.6 Sampling efficiency

The main assumption in our sampling design was that all microplastics added on day 0 (August 4, 2017) were homogenously distributed throughout the tank at all times throughout the study. Visual observations and weekly photos taken throughout the study showed a heterogeneous distribution of microplastics in the surface water. Microplastics also formed aggregates, were stuck to emergent and submergent plants, were found under the lip of the mesocosms, and were also found in high densities pressed to the edge of the mesocosm where the water and mesocosm walls met (Appendix B; Figures B7- B10). Nothing was done to overcome this behaviour.

2.3.7 Biofilm sampling and analysis

To measure biofilm growth in each mesocosm, unglazed, white, ceramic tiles were purchased at a multinational retailer (Appendix B; Figure B11 a). A total of four tiles were glued together using Marineland aquarium sealant (100% clear silicone

rubber), and cotton string was attached to the back of the tile. The finished tile had a surface area of 0.96 m^2 (0.098 m by 0.098 m). The tiles were deployed off the side of the tank using the string (suspended in the water column) and attached to the side of the tank (vertically) using a butterfly clip. The top of the tile was submerged approximately 0.065 m below the water's surface at all times throughout the study. Tiles were numbered, and a random number generator was used to determine which tile was sampled. A total of eight tiles were deployed in each tank on June 30, 2017 (day -35). Preliminary sampling occurred on July 29, 2017 (day -6). Biweekly sampling occurred in 2017 after dosing August 4, 2017 (exposure day 0) on August 11, 2017 (day 7) to October 13, 2017 (day 70). Biofilm sampling did not occur in the winter 2017-2018 (October 14, 2017 to June 16, 2018), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), on May 15, 2018 (day 284) five new tiles were placed in each mesocosm. Sampling occurred monthly in 2018 (Year two), June 16, 2018 (day 316) to October 16, 2018 (day 438). The tiles were removed by cutting the string near the tiles base, and gently placing it in a Ziploc bag and frozen in a deep freeze (-50°C).

Individually bagged frozen tiles were removed from the freezer and left to thaw. The biofilm was then rinsed/scraped off the tile into the original Ziploc bag in which they were stored. All biofilms were rinsed using (60 ml Milli-Q water) onto pre-ashed 47 mm diameter Whatman™ glass microfibre filters (GF/C™) using a vacuum pump onto a pre-weighed filter. The filters were then weighed on an analytical balance and placed into a drying oven at 70°C for 24 hours. The filters were then reweighed using the analytical balance, and ashed using an isotemp programmable forced draft furnace (650-750 series; Appendix B; Figure B11 b). The filters were placed in crucibles and put in the muffle furnace at 550ºC for 5 hours were then weighed again to calculate ash free dry weight mg/L (Ameel et al. 1998). Biofilm development was expressed as Ash Free Dry Weight (AFDW; mg/L).

2.3.8 Statistical Analysis

All statistical analyses were performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA. Water quality parameters, and biofilm data is represented as mean (±SE), unless otherwise indicated. Formulas and calculations used to determine microplastic/m^2 can be found Appendix B in Table B1. Trends (mean (±SE)) are reported in microplastic/m^2, and half-life of microplastics were calculated for each microplastic type (foam, film, fragment, microbead and fibre).

Water quality and biofilm

Trends for each water quality parameter were averaged across replicates (n=3) and mean (±SE) were plotted to evaluate changes with time that may have impacted microplastic settling rates (Appendix B; Figures B12-20; Table B2). A correlation ($p<0.05$) was conducted to determine whether densities of microplastics were correlated with biofilm densities (AFDW) in both the surface water and sediments (Table 2.4). Values were reported as mean±(SE) for each of the water quality parameters.

Microplastic kinetics

The microplastic densities for each type across replicates (n=3) were first plotted to see if they varied linearly or exponentially with time upon first inspection. If the data appeared to be linear, then a zero-order linear regression was explored as a possible fit. The residual plot was used as a method to determine whether the statistical approach was a “good fit”. If the residuals were randomly dispersed, with no apparent trend, it was considered a good fit. If the data was not randomly dispersed in the residual plot or curved, the y-axis was natural logged (ln) and a 1st order semi-logarithmic plot was applied to the data. The ln was taken of the densities of microplastics across replicates, and plotted over time. If the data had too many zero values, did not appear to be a “good fit” (following the above steps), or densities appeared to be increasing exponentially, then the data was then plotted using a 1st order exponential one-phase decay ($y=y_0$-plateau*e^{-k*x}+plateau). Y_0 is the y-value (density of microplastics) at time zero, k is the slope/rate constant, and plateau is the y-value (density of microplastics) at infinite times. The data were then inspected visually (as the statistical approach may not

fit or be ambiguous), then using the residual plot (same steps as above), and a unique statistical approach was then chosen for the each microplastic type for both the surface water and sediments. Again, if it was not a good fit, then the data were then plotted using another 1st order exponential growth trend (the trend was assessed based on the above previous steps). When none of the statistical approaches appeared to accurately capture the behaviour of the microplastic, then no statistical approach was fit to the data, and the behaviour of microplastic particle was described qualitatively. The average microplastic densities (mean (±SE)) of replicates (n=3) were plotted and both 2017 and 2018 were compared to see potential patterns.

Half-life

Half-lives were estimated to help evaluate microplastic settling and deposition in both surface water and sediments. When a statistical approach was fit, slope (k), plateau, y_0, and half-life were estimated using the appropriate statistical approach (Table 2.2). Microbeads in the surface water (2017), and fibres in the sediments (2017) both fit an exponential growth trend, where a doubling time (rate at which microplastic densities double) was instead calculated (Table 2.2). For microbeads in the surface water of 2017, the exponential growth rate was the resuspension rate of microbeads re-entering the surface water. For fibres in the sediments of 2017, the exponential growth rate was the settling/deposition rate of fibres entering the sediments.

Ice formation and microplastic densities

To determine influence of ice formation on microplastic densities (both films and fibres), the microplastic densities (mean±(SE) either surface water or sediments) were compared before ice formation to those after ice formation combining both winters 2017-2018 (day 70 versus day 278), and 2018-2019 (day 430 versus day 622). A two tailed paired t-test was conducted to evaluate differences, and due to our small sample size (n=3), results were considered statistically significant at $p \leq 0.06$ (Table 2.5).

2.4 Results

2.4.1. Water quality parameters

Temporal trends mean (±SE) of measured water quality parameters taken during the ice-free period (June 2017 to October 2018) can be found in Appendix B (Figures B12 to B20; Table 2.3 and Table B2).

2.4.2 Long-term microplastic trends

Overall trends

All microplastic types decreased in the surface waters throughout the study (Table 2.4; Figure 2.3 and 2.4 a, c, e). Microbeads, and fibres were the only microplastic types that increased in the surface water (2017) which was likely driven by the second dosing (Figures 2.6 a, and 2.7 a). Half-lives (days) ranged for the microplastic types from fastest to slowest based on the chosen statistical approach: foams ($t_{1/2}$=13) > fragments ($t_{1/2}$=166) > films ($t_{1/2}$=224; Table 2.3; Figure 2.3 and 2.4 a, c, e).

Neither foams nor fragments were detected in the sediments during the two-year study. Films, microbeads, and fibres were detected in the sediments, and did settle (Figure 2.3 b, d, f). No statistical approaches were fit over both years (2017-2019) for films, microbeads, or fibres in the sediments, as the statistical approaches I considered did not reasonably capture the temporal trends observed over the course of the whole study (Table 2.4; Figure 2.3 b, d, f). Films and fibres followed a similar trend in the sediments; initially microplastic deposition rates increased during the open water season, then decreased just before freeze-up (late fall) in both 2017 and 2018 (Figures 2.4 b, f). Microbeads in the sediments were low on average, except for day 374 in 2018, and the last sampling day 622 in 2019 (Figure 2.4 d).

Foams

The loss rate of foams from the surface waters of the mesocosms was described by an exponential one-phase decay trend (Table 2.4; Figure 2.3 a). Initially, densities of foams in the surface water decreased quickly in 2017 until ice on (day 71), then

densities leveled off and stayed consistent in 2018 (Figure 2.3 a). Densities of foams were higher on average in 2017 (3±1 foams and ranged from 1 to 5 foams; 3-fold increase) compared to the densities after ice off in 2018 (1±0 foams, and ranged from 0 to 1 foams; Figure 2.3 a). The overall density of foams in the surface water was 2±0 foams, ranged from 0 to 5 foams, and foams were detected in all three replicate tanks. Foams had a half-life of 15 days (Figure 2.3 a), yet were not detected in the sediments throughout the study. Some of the overall visual observations of foams included: uneven spatial distribution on the surface water, aggregation, found outside of the tanks on the ground (likely blew out when it was windy; or were fished out when deer drank from the tanks), squished against the sides of the tanks (due to the surface tension of the water), found under the lip of the mesocosm, weathering (particles became less uniformly spherical in shape and discoloured), and biofilm development (Appendix B; Figure B7).

Fragments

The loss rate of fragments from the surface water was described by an exponential one-phase decay trend (Table 2.4; Figure 2.3 b). Densities of fragments in the surface water decreased quickly initially (day 14), then decreased slowly and steadily over time (Figure 2.3 b). Densities of fragments were higher in 2017 (15±1 fragments and ranged from 13 to 17 fragments; 5-fold increase) compared to the densities after ice off in 2018 (3±1 fragments, and ranged from 0 to 6 fragments; Figure 2.3 b). The overall density of fragments in the surface water was 3±1 fragments, ranged from 0 to 6 fragments, and were detected in all three replicate tanks. Fragments had a surface half-life of 166 days (Figure 2.3 b), yet were not detected in the sediments throughout the study. Some of the overall visual observations included: uneven spatial distribution in the surface water, being stuck on emergent aquatic plants, squished against the sides of the tanks (due to the surface tension of the water), found during the clean-up of the study under the lip of the mesocosms, neutrally buoyant just below surface water resting on submergent aquatic plants, and biofilm development (Appendix B; Figure B8).

Films

The settling rate of films from the surface water was continuous over time, and was described by an exponential one-phase decay trend (Table 2.4; Figure 2.4 a). In the surface water, densities of films were higher in 2017 (9±3 films and ranged from 1 to 14 films; 4.5-fold increase; Figure 2.5 a) compared to the densities after ice on in 2018 (2±1 films, and ranged from 0 to 5 films; Figure 2.5 c). The overall films for the whole study in the surface water was 4±1 films, ranged from 0 to 14 films, and were detected in all three replicate tanks. Films had an over half-life of 224 days (Figure 2.4 a) in the surface water. Some of the overall visual observations of films included: uneven spatial distribution in the surface water, “disappearing” from the experiment i.e., dried to the side of the tank, glued to the biofilm tiles, or under the lip of the mesocosm, hetero-aggregation with filamentous algae, films attached to aquatic plants, films floating by themselves in the surface water, weathering (particles became vibrantly coloured), and biofilm growth (Appendix B; Figure B9).

No statistical approach was fit for the deposition rate of films in the sediments over the duration of the study, as none of the approached could accurately capture the overall seasonal pattern (Table 2.4; Figure 2.4 b). Films were detected in the sediments at a ~40-fold increase compared to the surface water and were detected at densities ~1.3 times higher than the initial dosing densities (Figure 2.4 b; initial density ~125 films). Film densities seemed to follow a seasonal trend in both 2017 (Figure 2.5 b) and 2018 (Figure 2.5 d), where densities increased in spring, summer, and early fall and decreased at the final sampling day before ice on in the late fall (Figure 2.4 b). In both 2017 and 2018, film densities decreased significantly on the last sampling date right before ice-on (Figure 2.5 b, d). Films were detected in the sediments by day 22, and were detected in all three replicate tanks. Densities of films in the sediments were lower in 2017 (118±41 films and ranged from 0 to 243 films; 2-fold decrease; Figure 2.4 b) compared to the densities after ice on in 2018 (223±91 films, and ranged from 35 to 729 films; Figure 2.5 d). The overall density of films in the sediments for the whole study was 155±50 films, and ranged from 0 to 729 films.

Microbeads

No overall statistical approach was fit for the overall trend of microbeads in the surface water (Figure 2.4 c), or for 2017 (Table 2.4;Figure 2.6 a), a linear regression trend was fit for 2018 (Figure 2.6 c). Microbeads increased exponentially in the surface water in 2017 until ice on, which was likely driven by the second dosing ($t_{1/2}$= 11 days; Table 2.4; Figure 2.4 c; Figure 2.6 a), then in 2018, microbeads began to settle as densities decreased from the surface water ($t_{1/2}$= 67 days; Table 2.4; Figure 2.4 c; Figure 2.6 c). Densities of microbeads in the surface water were similar in 2017 (51±30 microbeads and ranged from 0 to 162 microbeads; Figure 2.6 a) compared to the densities after ice off in 2018 (40±13 microbeads, and ranged from 0 to 88 microbeads; Figure 2.6 c). The overall densities of microbeads for the whole study in the surface water was 40±15 microbeads, and ranged from 0 to 162 microbeads, and were detected in all three replicate tanks. Some of the overall visual observations of microbeads included: uneven spatial distribution, homo-aggregation (i.e., microbeads only aggregating with other microbeads) on the surface water, and hetero-aggregation with foams against the side of the tank (Appendix B; Figure B10).

No statistical approach was fit for the deposition rate of microbeads in the sediments, as none of the approaches were able to accurately capture the overall trend of low densities, followed by a few extremely high densities (Table 2.4; Figure 2.4 d). Microbeads were detected in the sediments at a ~9-fold increase compared densities detected in the surface water (Figure 2.4 c, d). Microbeads were detected in the sediments by day 51 (Figure 2.4 d). Microbeads were only found in the sediments of one replicate (tank 8) throughout the study, except on the final day of the study (day 622), where microbeads were found in the sediments of all three replicate tanks (Figure 2.4 d). Microbeads were only detected in the sediments once in 2017 (day 51), and twice in 2018 (days 347, and 374; Figure 2.6 b, d) in tank 8. Densities of microbeads in the sediments were lower in 2017 (97±97 microbeads and ranged from 0 to 486 microbeads; 3-fold decrease Figure 2.6 b) compared to the densities after ice on in 2018 (283±271 microbeads, and ranged from 0 to 1910 microbeads; Figure 2.6 d).

Average microbead densities in the sediments was 365±217 microbeads, and ranged from 0 to 2280 microbeads.

Fibres

No statistical approach was fit for the settling rate of fibres from the surface water over 2017-2019 (Table 2.4; Figure 2.4 e). Densities of fibres were higher in 2017 (60±50 fibres and ranged from 2 to 208 fibres; 60-fold increase; Figure 2.7 a), compared to the densities after ice off in 2018 (1±0 fibres, and ranged from 0 to 2 fibres; Figure 2.7 c). The overall density of fibres in the surface water was 22±19 fibres, and ranged from 0 to 210 fibres. Fibres were detected in all three replicate tanks, and decreased continuously over time from the surface water until day 377 when fibres were not detected again in the surface water (Figure 2.4 e; Figure 2.7 c). Fibres were detected in the sediments at a ~850-fold increase compared to the surface water (Figure 2.4 e, f). There were no overall observations of fibres in the surface water as they were not found.

No statistical approach was fit for the deposition/settling rate of fibres/m^2 in the sediments, as the models were not able to accurately capture the overall trend (Table 2.4; Figure 2.4 f). Fibres were detected in the sediments on average at densities ~80 times higher than the initial dosing densities (Figure 2.4 f; ~250 fibres/m^2). Fibre densities seemed to follow a seasonal trend in both 2017 (Figure 2.7 b), and 2018 (Figure 2.7 d), where densities increased in spring and summer and then densities decreased significantly on the last sampling date right before freeze-up (Figure 2.7 b, d). Fibres were detected in the sediments by day 7, and were detected in all three replicate tanks. Densities of fibres in the sediments were lower in 2017 (13443±7057 fibres and ranged from 0 to 39375 fibres; ~2-fold decrease) compared to the densities after ice off in 2018 (26047±7037 fibres, and ranged from 5347 to 56250 fibres; Figure 2.4 f). The overall fibres density for the whole study in the sediments was 18785±4954 fibres, and ranged from 0 to 56250 fibres. Some of the overall visual observations included: fibres sampled from the surface water were found both as single fibres, and as bundles (visual observations) which contrasts with fibres were sampled from the sediments, as they were usually in large clumps or bundles, not as single fibres (visual observations).

2.4.3 Ice formation

Overall trends for surface water

Microplastic densities (films and fibres combined) were not statistically different in the surface water before versus after ice formation (p=0.2; Table 2.5). When considered separately, film densities declined significantly after ice formation in the surface water (p=0.06; Table 2.5; Figure 2.8), decreasing after the ice melted both winter 2017-2018 (3±2 films) and winter 2018-2019 (1±1 films; Figure 2.8 a).

Overall trends for sediments

Microplastic densities (films and fibres combined) were not statistically different in the sediments before versus after ice formation (p=0.3; Table 2.5). Film densities were not statistically different before or after ice formation in the sediments (p=0.6; Table 2.5). Fibre densities decreased significantly after ice formation in the sediments (p=0.05; Table 2.5). Fibre densities decreased in the sediments after the ice melted in both winter 2017-2018 (before minus after ice off; 21632±13862 fibres) and winter 2018-2019 (5347±2733 fibres; Figure 2.8 d). Fibres were not detected in the sediments of any of the three replicates on the last day of the study (day 622) after ice off (Figure 2.8 d).

2.4.4 Biofilm development

Mean biofilm AFDW in the microplastic treatment displayed a seasonal trend in both 2017, and 2018, as biofilm increased from spring to fall during open water season (Figure 2.9). Mean (±SE) AFDW for the microplastic treatment was 755±101 mg/L, and ranged from 227 to 1294 mg/L (Table 2.6; Figure 2.9). Densities of microplastics (foams, fragments, films and fibres) were negatively correlated with biofilm development in the surface water, yet microbeads were positively correlated (Table 2.6; Figure 2.10). Both films and fibres (microplastics that have settled) were positively correlated with AFDW in the sediments (Table 2.6; Figure 2.10).

2.5 Discussion

All microplastic types decreased in the surface waters during the experiment. Fragments appeared to be neutrally buoyant and attached to aquatic plants just below the surface water. Both films and fibres displayed seasonal patterns during the open water season in both 2017 and 2018, as densities increased over time in the sediments until just before ice on when sediment densities dropped, which supported with my first hypothesis that the unique sizes, polymers, shapes, and densities affected microplastic buoyancy, settling dynamic behaviour and fate (e.g.,foams, and fragments did not settle due to their buoyancy, and fibres, microbeads and films did settle due to their buoyancy, size, shape and polymer). As noted, I observed both visible weathering (microplastic colour less vibrant) and biofilm growth on the surface of the microplastic particles. Biofilm formation was a potential driver of microplastic settling (directions of the coefficients appear to be consistent), although not statistically significant, which weakly supported with my second hypothesis (biofilm enhanced settling). Ice formation enhanced settling/deposition of films, and enhanced resuspension of fibres in both the surface water and sediments in both winters (2017-2018 and 2018-2019), which supported with my third hypothesis as ice formation affected microplastic densities.

Seasonal patterns of microplastics

All microplastics displayed seasonal patterns in the surface water during the open water season of our study, as surface densities generally decreased from spring to fall. Within the sediments, both films and fibres displayed seasonal patterns during the open water season, as microplastic densities generally increased in the sediments from spring to fall until just before ice on (late fall) when densities suddenly dipped. The other microplastic types did not appear to behave similarly in the sediments as they were either not detected (foams and fragments), or detected only sparingly (microbeads) making seasonal patterns difficult to observe.

Biofilm development may be one of the most significant drivers of the seasonal pattern of microplastic behaviour during the open water season in both the surface water and sediments. During the spring, water temperatures increased, PAR values

increased (less shading due to senescence of aquatic plants in the winter), creating optimal conditions for biofilm growth which increased linearly during the open water season. Biofilm growth was negatively correlated with film and fibre densities in the surface water. Higher biofilm densities were positively correlated to film and fibre densities in the sediments. Together, these observations strongly suggest a role for biofilm (AFDW) as very likely driving the shift in the distribution of films and fibres throughout the open water season from surface waters to sediments.

Weathering of the microplastic particles may have also drove seasonal behaviour or both films and fibres in the open water season of the mesocosms. Films were noticeably weathered (became less vibrantly coloured) which may have caused tiny cracks to occur within the polymers surface, increasing the total surface area for biofilm growth, and ultimately driving the cyclical behaviour between years during the open water season by further enhancing settling. The dip in microplastic densities of both films and fibres in the sediments right before ice on may have been due to senescence of aquatic plants which enhanced sedimentation processes. As the water temperature decreased, the aquatic plants began to die (dense thick mass), freeing microplastics that were below the plants, therefore no longer restricting their vertical transport into the water column, enabling resuspension.

To our knowledge, no work has been conducted to date on seasonal patterns of microplastic behaviour in Canadian climates or any other climates, nor any evaluation of how seasonal patterns affect the distribution of microplastics which impacts their settling and fate within the water column. Observational microplastic studies have argued that seasonal fluctuations of point sources (e.g., WWTP input, urban traffic, or precipitation) might explain seasonal trends of microplastic densities (Anderson et al. 2017; Warrack et al. 2017; Mani and Burkhardt-Holm 2020; Wang et al. 2021). There are reported inconsistencies of microplastic source seasonality which was likely due to terrestrial anthropogenic sources and spring run-off between studies which have found either: no coherent pattern between microplastic density (surface water) and season (Mani and Burkhardt-Holm 2020), or a seasonal pattern, where densities in surface waters are higher in spring/early summer compared to fall (Warrack et al. 2017; Wang et al.

2021a). Consistency in these observational studies with our experimental observations suggests that seasonal patterns are likely driven by biofilm development.

Aggregation behaviour of microplastics

Not all microplastics displayed aggregation behaviour: foams, microbeads and fibres formed unique homo and hetero-aggregates, while fragments and films did not. Aggregation did not appear to be enough to alter overall density of foams, as they were not detected within the sediments. Microbead aggregates were detected in the sediments by the end of the 622-day study. Aggregation within this study likely occurred due to our experimental design (i.e., high number of particles dosed). Aggregation may not occur until a critical number of microplastics are added to a system, and it is currently unclear what the critical number is at this time. Microplastic aggregation behaviour within our study is consistent with other microplastic studies (Lagarde et al. 2016; Long et al. 2017; Alimi et al. 2018; Li et al. 2018; Michels et al. 2018; Cunha et al. 2019).

Wind may have also influenced aggregation behaviour in the mesocosms. Our mesocosms were set-up in an open field (average wind speed: 20 km/hour and ranged from 0 to 75 km/hour) and wind conditions created internal circulation within the tanks pushing microplastics together enabling them to form aggregates. Möhlenkamp et al. (2018) found that settled microbeads and phytoplankton aggregates can resuspend when there is enough flow (>1.5cm/s) in the water. Faster moving water may have the ability to resuspend settled microplastics. Biofouling of microplastic particles likely enhanced aggregation behaviour. Biofilm development on the surface of the microplastic particle made them sticky and able to attach to each other, and other suspended particles within the water column forming aggregates. Aggregation of microplastics occurs quicker (three hours compared to one day) when biofilm development has already occurred on the surface of the particle (Michels et al. 2018). Fibres forming homo-aggregate bundles was likely due to our initial dosing techniques, as it was hard to separate individual fibres from the fleece perfectly, and there was likely some error. Fibre bundles may also be a characteristic behaviour of the microplastic itself, as it tangles and knots around itself and other fibres within the water column.

Evidence of aggregation behaviour within our study provides insight into the long-term behaviour of foams, microbeads and fibres within freshwater systems. Aggregation of microplastics within our study likely impacted both their settling and resuspension behaviour. Aggregation can either increase or decrease settling rates of microplastics depending on their polymer and the buoyancy of the colonizing algal species (Long et al. 2015). Aggregation and fibre bundling behaviour likely increased the settling rate of fibres as the aggregates became heavier which enabled them to settle faster. Aggregation behaviour of fibres may have also enhanced their resuspension behaviour after ice formation, as the larger aggregate broke apart due to ice formation defouling, and algal decay, altering the aggregates buoyancy leading to microplastic resuspension.

An implication of aggregation behaviour is that it can alter microplastic settling rates. The sizes of microplastics, morphologies, polymers, organic (biofilm/agal densities and species compositions), or inorganic (sediments) material within the aggregate are unique (Alimi et al. 2018) which will all affect microplastic settling, and fate in aquatic ecosystems (Long et al. 2015). The aggregates specific location in the water column will also affect weathering processes (via photo-degradation; mechanical degradation, and biological degradation) and degradation rates of the microplastics (Alimi et al. 2018). More studies need to be conducted to understand the long-term aggregation behaviour of different (sizes, morphologies, polymers) microplastics which will further elucidate their behaviour and fate in Canadian freshwater systems.

Ice formation effects on microplastics

There were seasonal ice formation effects on both films and fibres (only two microplastic types assessed as densities were detected in both surface water and sediments consistently) within our study. Ice formation enhanced film settling/deposition from surface waters (densities decreased) in both winters. Ice formation had no effect on film densities in the sediments in the first winter, yet seemed to enhance resuspension of films in the second winter. Ice formation enhanced the resuspension of fibres in both winters in the surface water and sediments. To our knowledge, there is no literature to date on how ice formation and subsequent melting would affect microplastic

fate and behaviour. Further, no studies to my knowledge exist on resuspension of microplastics under “natural” freshwater conditions, as microplastic resuspension is captured within laboratory experiments (Möhlenkamp et al. 2018).

The warming water temperatures in spring following ice formation may have enhanced film deposition from the surface waters. After ice melted in spring, the mesocosms water temperature increased, which decreased water’s density. Biofouling and the water’s lower density led to films becoming either neutrally and/or negatively buoyant enabling deposition. The specific way ice melted within the mesocosm in the spring, likely led to fibre resuspension in both the surface water and sediments. In spring, the tanks thawed, and the ice separated from the sediment-water interface. Any fibres at the sediment-water interface remained in the ice, which floated within the liquid water. As the ice melted from the bottom up, fibres were discharged back into the water column (resuspended), which lead to lower densities in the sediments in the spring.

Both biofilm decay and water density changes in the fall may have also enhanced the resuspension of fibres within the mesocosms. As water became cooler in the fall, biofilm on the microplastics likely started to decay. Water also became denser, and therefore fibres and films within the sediments were resuspended back into the water column. Ice crystal formation may have also driven microplastic resuspension. Within the tanks, ice crystals could form around the microplastics within the water column or sediments. As the ice crystal formed around the microplastic, it become positively buoyant and floated back to the surface water and froze. This behaviour could account for the decrease in densities in the sediments for films and fibres in the spring. Fibre densities increased in the surface water in the spring, which may be the result of the fibres frozen within the surface water, awaiting biofouling or aggregation before they can settle again. In the sediments there was evidence of lower amounts of fibres resuspending each subsequent winter, which was likely due to entrainment within the sediments. Over time, fibres were buried and entrained deeper into the sediment layer. Once fibres were entrained under a certain amount of sediments, they were unable to resuspend back into the water column, and therefore fewer fibres were resuspended each subsequent winter as a result (Nakki et al. 2017).

Foams and films did not settle

Neither foams (PS) and fragments (PE) settled within the 622-day study, yet densities decreased over time in surface waters. Both microplastic types were found lost under the lip of the mesocosms during the takedown of the experiment, and fragments were found stuck to dense aquatic vegetation (neutrally buoyant), just out of reach of surface water sampling. Foams blew out of the mesocosms, and were stuck to the sides of tank (due to water surface tension), which created inaccuracies in our density estimates. However, these aggregations and unquantified losses are likely responsible for the observed decreasing trends in surface waters. The absence of settling of foams and fragments in our study was not consistent with other freshwater studies which have found evidence of both polystyrene foams and polyethylene fragments within aquatic sediments (Corcoran et al. 2015; Di and Wang 2018; Wen et al. 2018; Alam et al. 2019; Ding et al. 2019; Turner et al. 2019; Eitzen and Ruhl 2020). Foams have also been found in sediments of an urban UK lake dating back to 1950's (Turner et al. 2019), and fragments have compromised up to 20% of the total microplastics found within the the sediments of Lake Ontario (Corcoran et al. 2015).

Dense aquatic plant growth in the tanks may have also impaired the settling dynamics of fragments specifically. Aquatic plant densities were high and likely blocked the pathway for vertical transport of all microplastic particles to the sediments. Fragments were observed to be the most attracted to aquatic plants as they were often stuck below the surface of the water adhered to them. The plants also appeared to have a biofilm layer that trapped fragments (more so than other microplastic particles used within our study), further impacting their vertical transport within the mesocosm. Winnipeg's climate may have also affected the settling rate of both foams and fragments. Both algae and other biofilm species may have preferences for which microplastic types they will attach to forming biofilm layers and/or hetero-aggregates with (Cunha et al., 2019). Our mesocosms may have not had the right species and/or enough time to form a thick enough biofilm layer to alter fragment or foam buoyancy, or to form hetero-aggregates with foams or fragments, and therefore did not settle to the bottom of our tanks within our study's time frame. Foams and fragments may have not

settled during the study (622-days) as the time frame may not have been long enough to undergo processes that would enable them to settle. Potentially fragments (PE) and foams (PS) need more time (>622 days) to grow a thick biofilm layer, form hetero-aggregates, and settle within our mesocosms. Yet this conflicts with experimental settling velocities which have calculated variable rate of polystyrene foams (9-34 cm/s), and polyethylene fragments (1-9 cm/s; Waldschläger and Schüttrumpf 2019) where they settle quickly in motionless water.

Water quality within the mesocosms may have also affected the ability of both foams and films settling. The water quality within our mesocosms was pristine compared to “polluted” (i.e., sewage overflow, WWTP discharge, heavy metals, organic pollutants, heavy urban traffic) freshwater systems that have found foamed polystyrene within sediment samples (Di and Wang 2018; Ding et al. 2019; Turner et al. 2019; Eitzen and Ruhl 2020). Poor water quality in these freshwater systems may have led to foams adsorbing substances, minerals, and enhanced biofouli ng, all of which could alter particle buoyancy and hydrophobicity, enabling them to sink. The water quality within our mesocosms (no nutrient inputs, sewage overflow, WWTP discharge, heavy metals, organic pollutants, heavy urban traffic; water from local municipal source) likley created conditions where the microplastics ultimately need more time to undergo processes that would enable them to settle.

The small size of the mesocosms may have also contributed to foams and fragments inability to settle. The mesocosms were small (surface area= 5.7 m^2) and were not truly realistic compared to freshwater rivers, lakes, reservoirs that have found both foams and fragments to settle (Di and Wang 2018; Ding et al. 2019; Turner et al. 2019; Eitzen and Ruhl 2020). These larger water bodies have non-quiescent flow, which can lead to microplastic entrainment and mechanical weathering, which alters their hydrophobicity, polarity and denisty, enabling them to sink (Eitzen and Ruhl 2020).

The hydrophobicity of foams also appeared to contribute to their inability to settle within our study. The foams were so hydrophobic that they floated on top of the surface water, and even seemed to be repelled by it. This enabled strong winds to easily blow foams out of the mesocosms (witnessed while dosing). Methods such as physical

shaking (sonification), or chemical additions (surfactants) are often used to force foams into the water column (Eitzen and Ruhl 2020) during toxicology and settling velocity studies. However, I relied solely on natural processes occurring within the tanks to settle; as such, our results may provide a more realistic timeline of settling (e.g., > 622 days) compared to other studies which force particles into the water column by artificial means. My work indicates that microplastics (foams and fragments in particular) may persist on water surfaces and/or in the water column longer than previously thought within Winnipeg's freshwater ecosystems.

Implications of the study

Microplastics displayed seasonality during the open water season in both the surface water and sediments, with significant correlations between settling (changes in microplastic densities from surface to sediments) with biofilm development. Consistency in our experimental observations suggests that seasonal patterns are likely driven by biofilm development. Seasonal microplastic behaviour may be a predictable pattern, and we may be able to track and predict microplastic behaviour in the water column through biofilm development. Some (not all) microplastic types form aggregates (microbeads, foams, fibres), which could be used as a potential removal technique in WWTP (Zhang and Chen 2020; Wang et al. 2021).

Complex microplastic behaviours driven by ice formation, were likely caused by ice crystal formation, and the changing temperature (density) of the water causing both biofilm decay and changes in the particle's buoyancy. Ice formation enhancing both resuspension and deposition of some microplastic types within both the surface water and sediments. The overall long-term behaviour of all microplastic types (foams, films, fragments, microbeads, and fibres) decreased in the surface waters, but that not all settled to the sediments (foams and fragments). Microplastics (foams and fragments in particular) may persist on water surfaces and/or in the water column longer than previously thought within Canadian freshwater ecosystems, and that microplastic fate and behaviour in freshwater systems may not be as straight forward as their inevitable settling and entrainment as previously thought, factors such as water quality, weathering, and climate likely play a role in their ability to settle.

While significant gaps exist in freshwater microplastic research with regards to how microplastics behave in the water column throughout distinct seasons (including ice formation) especially within our Canadian climate, my research has begun to advance our understanding of these processes. Sediments may not be the ultimate sink for microplastics as previously thought, as their behaviour within the water column is far more complex (aggregation, biofouling, ice crystal formation, water temperature-density effects) and factors of the water body (i.e., climate and water quality) will also affect both behaviour and their ultimate fate.

2.6 References

Alam, F.C., Sembiring, E., Muntalif, B.S., and Suendo, V. 2019. Microplastic distribution in surface water and sediment river around slum and industrial area (case study: Ciwalengke River, Majalaya district, Indonesia). Chemosphere **224**: 637–645.

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., and Tufenkji, N. 2018. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. **52**(4): 1704–1724.

Anderson, J.C., Park, B.J., and Palace, V.P. 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environ. Pollut. **218**: 269–280.

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. **225**: 223–231.

Andrady, A.L. 2011. Microplastics in the marine environment. Mar. Pollut. Bull. **62**(8): 1596–1605.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., and Longstaffe, F.J. 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. **110**(1): 383-395.

Barnes, D.K.A., Galgani, F., Thompson, R.C., and Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. **364**(1526): 1985–1998.

Baxter, L.R., Sibley, P.K., Solomon, K.R., and Hanson, M.L. 2013. Interactions between atrazine and phosphorus in aquatic systems: effects on phytoplankton and periphyton. Chemosphere **90**(3): 1069–1076.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., and Thompson, R. 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. **45**(21): 9175–9179.

Camenen, B. 2007. Simple and general formula for the settling velocity of particles. J. Hydraul. Eng. **133**(2): 229–233.

Campbell, S.H., Williamson, P.R., and Hall, B.D. 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. Facets **2**(1): 395–409.

Cardinal, P. 2013. Assessing nutrient and pharmaceutical removal efficiency from wastewater using shallow wetland treatment mesocosms. Masters thesis submitted to the University of Manitoba. pp. 1-229.

Cardinal, P., Anderson, J.C., Carlson, J.C., Low, J.E., Challis, J.K., Beattie, S.A., Bartel, C.N., Elliott, A.D., Montero, O.F., Lokesh, S., Favreau, A., Kozlova, T.A., Knapp, C.W., Hanson, M.L., and Wong, C.S. 2014. Macrophytes may not contribute significantly to removal of nutrients, pharmaceuticals, and antibiotic resistance in model surface constructed wetlands. Sci. Total Environ. **482**–**483**(1): 294–304.

Cole, M., Lindeque, P., Halsband, C., and Galloway, T.S. 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. **62**(12): 2588–2597.

Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., and Marvin, C.H. 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environ. Pollut. **204**: 17–25.

da Costa, J.P., Duarte, A.C., and Rocha-Santos, T.A.P. 2017. Microplastics – occurrence, fate and behaviour in the environment. Compr. Anal. Chem. **75**: 1–24.

Cunha, C., Faria, M., Nogueira, N., Ferreira, A., and Cordeiro, N. 2019. Marine vs freshwater microalgae exopolymers as biosolutions to microplastics pollution. Environ. Pollut. **249**: 372–380.

Di, M., and Wang, J. 2018. Microplastics in surface waters and sediments of the Three Gorges Reservoir, China. Sci. Total Environ. **616**–**617**: 1620–1627.

Dietrich, W.E. 1982. Settling velocity of natural particles. Water Resour. Res. **18**(6): 1615–1626.

Ding, L., Mao, R. fan, Guo, X., Yang, X., Zhang, Q., and Yang, C. 2019. Microplastics in surface waters and sediments of the Wei River, in the northwest of China. Sci. Total Environ. **667**: 427–434.

Dris, R., Gasperi, J., Saad, M., Mirande, C., and Tassin, B. 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. **104**(1–2): 290–293.

Eitzen, L., and Ruhl, A.S. 2020. Particle size and pre-treatment effects on polystyrene microplastic settlement in water: implications for environmental behavior and ecotoxicological tests. Water (Switzerland) **12**(3436): 1–12.

Environment and Climate Change Canada. 2019. Economic study of the Canadian plastic industry, markets and waste: summary report to Environment and Climate Change Canada. pp. 1-43.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., and Amato, S. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. **77**(1–2): 177–182.

Falco, F. De, Pia, M., Gentile, G., Di, E., Escudero, R., Villalba, R., Mossotti, R., Montarsolo, A., Gavignano, S., Tonin, C., and Avella, M. 2018. Evaluation of microplastic release caused by textile washing processes of synthetic fabrics. Environ. Pollut. **236**: 916–925.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., and Boldgiv, B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. **85**(1): 156–163.

Geilfus, N., Munson, K.M., Sousa, J., Germanov, Y., Bhugaloo, S., Babb, D., and Wang, F. 2019. Distribution and impacts of microplastic incorporation within sea ice. Mar. Pollut. Bull. **145**(June): 463–473.

Georg Mehlhart, M.B. 2012. Study on land-sourced litter(LSL) in marine environments: review of sources and literature in the context of initiative of Declaration of the Global Plastics Associations for Solutions on marine Litter. Öko-Institut eV, Darmstadt/freibg. **49**(0): 30–40.

Govender, J., Naidoo, T., Rajkaran, A., Cebekhulu, S., Bhugeloo, A., and Naidoo, S. 2020. Towards characterising microplastic abundance, typology and retention in mangrove-dominated estuaries. Water (Switzerland). **12**(10): 1-24.

Helm, P.A. 2020. Occurrence, Sources, transport, and fate of microplastics in the Great Lakes--St. Lawrence River Basin. *In* Contaminants of the Great Lakes. *Edited by* J. Crossman and C. Weisener. Springer International Publishing, Cham. pp. 15–47.

Henry, B., Laitala, K., and Klepp, I.G. 2019. Microfibres from apparel and home textiles: Prospects for including microplastics in environmental sustainability assessment. Sci. Total Environ. **652**: 483–494.

Hernandez, E., Nowack, B., and Mitrano, D.M. 2017. Polyester Textiles as a Source of Microplastics from Households: a mechanistic study to understand microfiber release during washing. Environ. Sci. Technol. **51**(12): 7036–7046.

Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., and Law, K.L. 2015. The ocean, **347**(6223): 3–6.

Kaiser, D., Kowalski, N., and Waniek, J.J. 2017. Effects of biofouling on the sinking behavior of microplastics. Environ. Res. Lett. **12**(12):1-10.

Khatmullina, L., and Isachenko, I. 2017. Settling velocity of microplastic particles of regular shapes. Mar. Pollut. Bull. **114**(2): 871–880.

Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., and Caruso, A. 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. **215**: 331–339.

Lambert, S., Sinclair, C., and Boxall, A. 2014. Occurrence, degradation and effect of polymer-based materials in the environment. Rev. Environ. Contam. Toxicol. **227**: 1–53.

Li, S., Liu, H., Gao, R., Abdurahman, A., Dai, J., and Zeng, F. 2018. Aggregation kinetics of microplastics in aquatic environment: Complex roles of electrolytes, pH, and natural organic matter. Environ. Pollut. **237**: 126–132.

Lobson, C. 2018. Aquatic insects as a vector for antibiotic resistant gene-bearing bacteria. Masters thesis submitted to the University of Manitoba. pp 1-114.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., and Soudant, P. 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. **175**: 39–46.

Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., and Soudant, P. 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. **228**: 454–463.

Magnusson, K., Eliasson, K., Fråne, A., Haikonen, K., Hultén, J., Olshammar, M., Stadmark, J., and Voisin, A. 2016. Swedish sources and pathways for microplastics to the marine environment. A review of existing data. IVL Sven. miljöinstitutet (C 183): 1–89. Available from www.ivl.se.

Mani, T., and Burkhardt-Holm, P. 2020. Seasonal microplastics variation in nival and pluvial stretches of the Rhine River – from the Swiss catchment towards the North Sea. Sci. Total Environ. **707**: 135579.

Masura, J., Baker, J., Foster, G., and Arthur, C. 2015. Laboratory methods for the analysis of microplastics in the marine environment. NOAA Mar. Debris Progr. **Technical**(July), pp. 1-31.

Van Melkebeke, M., Janssen, C., and De Meester, S. 2020. Characteristics and sinking behavior of typical microplastics including the potential effect of biofouling: implications for remediation. Environ. Sci. Technol. **54**(14): 8668–8680.

Michels, J., Stippkugel, A., Lenz, M., Wirtz, K., and Engel, A. 2018. Rapid aggregation of biofilm-covered microplastics with marine biogenic particles. Proc. R. Soc. B Biol. Sci. **285**(1885):1-9.

Möhlenkamp, P., Purser, A., and Thomsen, L. 2018. Plastic microbeads from cosmetic products: An experimental study of their hydrodynamic behaviour, vertical transport and resuspension in phytoplankton and sediment aggregates. Elementa. **6:** 1-16.

Napper, I.E., and Thompson, R.C. 2016. Release of synthetic microplastic plastic fibres from domestic washing machines: effects of fabric type and washing conditions. Mar. Pollut. Bull. **112**(1–2): 39–45.

Nguyen, T.H., Tang, F.H.M., and Maggi, F. 2020. Sinking of microbial-associated microplastics in natural waters. PLoS One **15**(2): 1–20.

Pichel, W.G., Churnside, J.H., Veenstra, T.S., Foley, D.G., Friedman, K.S., Brainard, R.E., Nicoll, J.B., Zheng, Q., and Clemente-Colón, P. 2007. Marine debris collects within the North Pacific subtropical convergence zone. Mar. Pollut. Bull. **54**(8): 1207–1211.

Pruter, A.T. 1987. Sources, quantities and distribution of persistent plastics in the marine environment. Mar. Pollut. Bull. **18**(6 SUPPL. B): 305–310.

Randell, M. 2019. Chitobiase as a surrogate measure of aquatic invertebrate biomass and secondary production in an environmental effects monitoring context. Masters thesis submitted to the University of Manitoba. pp. 1-139.

Rillig, M.C. 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. **46**(12): 6453–6454.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H. De, Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., and Hung, C. 2019. Rethinking microplastics as a

diverse contaminant suite. Environ. Toxicol. Chem. **38**(4): 703–711.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., and Schmitt-Jansen, M. 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. **4**(7): 258–267.

Solomon, K.R., Smith, K., and Stephenson, G.L. 1982. Depth integrating samplers for use in limnocorrals. Hydrobiologia **94**(1): 71–75.

Turner, S., Horton, A.A., Rose, N.L., and Hall, C. 2019. A temporal sediment record of microplastics in an urban lake, London, UK. J. Paleolimnol. **61**(4): 449–462.

Vanderpont, A.K. 2018. Otoliths as Indicators of Trace Element Exposure in Freshwater Fish: a mesocosm experiment with manganese and an examination of hydro-impoundment on otolith trace element signatures. Masters thesis submitted to the University of Manitoba. pp. 1-360.

Vermaire, J.C., Pomeroy, C., Herczegh, S.M., and Haggart, O. 2017. Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. Facets. **2**: 301–314.

Villeneuve, A., Montuelle, B., Pesce, S., and Bouchez, A. 2013. Environmental river biofilms as biological indicators of the impact of chemical contaminants. *In* Encyclopedia of Aquatic Ecotoxicology. *Edited by* J.-F. Férard and C. Blaise. Springer Netherlands, Dordrecht. pp. 443–456.

Waldschläger, K., and Schüttrumpf, H. 2019. Effects of particle properties on the settling and rise velocities of microplastics in freshwater under laboratory conditions. Environ. Sci. Technol. **53**: 1958–1966.

Wang, G., Lu, J., Li, W., Ning, J., Zhou, L., Tong, Y., Liu, Z., Zhou, H., and Xiayihazi, N. 2021a. Seasonal variation and risk assessment of microplastics in surface water of the Manas River Basin, China. Ecotoxicol. Environ. Saf. **208**: 111477.

Wang, W., Ndungu, A.W., Li, Z., and Wang, J. 2017. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China.

Sci. Total Environ. **575**: 1369–1374.

Wang, X., Bolan, N., Tsang, D.C.W., Sarkar, B., Bradney, L., and Li, Y. 2021b. A review of microplastics aggregation in aquatic environment: Influence factors, analytical methods, and environmental implications. J. Hazard. Mater. **402**(March 2020): 123496.

Warrack, S., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastics flowing into Lake Winnipeg: densities, sources, flux, and fish exposures. Proc. Manitoba's Undergrad. Sci. Eng. Res. **3**: 5–15. University of Manitoba Libraries.

Wen, X., Du, C., Xu, P., Zeng, G., Huang, D., Yin, L., Yin, Q., Hu, L., Wan, J., Zhang, J., Tan, S., and Deng, R. 2018. Microplastic pollution in surface sediments of urban water areas in Changsha, China: Abundance, composition, surface textures. Mar. Pollut. Bull. **136**(June): 414–423.

Wetzel, R. 1975. Limnology: lake and river ecosystems. Academic press, pp. 1-1006.

Wright, S.L., Thompson, R.C., and Galloway, T.S. 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. **178**: 483–492.

Yang, L., Qiao, F., Lei, K., Li, H., Kang, Y., Cui, S., and An, L. 2019. Microfiber release from different fabrics during washing. Environ. Pollut. **249**: 136–143.

Ye, S., and Andrady, A.L. 1991. Fouling of floating plastic debris under Biscayne Bay exposure conditions. **22**(12): 608-613.

Zhang, Z., and Chen, Y. 2020. Effects of microplastics on wastewater and sewage sludge treatment and their removal: a review. Chem. Eng. J. **382**(July 2019): 122955.

Zhiyao, S., Tingting, W., Fumin, X., and Ruijie, L. 2008. A simple formula for predicting settling velocity of sediment particles. Water Sci. Eng. **1**(1): 37–43.

2.7 Tables

Table 2.1. Microplastic type, shape, colour, size, polymer, source, photo, number added, and density for microplastics added to mesocosm tanks in both the long-term and short-term study.

Morphology	Shape	Colour	Size (mm)	Polymer	Attributes	Source	Photo	Number added	MP/L	MP/m^2
Foam	Sphere	white	<5	foamed polystyrene	sponge-like	Bean bag chair beads		2,200	1	125
Film	Square	blue, yellow, orange, pink, green	<5	polyethylene	thin and flimsy	Post-it tags		2,200	1	125
Fragment	irregular	multi-coloured	<5	polyethylene	hard with jagged edges	Melty beads		2,200	1	125
Microbead	Sphere	white and blue	0.33	polyethylene	sphere	Face wash		81,492	29	4,641
Fibres	Line	orange	<5	polyester	thin lines	Fleece fabric		8,800	3	501
							Total	96,892	35	5,518

Table 2.2. Trends of microplastics and the subsequent kinetics, equation of line, half-life, and slope.

Kinetics	**Trend**	**Equation of line (y=mx+b)**	**Half-life ($t_{1/2}$) or doubling time**	**slope**
Zero order	Linear regression	$[A]=-kt+[A]_0$	$t_{1/2}= [A]_0/2*k$	-k
1st order	Exponential one-phase decay	$[A]=[A]_0*e^{-kt}$	$t_{1/2}=Ln(2)/k$	-k
Growth	Exponential growth	$[A]=[A]_0*e^{kt}$	Doubling time= Ln(2)/k	k

Table 2.3. Mean (±SE) water quality parameters for the long-term study.

Water quality parameter	**Mean (±SE)**	**Minimum**	**Maximum**
Temperature (℃)	16±0	2	25
pH	10±0	9	11
Dissolved Oxygen (mg/L)	9±0	3	16
Chlorophyll-a (µg/L)	7±0	0	18
PAR (µmol/m^2/s)	443±47	133	867
Depth (cm)	37±0	32	41
General Hardness (mg/L)	190±8	127	260
Alkalinity (mg/L)	108±6	73	177
Conductivity (uS/cm) 2017	1±0	0	1
Conductivity (uS/cm) 2018	626±5	386	747
Filamentous Algae	1±0	1	1
AFDW (mg/L)	755±101	227	1294

Table 2.4. Summary of statistical type, microplastic behaviour (resuspension or settling), and half-life ($t_{1/2}$) in three (n=3) mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Microplastic densities were averaged with in the replicates, and then trends were assessed.

Microplastic type	**Surface Water or Sediments**	**Year**	**Figure**	**Statistical Type**	**Resuspension or settling**	**R^2**	**Equation of Line**	**Slope (k)**	**$t_{1/2}$ (days)**
Foams/m^2	Surface Water	2017-2019	2.4 a	One-phase decay	Settling*	0.4	$y=7.8*e^{-0.05x}+0.8$	0.05	15
Fragments/m^2	Surface Water	2017-2019	2.4 b	One-phase decay	Settling*	0.4	$y=18.9*e^{-0.004x}-1.4$	0.004	166
Films/m^2	Surface Water	2017-2019	2.5 a	One-phase decay	Settling	0.3	$y=11.9*e^{-0.003x}-1.8$	0.003	224
Films/m^2	Surface Water	2017	2.6 a	No fit	Settling				
Films/m^2	Surface Water	2018	2.6 c	One phase decay	Settling	0.2	$y=289*e^{-0.02x}+0.6$	0.02	45
Films/m^2	Sediments	2017-2019	2.5 b	No fit	Both				
Films/m^2	Sediments	2017	2.6 b	No fit	Settling				
Films/m^2	Sediments	2018	2.6 d	No fit	Settling				
Microbeads/m^2	Surface Water	2017-2019	2.5 c	No fit	Both				
Microbeads/m^2	Surface Water	2017	2.7 a	Exponential Growth	Resuspension**	0.7	$1.9*e^{0.06x}$	0.06	11
Microbeads/m^2	Surface Water	2018	2.7 c	Linear regression	Settling	0.3	$y= -0.4x+175.5$	0.4	67

Table 2.4. Continued.

Microplastic type	Surface Water or Sediments	Year	Figure	Statistical Type	Resuspension or settling	R^2	Equation of Line	Slope (k)	$t_{1/2}$ (days)
Microbeads/ m^2	Sediments	2017-2019	2.5 d	No fit	Both				
Microbeads/ m^2	Sediments	2017	2.7 b	No fit	Both				
Microbeads/ m^2	Sediments	2018	2.7 d	No fit	Both				
Fibres/m^2	Surface Water	2017-2019	2.5 e	No fit	Settling				
Fibres/m^2	Surface Water	2017	2.8 a	No fit	Both				
Fibres/m^2	Surface Water	2018	2.8 c	One-phase decay	Settling	0.4	$y=68.3*e^{-0.01x}-0.7$	0.01	62
Fibres/m^2	Sediments	2017-2019	2.5 f	No fit	Both				
Fibres/m^2	Sediments	2017	2.8 b	Linear Regression	Settling	0.4	$y= 556.8x-5816$	556.8	
Fibres/m^2	Sediments	2018	2.8 d	No fit	Settling				

*both foams and fragments were not detected in the sediments, and therefore the term settling is likely a loss of microplastics due to an inability to sample.

** The term resuspension used here for microbeads was not likely resuspension but the increase in densities in the surface was due to our second dos

Table 2.5. Comparison of densities of films/m^2 and fibres/m^2 before versus after ice-off in both winters 2017-2018 and 2018-2019 (before= last sampling day before freeze-up, and after= first sampling date after ice melt), using a two tailed paired t-test. Data was transformed (ln(x+1)), and variation within the treatment was considered statistically significant when $p \leq 0.06$ due to our small sample size (n=3).

Microplastic Type	Location	p-value	Statistically Significant	t	DF
Films/m^2 and Fibres/m^2	Surface Water	p=0.2	No	1.3	11
Films/m^2 and Fibres/m^2	Sediments	p=0.3	No	1.1	11
Films/m^2	Surface Water	p=0.06	Yes	2.5	5
Films/m^2	Sediments	p=0.6	No	0.6	5
Fibres/m^2	Surface Water	p=0.4	No	1.0	5
Fibres/m^2	Sediments	p=0.05	Yes	2.6	5

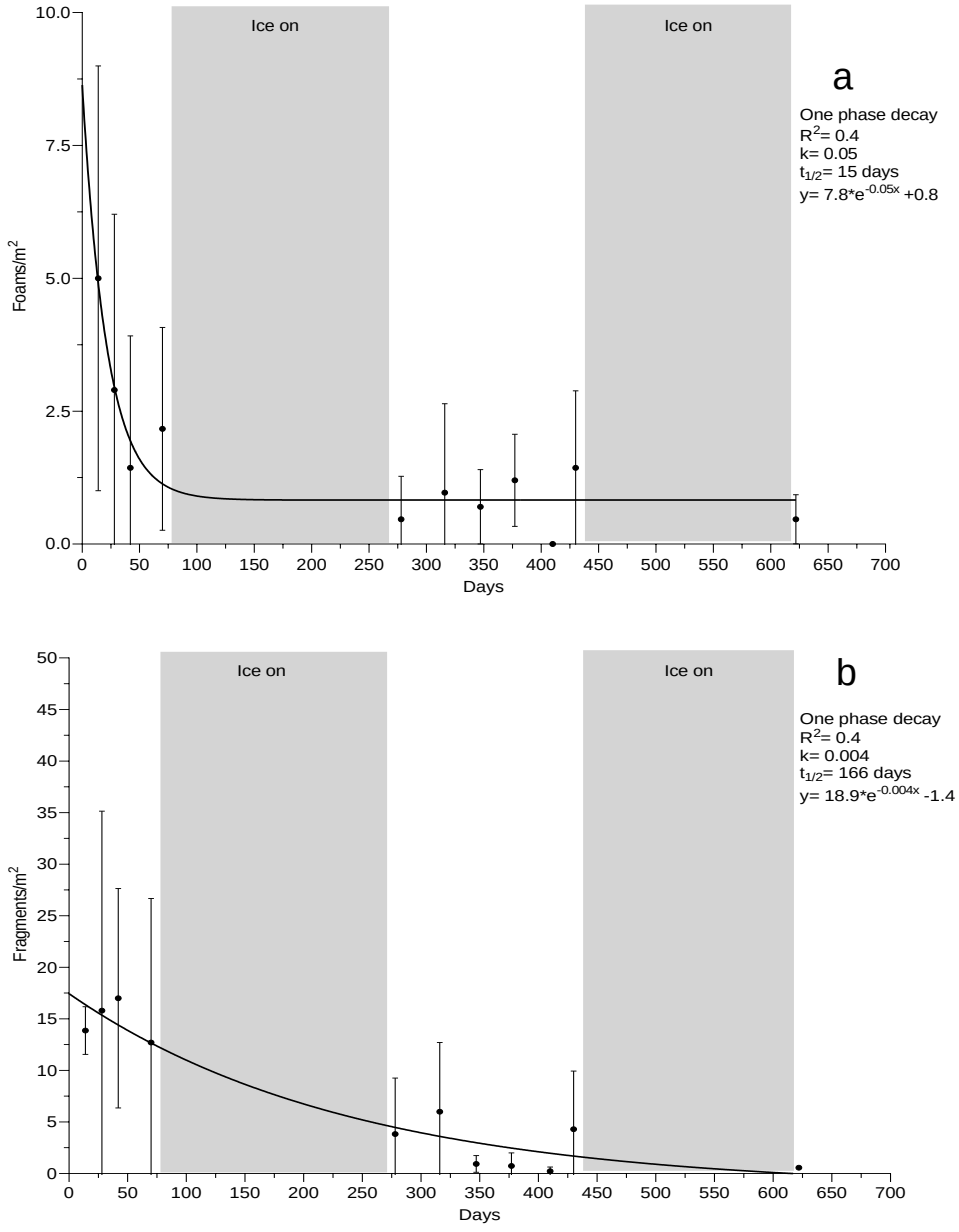
Table 2.6. Comparison between densities of microplastics (films/m^2 and fibres/m^2) in the surface water or sediments and AFDW using a correlation matrix. Correlation was considered statistically significant when $p<0.05$.

Microplastic Type	Location	Pearson r	p-value	Statistically Significant
Foams/m^2	Surface Water	-0.27	0.44	No
Fragments/m^2	Surface Water	-0.30	0.39	No
Microbeads/m^2	Surface Water	0.29	0.42	No
Films/m^2	Surface Water	-0.27	0.45	No
Films/m^2	Sediments	0.35	0.32	No
Fibres/m^2	Surface Water	-0.20	0.57	No
Fibres/m^2	Sediments	0.59	0.07	No

2.8 Figures

Figure 2.1. Mesocosm dosing day one (August 4, 2017). (a) Microplastics were added to mesocosms using four quadrants simultaneously by four individuals to ensure even distribution of particles. (b) A seeder was used to evenly disperse fragments into mesocosms.

Figure 2.2. (a) Surface water sampling using a surrogate manta trawl. (b) Sediment box in Long-term study. (c) Aquarium vacuum used to collect sediment samples in the short-term study.



Figures 2.3. Overall trend of mean (±SE) microplastics/m^2 in the surface water of (a) foams/m^2, and (b) fragments/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Microplastic/m^2 densities were averaged across replicates (n=3) the microplastic treatment. Microplastics/m^2 densities were measures un the surface water from August 18, 2017 to April 18, 2019 (days 14-622). Initial density of both foams/m^2 and fragments/m^2 was 125 microplastics/m^2.

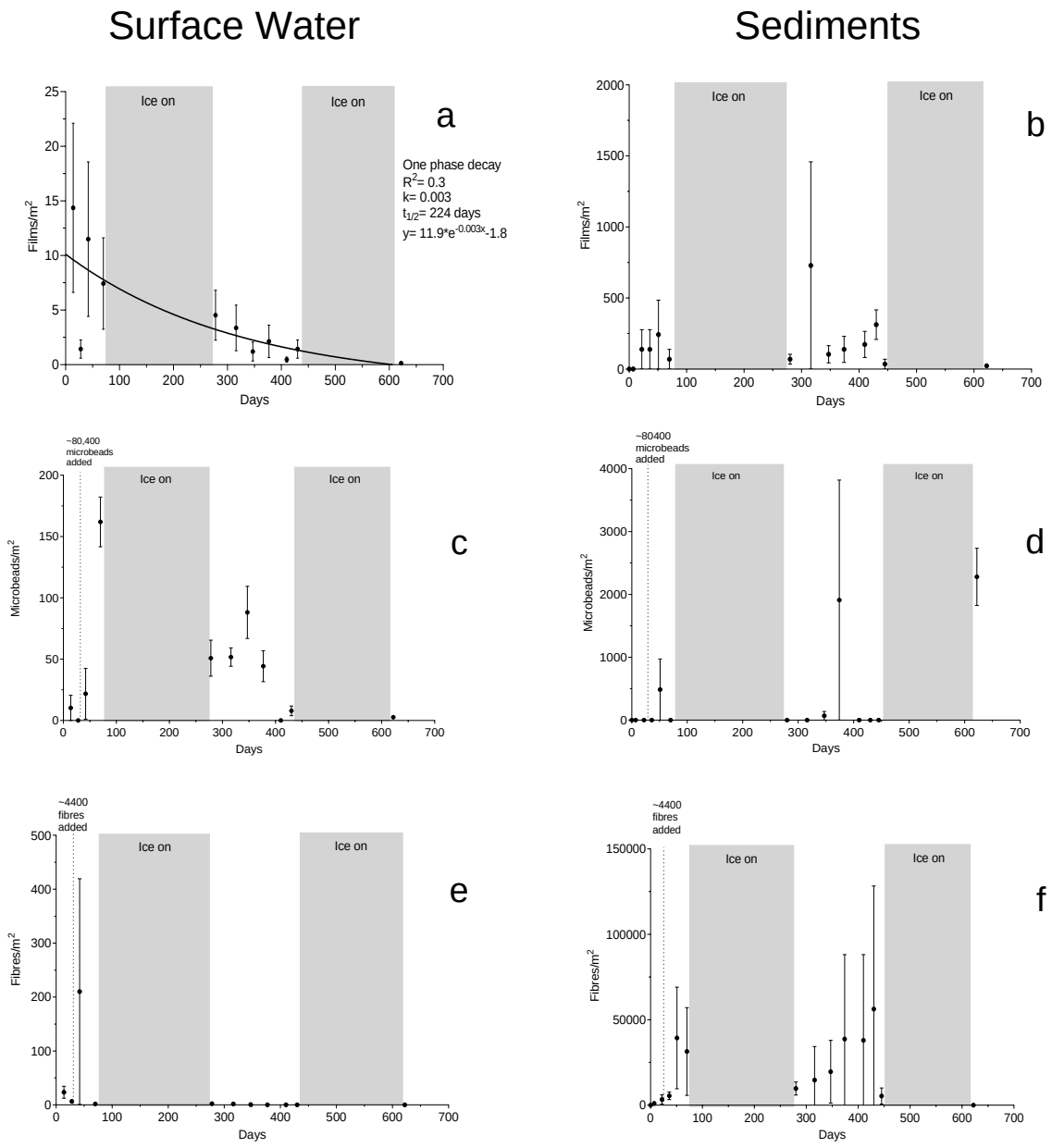


Figure 2.4. Overall trend of mean (±SE) microplastics/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Microplastic/m^2 densities were averaged across replicates (n=3). Microplastic/m^2 densities were measured in 2017 for the both the surface water: (a) films/m^2, (c) microbeads/m^2, (e) fibres/m^2, and sediments: (b) films/m^2, (d) microbeads/m^2, and (f) fibres/m^2.Sampling occurred from August 11, 2017 to October 23, 2018 (days 7-445). The initial density of ~125 films/m^2, ~60 microbeads/m^2, and ~250 fibres were added to the mesocosms.

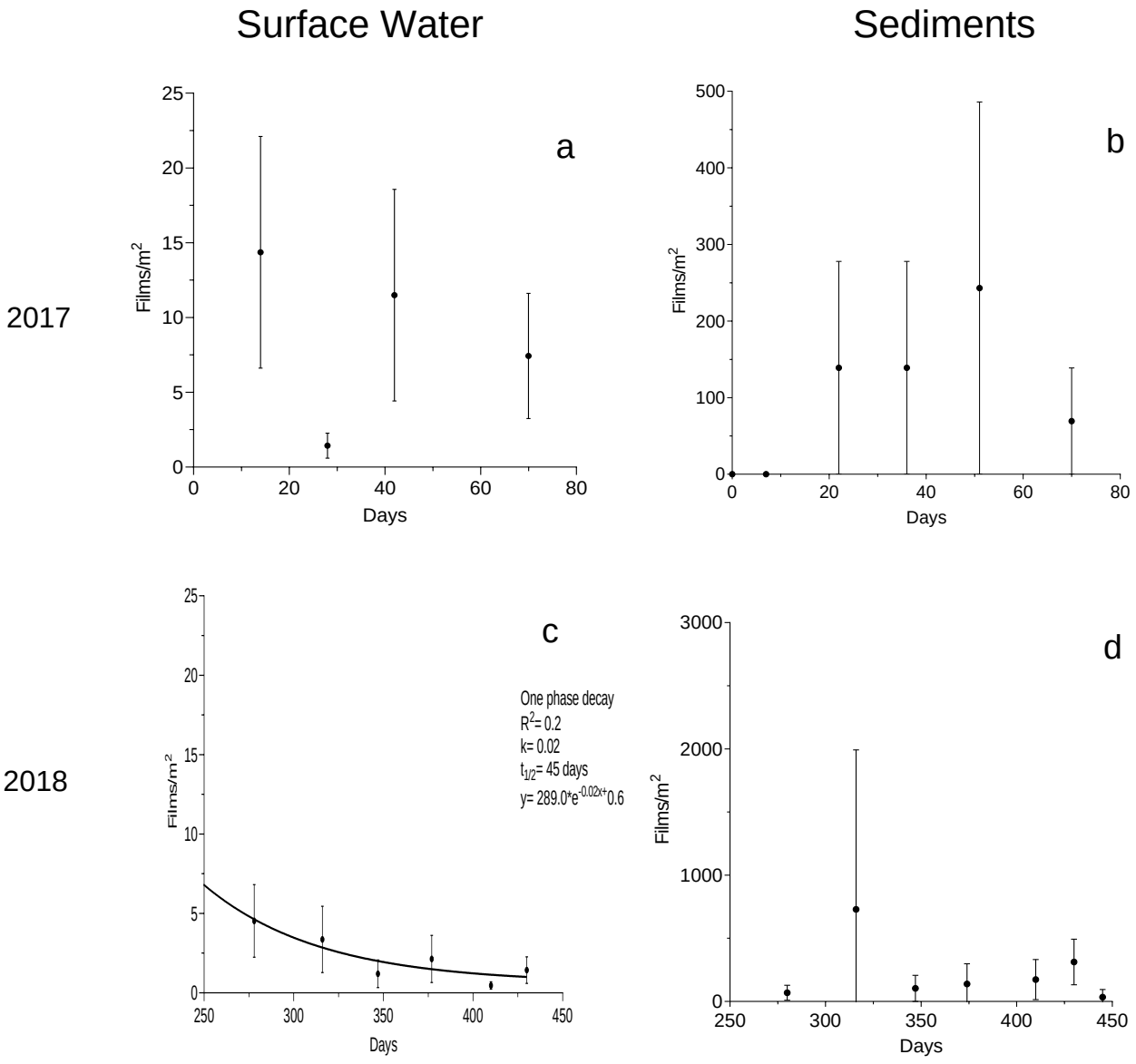


Figure 2.5. Overall trend of mean (±SE) films/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Film/m^2 densities were averaged across replicates (n=3). Film/m^2 densities were measured in 2017 for the both the (a) surface water (day 14 to 70) and (b) sediments (day 7 to 70), and in 2018 for both the (c) surface water (day 278 to 430) and (d) sediments (day 280 to 445). The initial density of ~125 films/m^2 was added to the mesocosms.

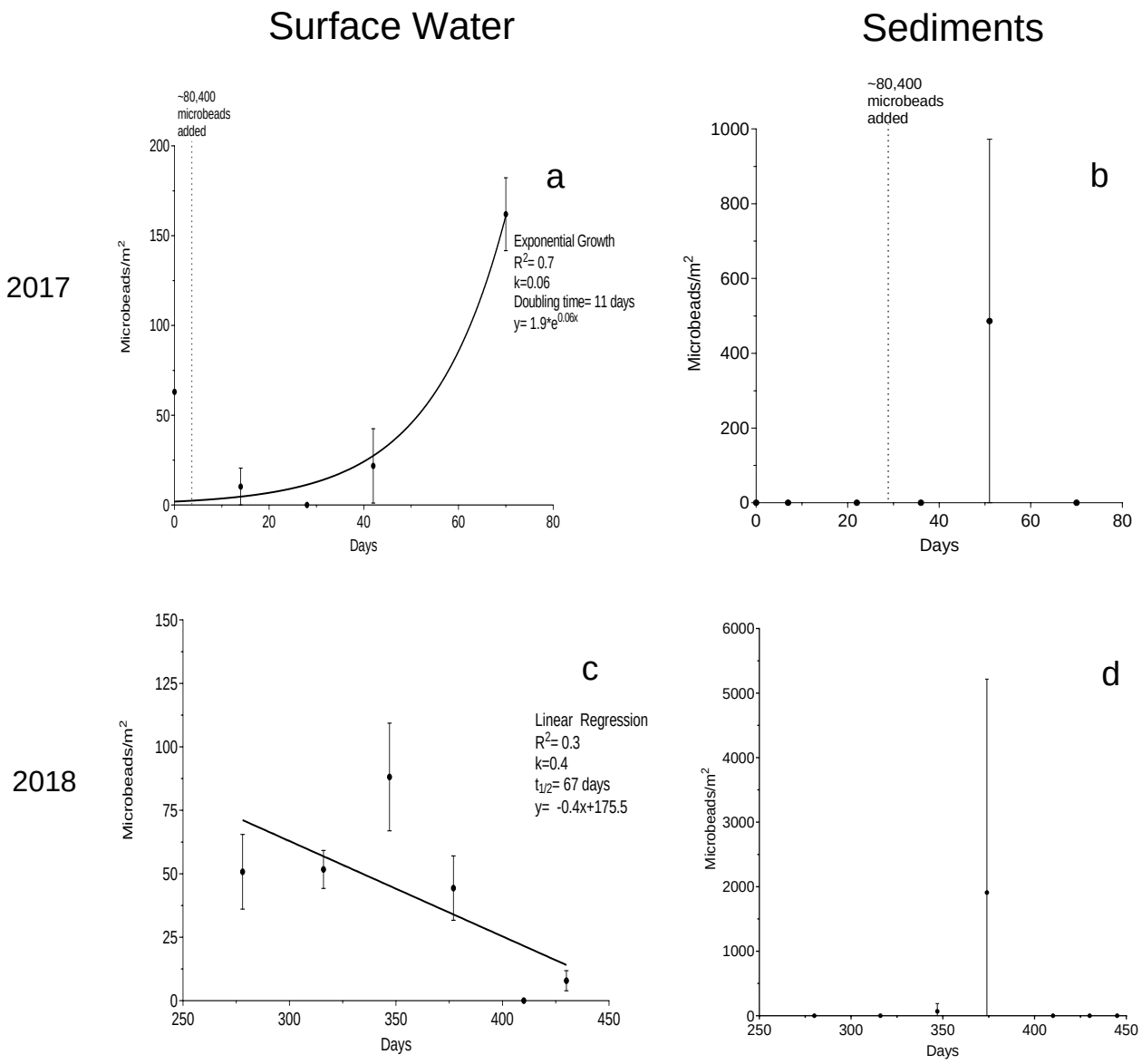


Figure 2.6. Overall trend of mean (±SE) microbeads/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Microbead/m^2 densities were averaged across replicates (n=3). Microbead/m^2 densities were measured in 2017 for the both the (a) surface water (day 14 to 70) and (b) sediments (day 7 to 70), and in 2018 for both the (c) surface water (day 278 to 430) and (d) sediments (day 280 to 445). The initial density of 60 microbeads/m^2 were added to the mesocosms, then a second dosing of ~4600 microbeads/m^2 was added (~4640 microbeads/m^2 in total).

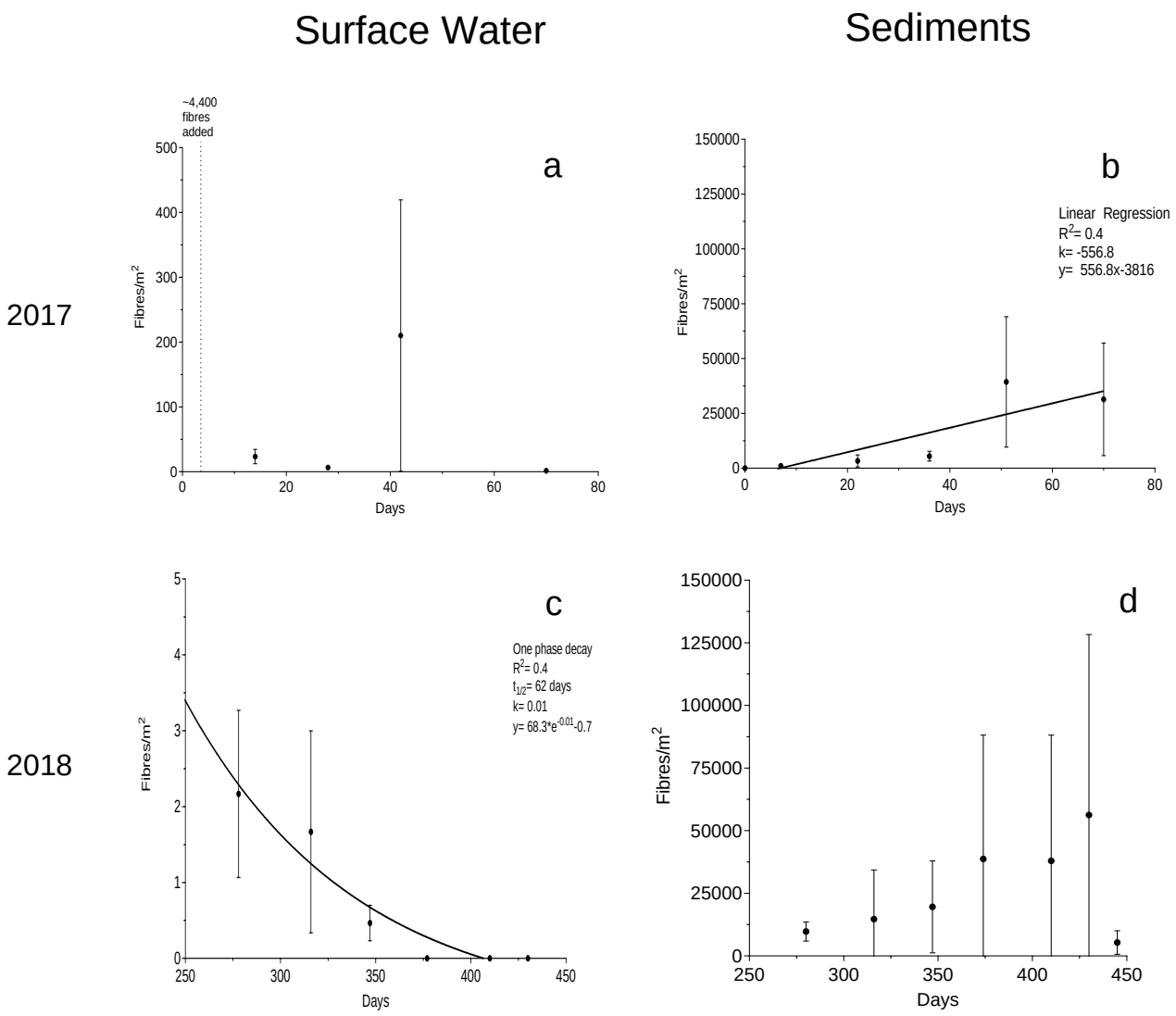


Figure 2.7. Overall trend of mean (±SE) fibres/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Fibre/m^2 densities were averaged across replicates (n=3). Fibre/m^2 densities were measured in 2017 for the both the (a) surface water (day 14 to 70) and (b) sediments (day 7 to 70), and in 2018 for both the (c) surface water (day 278 to 430) and (d) sediments (day 280 to 445). The initial density of 250 fibres/m^2 was added to the mesocosms, then a second dosing of 250 fibres/m^2 was added (~500 fibres/m^2 in total).

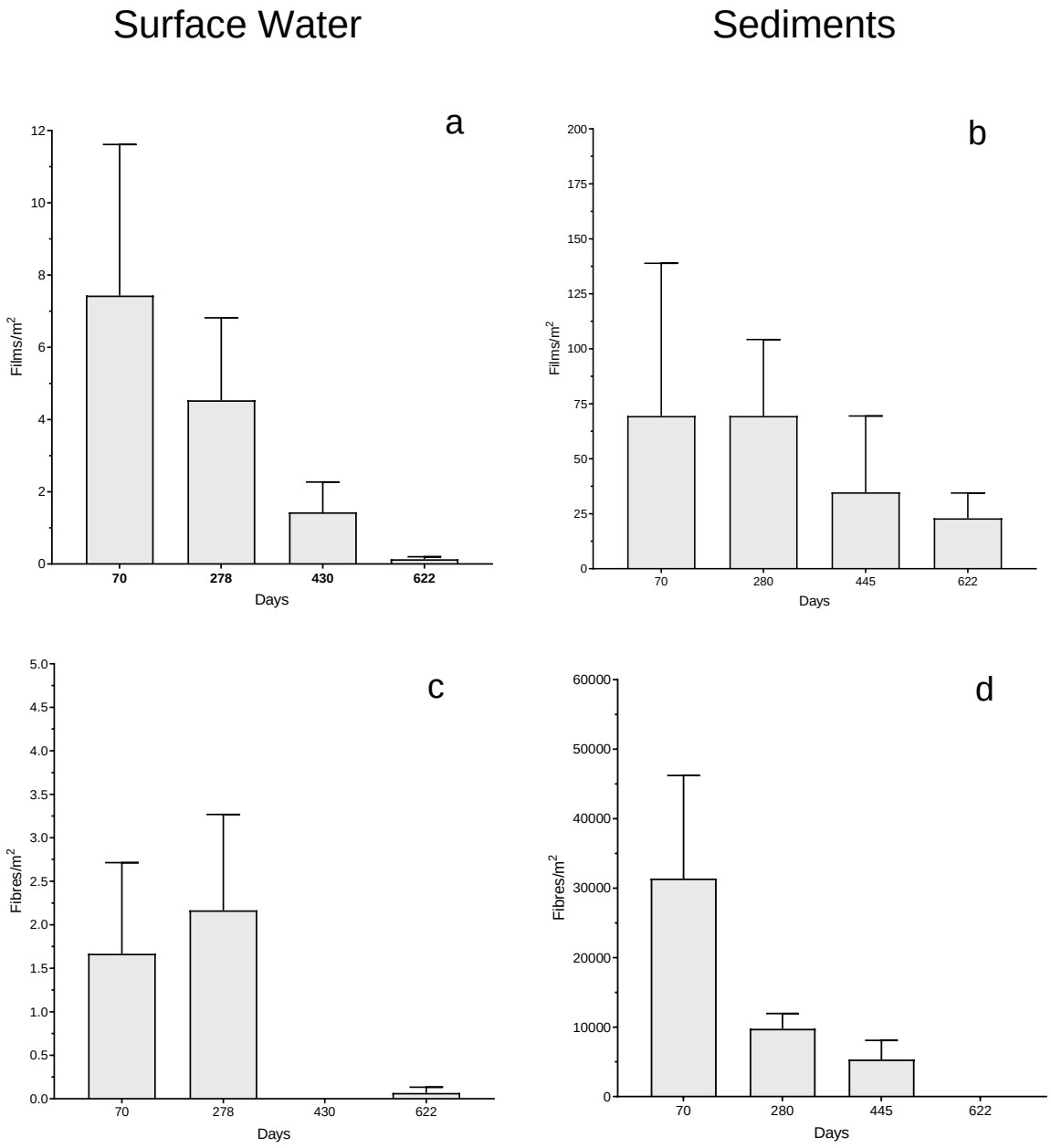


Figure 2.8. Plot of the mean (±SE) of microplastic/m^2 for before (days 70 and 430) versus after (days 278 and 622) ice cover for (a) surface water films/m^2, (b) sediments films/m^2, (c) surface water fibres/m^2, (d) sediments fibres/m^2 of three mesocosms at the Prairie Wetland Research Facility (PWRF). Microplastic/m^2 densities were averaged across replicates (n=3). The period of ice cover on the tanks were from days 71-277 (winter 2017-2018), and days 429-621 (winter 2018-2019).

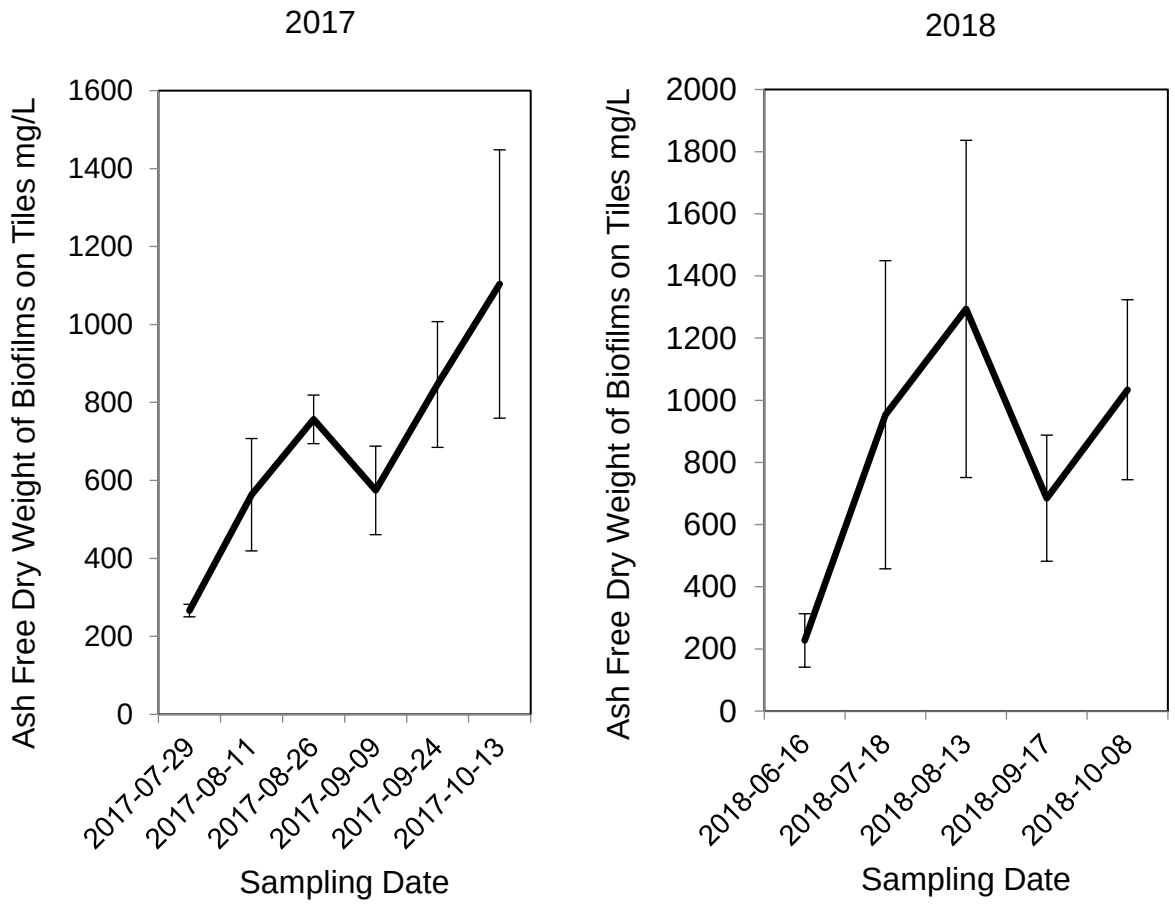


Figure 2.9. Mean(±SE) ash free dry weight (AFDW) densities (mg/L) of biofilm on tiles of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study in 2017 and 2018. AFDW values were averaged across replicates (n=3) for the microplastic treatment. Biofilm densities were measured biweekly from August 3, 2018 to October 8, 2018 by scraping biofilm from ceramic tiles and ashed in a muffle furnace. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

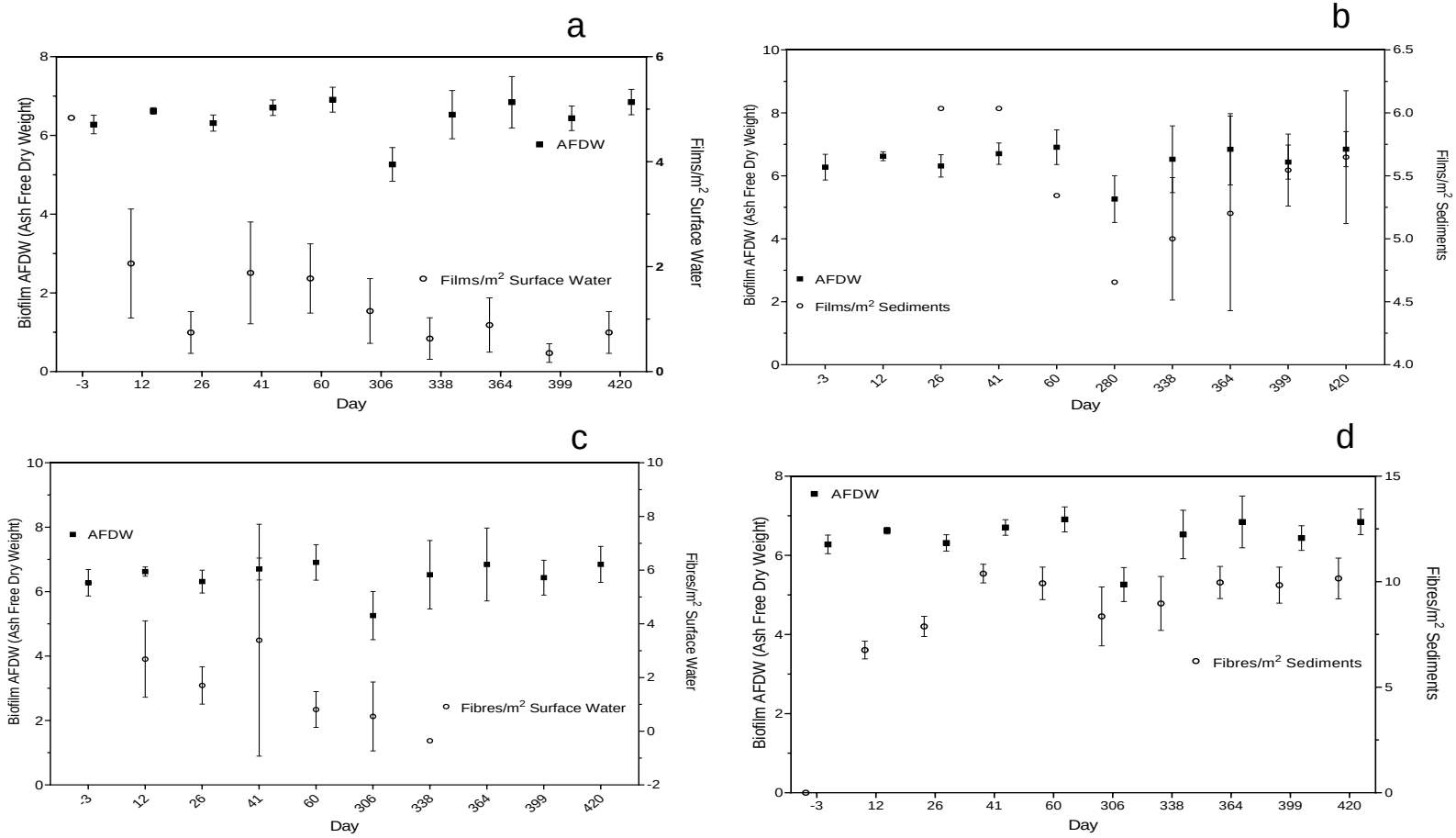


Figure 2.10. Microplastic (second y-axis; shape= open circles) and biofilm (first y-axis; shape= closed squares) densities from August 1, 2017, to September 28, 2018 plotted on a single figure (a) films/m^2 in surface water, (b) films/m^2 in sediments, (c) fibres/m^2 in the surface water, and (d) fibres/m^2 in the sediments.

Chapter 3. Short-term settling dynamics of microplastics with a nutrient addition in model constructed wetlands: a field-based mesocosm study

3.1 Abstract

Microplastics (plastic particles <5.0 mm in diameter) have been detected in freshwater ecosystems worldwide with little understanding on their fate and behaviour in Canadian freshwater systems. Specific knowledge gaps include understanding how ice formation, water quality (i.e., nutrients) and the presence of emergent aquatic plants (e.g. cattails) influences microplastic fate and behaviour. To address these questions, freshwater mesocosms (n=9) were dosed with microplastic films and fibres. The study consisted of three treatments: Control, Nutrient, or Nutrient+Plant treatments with triplicate replicates, monitored over a 72-day open water period, and left to over winter for 179-days. An initial nutrient spike was added to both the Nutrient and Nutrient+Plant treatments, and cattails were established prior to the commencement of the study in the Nutrient+Plant treatment. Film densities in the surface water in all three treatments remained low and relatively stable over time. Film substrate densities decreased over time, appeared to resuspend, and the Control treatment had the fastest resuspension rate, followed by the Nutrient treatment, and lastly the Nutrient+Plant treatment. Fibre behaviour in surface water was not consistent across treatments, as fibres either decreased over time (Control and Nutrient+Plant), or increased (Nutrient treatment). Fibres in the substrate followed a decreasing trend across all three treatments, until fibres either reached a threshold density (Control and Nutrient treatments), or continued to decrease over time (Nutrient+Plant treatment). In substrate, cattails appeared to delay resuspension of microplastics. Both films and fibres settled (>99%) within 14 days, and our results indicate that biofilms were the most likely driver of microplastic deposition/resuspension behaviour based on correlation analysis. Ice formation enhanced film deposition across all three treatments in both the surface water and

substrates. Ice formation had different behavioural effects in each treatment, as both settling and resuspension occurred in both the surface water and substrate. It appears as though fibres are more sensitive to treatment effects (i.e., nutrient and aquatic plants) compared to films following ice formation.

3.2 Introduction

Microplastics (plastic particles <5.0 mm in diameter) have been detected in freshwater ecosystems worldwide. Sources of microplastics into freshwater systems include landfill seepage to groundwater (Environment and Climate Change Canada 2019), sludge applied to agricultural fields (Free et al. 2014; Magnusson et al. 2016), fishing gear (Pruter 1987), and synthetic textiles (Browne et al. 2011; Rillig 2012). Microplastics can enter freshwater ecosystems through pathways based on their originating source. For example, microbeads (primary microplastic) enter primarily via effluent discharge (Browne et al. 2011) and fibres (secondary microplastic) via aerial deposition (Dris et al. 2016). Microplastics have been found in both surface water and sediments of freshwater systems (lakes, rivers, streams) in Canada (Eriksen et al. 2013; Corcoran et al. 2015; Ballent et al. 2016; Anderson et al. 2017; Campbell et al. 2017; Vermaire et al. 2017; Warrack et al. 2017; Helm 2020). Once in freshwater systems, microplastics can undergo a range of abiotic (e.g., degradation, settling, resuspension, aggregation) and biotic (e.g., ingestion, excretion, trophic transfer, bioaccumulation, biofouling) processes and behaviours (Lambert et al. 2014; Anderson et al. 2016; da Costa et al. 2017; Rochman et al. 2019) which ultimately affect their fate.

The fate of microplastic particles, and their behaviour in freshwater systems is not well understood. Microplastic resuspension behaviour can affect where microplastics are transported vertically in the water column (vertically) and aggregation behaviour where abiotic (other microplastics, sediment, rocks), and/or biotic (free floating algal species, or macrophytes) particles cause microplastics to attach together (Long et al. 2015; Lagarde et al. 2016; Long et al. 2017). As aggregation occurs, microplastic density will change depending on the particles within it, which will affect settling dynamics in aquatic systems. Biofilm development on the microplastic particle can also alter the microplastic particle density, potentially enhancing settling and ultimately its fate and behaviour in the water column. Water quality parameters (e.g., nutrients, DO, pH, conductivity, water clarity) can affect the biofilm species composition in freshwater ecosystems (Villeneuve et al. 2013) and potentially therefore the fate and behaviour of microplastics via enhanced or reduced biofouling rates depending on the

water quality of the environment under study. Freshwater systems with high nutrient levels (i.e., phosphorus) are likely to have higher biofilm growth rates (Fang et al. 2009), and microplastics that enter these systems may biofoul and settle more quickly compared to low nutrient freshwater systems.

Few studies to date exist on the interaction of microplastics and aquatic plants in freshwater ecosystems. Microplastic surface texture, morphology and density play an important role in microplastic-plant interactions (Kalčíková 2020), as regulators of potential biofilm growth (Ye and Andrady 1991; Rummel et al. 2017); in turn, biofilms will make microplastics more ‘sticky’, further enhancing their ability to interact and adhere to aquatic plants (Goss et al. 2018). Microplastics have been found to reduce population growth, decrease photosynthetic activity of freshwater microalgae (Lagarde et al. 2016), and have also been found to attach to roots and the underside of duckweed leaves, negatively affecting their growth rates (Bhattacharya et al. 2010). Plants are able to take up microplastics (within smaller size range 0.2 to 0.1 µm) into their root systems and transport them into their tissues via intercellular spaces, and once in their vascular systems, microplastics can be transported from roots to stems and leaves (Li et al. 2019). Emergent macrophytes may enhance settling and sedimentation of microplastics by stabilizing sediments, reducing suspended particle resuspension (including plastic) and turbidity in aquatic ecosystems (Madsen et al. 2001). Emergent macrophytes also can dampen wind effects, further decreasing turbidity and resuspension of suspended particles (including microplastics) within aquatic ecosystems.

This thesis chapter examines the short-term fate and behaviour of microplastics in freshwater ecosystems, and the potential impact of nutrient and plant additions. This work will aid in better understanding the drivers of microplastic settling rates e.g., ice formation effects on microplastics, seasonal settling trends, dynamics of the settled microplastics (i.e. do they stay in the sediment or resuspend), and whether nutrient additions, with or without the presence of aquatic plants enhance settling rates though biofouling and/or enhanced sedimentation of microplastic particles. To investigate this topic, I conducted a mesocosm study at the Prairie Wetland Research Facility (PWRF)

at the University of Manitoba, to understand whether nutrient and plant additions affect microplastic fate and behaviour in freshwater ecosystems. This mesocosm study monitored two microplastics (films and fibres) to determine whether there were treatment effects (nutrient and cattail additions) on microplastic behaviour and settling dynamics through biofouling (biofilm growth as a predictor) and enhanced sedimentation (via cattails), and potential ice formation effects over a 251-day period. Fibres and films were the only microplastic type used in the short-term study.

Objectives for this study:

1. To investigate and evaluate effects, if any, between treatment (control; nutrient and cattail additions) and microplastic behaviour (settling/resuspension rate) of two microplastic types in aquatic mesocosm conditions over a 72-day period at the Prairie Wetland Research Facility at the University of Manitoba.
2. To investigate ice formation and treatment effects, if any, on microplastic densities before versus after ice melt in aquatic mesocosm conditions over a 179-day overwinter period at the Prairie Wetland Research Facility at the University of Manitoba.

Hypotheses:

1. Given that nutrients enhance productivity of aquatic systems, I hypothesize that an initial nutrient addition will stimulate periphyton (biofilm) growth initially (until nutrients are all used up) within the mesocosm, leading to enhanced biofouling and settling rates of the microplastics in both the Nutrient and Nutrient+Plant treatments.
2. Since cattails enhance sedimentation, I hypothesize that the addition of cattails in the Nutrient+Plant treatment will enhance microplastic settling rates relative to the other two treatments, and reduce resuspension.
3. Given that filamentous algal growth will range from low to high densities from the Control, Nutrient to Nutrient+Plant treatments due to the cattails and nutrient addition, I hypothesize that films will resuspend after they have settled the quickest in the Control treatment, intermediate in the Nutrient treatment, and

slowest in the Nutrient+Plant treatment, as the filamentous algae will trap microplastics delaying their resuspension.

4. I hypothesize that fibres will not resuspend, and once fibres settle they will remain in the crushed glass, due to the fact that fibres will form aggregates that settle, trapping the fibres, which are then unable to resuspend.
5. I hypothesize that ice formation will enhance settling of films in both the surface (densities decrease), and in the crushed glass (densities increase), which was the same trend in the long-term study (Chapter 2).
6. I hypothesize that due to microplastics forming aggregates which mainly consist of fibres, there will be treatment effects of fibres after ice formation leading to different settling and resuspension behaviours compared to dynamics prior to ice off.

3.3 Materials and methods

3.3.1 Mesocosm study experimental design

Test facility and mesocosm preparation

A total of nine mesocosms with crushed glass as a substrate were used to evaluate the role of nutrients and plants on the settling rates of microplastics (Appendix B; Figure B1). Each of the nine tanks were randomly assigned to three different treatments (three replicates per treatment) as a Control (microplastics only), Nutrient (synthetic wastewater addition and microplastics), or Nutrient+Plant (synthetic wastewater addition, cattails and microplastics; Figure B1). This study was part of larger study understanding pharmaceuticals in constructed wetlands. All nine tanks were dosed with films and fibres, using the same methods as the long-term study (refer to Chapter 2 section 2.3.1 under *Microplastic additions* for more specific details). Films and fibres were the only microplastics used based on the results from our long-term study, which indicated both had the fastest settling rates, which was desirable as I had a limited time frame (72 days before freeze-up). The long-term study results showed that films were detected in the sediments by day 22 ($t_{1/2}$= 4 days in the surface water), and that fibres were detected in the sediments by day 7 ($t_{1/2}$= 4 days). Foams and fragments

were not detected in the sediments (over 622 days) in our long-term study and therefore were excluded from the short-term study. Microbeads were banned for use in Canada on July 1, 2018 and therefore were no longer relevant to the context of our study, and not used.

The study commenced on August 14, 2018, with 29 days pre-treatment monitoring (water quality parameters and baseline biofilm development) and regular monitoring following the microplastic and nutrient additions on August 14, 2018, which continued until October 25, 2018, after which ice formation limited sampling. Regular monitoring resumed for a final observation in the spring when tanks thawed completely on April 22, 2019. Monitoring of mesocosms consisted of measuring water quality parameters, taking weekly photos of mesocosms, collecting surface and crushed glass substrate samples for microplastics, and removal of a biofilm tile to estimate biofilm development.

Preparation of microplastics

The short-term study was conducted at the Prairie Wetland Research Facility (PWRF), at the University of Manitoba (refer to Chapter 2 section *2.3.1* under *Test facility and mesocosm preparation* for more specific details). This research was part of a larger study to better understand the effectiveness of crushed glass as a substrate in constructed wetlands (see Humeniuk et al. 2019 for more details). A total of nine clean and dry mesocosms were filled to a depth of approximately 30 cm of crushed glass with size of glass particles ranging from 1.5 cm-2.5 cm (May 26, 2018, day -80). Tap water from the City of Winnipeg was used to fill the mesocosms to a volume of approximately 2400 L. No water was added throughout the study, and water levels fluctuated about 0.13 m on average in each tank due to precipitation and evaporation.

Cattails (*Typha* spp.) were planted in the three “Nutrient+Plant” replicates, by placing roots deep into the crushed glass. The cattail shoots were greater than 0.5 meters out of the water at the time of planting. Cattails were placed in the mesocosm at a density of five plants per square metre, for a total of 25 plants per tank. The macrophytes were acclimated in the tanks for 26 days (July 20, 2018) prior to the start

of the study. A large deer fence was also built around the tanks to deter animals from consuming macrophytes and/or drinking out of the mesocosms.

Two microplastic types (fibres and films) were deployed. Films and fibres were sourced and prepared in the same way as in the long-term study (refer to Chapter 2 section *2.3.1* under *Preparation of microplastics* for more specific details). Films (polyester) were plastic post-it flags purchased from a multinational retailer (Table 3.1). The films were pink, orange, yellow, green, and blue in colour. The post-it flags were sliced into squares (<5 mm) by hand using a rotary cutter. Fibres (polyester) were fleece fabric purchased from a multinational sewing store (Table 3.1). The colour of the fibres used in this study was orange, the same material used in the long-term study. During previous analyses of samples taken from Lake Winnipeg and the Red, Assiniboine, and Nelson Rivers (Anderson et al. 2017; Warrack et al. 2017), orange fibres were never encountered and therefore could be differentiated from other colours of fibres (blue, clear, black) that may be introduced via aerial deposition, experimenter clothing, and sample processing. Fibres were sliced off the main fabric by hand using razors and scissors. The fibres were then measured to make sure they were <5mm in length.

The nominal amount of films ~8,575 and fibres ~32,000 (~21,412 added day 0, and ~10,565 added day 7) were estimated by weight (using our density curve; Appendix B; Figure B3 and B6), divided into four equal amounts (as films ~2,140; fibres ~8,000) and placed into separate plastic bags to facilitate dosing (see below). The density of microplastics added to the tanks was higher than would be considered environmentally relevant but given that the focus of this experiment was on fate of microplastics, a high rate of encounter for repeated sampling was required. Densities were chosen based on preliminary results from our long-term study. During the long-term study, films would often stick to the side of the tanks (visual observation) requiring additional particles to counter these potential losses, as well as anticipated sampling error in both the surface waters and crushed glass substrate. A high density of fibres was added to the tanks (triple the amount added in the long-term study) as fibres tended to clump together and were not evenly distributed throughout the tank while dosing, despite best efforts to maintain a homogeneous distribution. This high density of fibres was added to try to

combat the initial heterogeneity in dosing and help with creating a more even distribution for the duration of the experiment.

Microplastic additions

Films were added to the nine mesocosms using the same techniques as the long-term study (refer to Chapter 2 section *2.3.1* under *Microplastic additions* for more details). To dose the tanks, four equal quadrants were made using cotton string, and duct tape to tact the string down were temporarily placed over the tanks. The quadrants were used to ensure microplastics were evenly distributed throughout the tanks. Each quadrant received equal amounts of each of the pre-weighed microplastic type. The microplastics were added by four individuals to each quadrant simultaneously (Figure 3.1). Before fibres were added to each quadrant in a tank, they were added to a Magic Bullet blender, with 250 ml of Milli-Q water, where contents were blended for 15 seconds, then poured into each quadrant a zigzag pattern (Figure 3.1). This method was used to reduce aggregation of fibres that seemed to occur during dosing in the long-term mesocosm study.

A total of ~30,000 microplastics were added on August 14, 2018 (exposure day 0) to each of the nine mesocosms (~8,575 films, and ~21,412 fibres). The mesocosms were dosed again with ~10,565 fibres (each) on August 21, 2018 (day 7), as the density of fibres did not seem to be sufficiently high to evenly distribute them throughout the tank (hetero-aggregation was occurring with some of the fibres), based on qualitative observations. After this second round of additions, each tank contained a total of ~40,552 microplastics (fibres: ~31,977, and films: ~8,575; Table 3.1).

Synthetic wastewater addition

After the microplastics were added, the Nutrient and Nutrient+Plant treatments received one litre of synthetic wastewater, and one litre of secondary wastewater on August 14, 2018 (exposure day 0). The synthetic wastewater contained (per litre): 32.0 g peptone, 19.0 g Lab Lemco powder meat extract, 6.7 g $(NH_4)_2SO_4$, 3.0 g urea, 3.0 g yeast extract, 2.9 g K_2HPO_4, 2.3 g KH_2PO_4, 0.27 g $CaCl_2{\cdot}2H_2O$, and 0.2 g $MgSO_4{\cdot}2H_2O$. One litre of secondary wastewater from Dunnottar, Manitoba

(50°27'16.9"N 96°57'06.5"W) was also added to the mesocosms to provide microorganism colonies that are pre-established within the wastewater from Dunnottar.

3.3.2 Water quality parameters

YSI measurements

A YSI 6600 V2 Sonde (Yellow Springs, OH) was used to measure temperature (˚C), specific conductivity (mS/cm), pH, chlorophyll content (µg/L), and dissolved oxygen (mg/L) which were monitored daily between 8-9:30am, and once a week between 1:30-3:30pm at the same spot in each tank (marked by a piece of duct tape), at a depth of ~0.40 m to characterize fluctuations of water quality throughout the study. Pre-exposure YSI monitoring occurred from July 16, 2018 (day -30) to August 14, 2018 (day 0), and post exposure monitoring occurred from August 15, 2018 (day 1) to October 10, 2018 (day 58).

Photosynthetically active radiation (PAR)

Photosynthetically active radiation (PAR) was measured on clear days (without cloud cover) with an Apogee MQ-200 quantum sensor (in µmol*m^2/s) with an AL-100 sensor levelling plate (Hoskin Scientific, Burlington, ON). Measurements were taken weekly around noon (between 11:45 am to 1:00 pm), at the crushed glass level (the same spot was used each time, marked with a flag) in each mesocosm, and values were rounded to the nearest hundredth. Pre-exposure monitoring occurred on July 27, 2018 (day -19), August 3, 2018 (day -1) and August 10, 2018 (day -5). Post-exposure monitoring occurred after dosing (August 14, 2018; exposure day 0) to September 18, 2018 (day 36). September 18, 2018 was the last day PAR was measured as it was cloudy every other week when trying to monitor.

Filamentous algae

Qualitative filamentous algae assessments were conducted weekly by the investigator, and other trained individuals. Each tank was assessed using a scale of 1 to 3 (1= no algae present, 2= distinct algal masses visible, 3= full algal colonization), to approximate algal growth or productivity (Baxter et al. 2013). Pre-exposure monitoring

occurred July 19, 2018 (day -27) to August 14, 2018 (day 0). Post exposure monitoring occurred after August 14, 2018 (exposure day 0) on August 21, 2018 (day 7) to October 25, 2018 (day 72).

Depth

A total of six depth measurements were taken at random locations within each tank weekly, where average depth was then determined. Depths were used to calculate water volume for the study. Pre-exposure monitoring occurred from July 19, 2018 (day -27) to August 13, 2018 (day -1). Weekly post exposure monitoring occurred after August 14, 2018 (exposure day 0) on August 21, 2018 (day 7) to October 25, 2018 (day 72).

General hardness and alkalinity

General hardness and alkalinity were measured biweekly in the lab using the same integrative sampling technique (Solomon et al. 1982) and methods employed in the long-term study (refer to Chapter 2 section *2.3.2* under *General hardness, and alkalinity* for more details). Pre-exposure monitoring occurred from July 19, 2018 (day -27) to August 3, 2018 (day -11). Biweekly post exposure monitoring occurred after August 14, 2018 (exposure day 0) on August 29, 2018 (day 15) to October 25, 2018 (day 72). Measurements were not taken in the winter October 26, 2018 day 72 to April 17, 2019 day 246), as the mesocosms had ice cover on the surface. As soon as the mesocosms were thawed (0% ice cover), monitoring continued for a final sample on April 18, 2019 (day 247).

Phosphorus

To measure phosphorus levels from the nutrient addition, sterile 250 mL Nalgene HDPE bottles (metal analysis grade) were used to collect water samples using the integrative technique for total phosphorus (TP; for more details refer to Solomon et al. 1982). Zooplankton mesh (200 μm) was placed on the end of the hose, so no microplastics were lost while sampling. To ensure QA/QC, each treatment used its own integrative sampler, which was rinsed with DI water between each use. Integrative

sampling consisted of six grab samples from different depths and places within each mesocosm. Each grab sample (~0.9 L) was poured into a clean plastic bucket (~5.4 L in total). The bottles were filled with 125mL of water and frozen in a freezer (-50ºC). TP samples were collected three times, pre-addition on August 14, 2018 (day 0), post-addition on August 14, 2018 (day 0), and on August 28, 2018 (day 14). The samples were later processed by ALS Environmental lab in Winnipeg, Canada (Appendix C; Table C2). Samples were measured for suspended phosphorus and total dissolved phosphorus which were later totaled for TP.

3.3.3 Surface water microplastic sampling and analysis

Microplastic sampling in the surface waters employed the same methods (quadrants, circular sampler) and sample processing as in the long-term study (refer to Chapter 2 section *2.3.3 Surface water microplastic sampling and analysis* for more details; Figure 2.2 a). Pre-exposure surface water samples were taken on July 19, 2018 (day -27), and post exposure monitoring occurred biweekly August 29, 2018 (day 15) to October 25, 2018 (day 72). Final surface water samples were taken in triplicate and averaged on April 22, 2019 (day 251) after the mesocosms had thawed. Microplastics have not been found to affect the rate of ice growth over a body of water, and the densities used in our study were too low to affect albedo; therefore I can assume microplastics did not alter the rate of ice formation within our study (Geilfus et al., 2019). Surface water samples were processed using the WPO method (Masura et al. 2015).

3.3.4 Crushed glass microplastic sampling and analysis

To detect microplastics in the crushed glass substrate, I first created a random sampling map of the mesocosm before sampling to ensure I did not sample the same area more than once. Using that map, a sinking ring (internal diameter of the ring: diameter= 0.11 m, height= 0.03 m, area= 0.0095 m^2) was tossed into the pre-determined area using the map. A battery-operated aquarium gravel vacuum siphoned the microplastics off the glass substrate within the ring's diameter, which was placed into a one litre mason jar and preserved with 70% ethanol for later processing (Figure 2.3 c). Pre-exposure monitoring occurred in triplicate within each tank, and microplastic

densities were averaged within each tank on July 19, 2018 (day -26). Post-exposure sampling occurred biweekly from August 28, 2018 (day 14) to October 25, 2018 (day 72). Final crushed glass samples were taken in triplicate within each tank and then values were averaged within each tank on April 22, 2019 (day 251) after the mesocosms had thawed. Crushed glass samples were processed using the WPO method (refer to Chapter 2 section *2.3.3 Surface water sampling and analysis* for more details).

3.3.5 Quality assurance and quality control (QA/QC)

QA/QC involved being able to quantify potential microplastic contamination through aerial deposition in the lab, surface water, DI water, and sediment blanks (see below). I also ensured that no investigators wore orange fleece during the study, to eliminate possible contamination sources of fibres. Blanks were only conducted at the beginning of the study to give a sense whether more blanks should have been employed throughout the study. I used specific microplastics (orange fibres and brightly coloured square-shaped films) to make sure I could easily identify the microplastics added to the study, compared to the microplastics introduced through aerial deposition, surface water sampling, DI water, or sediment sampling. The use of specific microplastics enabled me to do minimal QA/QC as all microplastics introduced through sampling and processing were completely different visually from those initially spiked.

Aerial deposition blanks

Two air blanks were employed to understand whether microplastics (same colour and shape as our experiment) were being introduced into our samples while I was processing and enumerating under the dissecting microscope. Two aerial deposition blanks were deployed in the lab by leaving one liter of Milli-Q water in glass mason jars out on the lab counter for 24 hours. After 24 hours, lids were placed on the jars, and the blank was processed in the same way that the other samples were using the WPO method (Masura et al. 2015). Within the two blanks, a total of eight and seven fibres (clear and blue in colour) were introduced over the 24-hour time-period, or 0.3 fibres/hour. Since the average time for sorting of samples under the dissection

microscope was four hours, I estimate that on average 1.25 microplastic particles were introduced from the lab air. Since I used orange-coloured fibres, I can assume fibres from aerial deposition did not influence our microplastic counts.

Surface water blank

Preliminary surface water samples were taken to determine what types of microplastics were already within the system. Floating debris (e.g., paint, films, clear fibres, plastic bottle caps, labels) were found in the crushed glass substrate. Any debris was skimmed from the surface water in each tank prior to the commencement of the experiment. Three surface water blanks were employed for each tank where I used the same sampling method for surface water sampling. Each sampler was rinsed using one litre of Milli-Q water, and visually inspected before the next sample was taken. Pre-exposure surface water blanks were taken on July 19, 2018 (day -27), after the tanks were skimmed initially, in order to characterize any floating debris (plastic particles in the crushed glass) not captured during our clean-up efforts and remained within our mesocosms. Each treatment was assigned its own surface sampler, which helped avoid cross contamination between treatments. Samples were then processed using WPO method (Masura et al. 2015) and enumerated under the dissecting microscope. This characterization was vital to make sure that any microplastics already within the tanks were readily differentiated from the introduced multi-coloured films and orange fibres that were dosed. The introduced films and fibres were easy to identify, as they had unique characteristics; added films were square in shape with straight edges, and added fibres were orange in colour. By contrast, films within the glass debris were oblong shaped, and fibres were red, black, clear or blue in colour. Only films and fibres conforming to our attributes of those added were enumerated.

Deionized water blanks

Four lab blanks were used to determine whether microplastics were being introduced into our samples from DI water during processing in the lab. A total of four DI blanks were conducted. I ran the DI water tap at a rate of 8 L/minute (480 L total) at the University of Manitoba for 60 minutes on a clean 355 µm brass sieve. Any contents

within the sieve were then rinsed into a petri dish, and viewed under a dissecting microscope. Within the four blanks: 13, 5, 16 and 9 fibres (clear and blue in colour) were found. This suggests that on average one microplastic particle (fibre) was introduced for every 48 L of DI water used when processing the samples. The average rinse time of a sample is five minutes with DI water (at 8 L/minute), with reconstitution to 1.25 L prior to subsampling, I can estimate that on average, 0.85 fibres were introduced to our samples, from the DI water alone. Again, orange-coloured fibres were used, I can assume that these fibres did not influence our microplastic counts.

Crushed glass blank

Preliminary substrate samples were taken to determine what types of microplastics were already within the crushed glass substrate before dosing. The pre-microplastic exposure glass substrate blanks were sampled the same way as above, using a battery-operated aquarium gravel vacuum, which siphoned any microplastics off the glass substrate. Samples were taken on July 19, 2018 (day -27), after the tanks were skimmed initially to remove large particulate from the surface that were introduced from the crushed glass. Three crushed glass blanks were employed for each tank by first tossing the sinking ring, then using the aquarium vacuum to siphon any microplastics off the glass substrate within the ring's diameter. The sample was put into a one litre mason jar and preserved with 70% ethanol for later processing. Samples were then processed using WPO method (Masura et al. 2015) and enumerated under the dissecting microscope. There were no films and fibres initially within the tanks (shape, and colour) to those added, therefore corrections were not applied when microplastics were enumerated.

3.3.6 Sampling efficiency

A main assumption in our sampling design was that all microplastics added on day 0 (August 14, 2018) were homogenously distributed throughout the surface water of the tank at all times throughout the study. Visual observations and photos showed a heterogeneous distribution of both films and fibres in the surface water, aggregate formation, adhesion to emergent and submergent plants, and films were also found in

high densities pressed to the edge of tanks where the water and mesocosm meet (Appendix C; Figure C12-C15).

3.3.7 Biofilm sampling and analysis

The same methods for tile deployment to measure biofilm development were employed as in the long-term study (refer to Chapter 2 section *2.3.7 Biofilm sampling and analysis* for more details). The string on the back of the tile (surface area of 0.96 m^2, length 0.098 m and width 0.098 m) was glued to the base of a stake flag. The flags were numbered (1-5), and a random number generator was used to determine which tile was sampled at each biweekly sampling date. The tiles were laid carefully on the bottom of the tank, and the base of the flag was pushed down into the crushed glass, so it stood vertically in the water column. This made it easy to pull the flag (and attached tile) out of the tank (Figure 3.2). One tile per tank was deployed for 14 days on July 20, 2018 (day -25), and taken out of the tank on August 3, 2018 (day -11) to measure preliminary biofilm development. Five tiles were deployed in each tank on August 14, 2018 (day 0), prior to the microplastic dosing. Biweekly sampling occurred after dosing on August 14, 2018 (exposure day 0), from August 28, 2018 (day 14) to October 8, 2018 (day 55). Tiles were frozen and processed for AFDW the same way as in the long-term study (refer to Chapter 2 section *2.3.7 Biofilm sampling and analysis* for more details; Appendix B; Figure B11).

3.3.8 Statistical analysis

All statistical analyses were performed using GraphPad Prism version 9.0.0 for Windows, GraphPad Software, San Diego, California USA. Water quality parameters and biofilm data are presented as mean (±SE), unless otherwise indicated. Formulas and calculations used to determine microplastic/m^2 can be found in Appendix B in Table B1. Trends (mean (±SE)) of microplastic/m^2, and half-life of microplastics were calculated for each microplastic type (film, fibre) using a statistical approaches (see below).

Water quality and biofilm

Trends for each water quality parameter were averaged across replicates (n=3) and means (±SE) were plotted. A two-way ANOVA mixed-effects model (REML; fixed= treatment; random= tank) was used to determine whether treatments had different densities of filamentous algae, and if statistical significance was detected ($p<0.05$), appropriate post hoc tests were conducted (Tukey; Table 3.4). A Pearson correlation analysis ($p<0.1$; due to small sample size) was conducted to determine whether densities or microplastics in either surface waters or sediments were correlated with biofilm densities (AFDW; Table 3.5).

Microplastic kinetics

The microplastic densities across replicates within a treatment were first plotted to determine the overall pattern (e.g., linear or exponential (curved)) upon first inspection. If the data appeared to be linear (visual inspection), then a zero-order linear regression trend was explored as a possible fit. The residual plot was used to determine whether the statistical approach was a "good fit" (e.g., residuals were randomly dispersed, with no apparent trend). If the data were not randomly dispersed in the residual plot or curved, a 1st order semi-logarithmic trend was applied to the data. The natural log (ln) was taken of the densities of microplastics across replicates, and plotted over time. If the data had too many zero values, or did not appear to be a "good fit" (following the above steps), then the data was then plotted using a 1st order exponential one-phase decay:

$$y=y_0\text{-plateau}*e^{-k*x}+\text{plateau} \qquad \text{eq. 3.1}$$

where y_0 is the y-value (density of microplastics) at time zero, k is the slope/rate constant, and plateau is the y-value (density of microplastics) at infinite time. The trend was then inspected visually (as the statistical approach may not fit or be ambiguous) using the residual plot (same steps as above), and a unique statistical approach was then chosen for the each microplastic type for both the surface water and crushed glass substrate. When none of the statistical approaches appeared to accurately capture the behaviour of the microplastic, then no trend was fit, and the behaviour of microplastic

particles was described qualitatively. The average microplastic densities (mean (±SE)) of replicates (n=3) were plotted for 2018 were compared among treatments.

Half-life

Half-lives were used to help determine how both films and fibres behave over time in the three treatments in both the surface water and sediments. When a trend was fit, slope (k), plateau, y_0, and half-life were calculated using the statistical approach (Table 3.2). The equation of the line was then calculated based on the trend using the slope, plateau and y_0 (Table 3.2). Half-lives are often used in chemistry, and are the time required for half of something to decay. I adapted the concept of half-lives for microplastics in our study. A microplastic half-life is the time required for half of the microplastics to settle (microplastics leaving the surface water or accumulate in the sediments, as appropriate), and was used to estimate a settling rate. Similarly, half-lives were used to estimate resuspension rate, i.e., the time required for half the microplastics to resuspend from the substrate or accumulate at the surface (as appropriate).

Treatment effects on microplastic behaviour

To answer the question of whether Nutrient or Nutrient+Plant additions affected the behaviour of microplastics in both the surface water or crushed glass over time, average microplastic densities (mean (±SE)) of replicates (n=3) were plotted for 2018 until the tanks were frozen, and were compared.

Ice formation effects on microplastics

To answer the question whether ice formation alters densities of films and fibres before versus after ice melt in either the surface water or glass substrate, a paired t-test was used (two-tailed; $p<0.05$; Table 3.7).

Treatment effect on microplastic densities after ice formation

To test for differences in microplastic densities among treatments after ice formation a one-way ANOVA was used, and if statistical significance was detected ($p<0.05$), a Tukey post hoc test was conducted (Table 3.8).

3.4 Results

3.4.1. Water quality parameters

Temporal means (±SE) for measured water quality parameters (July to October 2018) can be found in Appendix C (Figures C2 to C10; Table 3.3 and 3.4). Water quality parameters were consistent (except filamentous algae) across treatments and therefore unlikely to have influenced settling/resuspension dynamics of microplastics. Filamentous algae ranged from low in the Control treatment, to intermediate in the Nutrient treatment to high in the Nutrient+Plant treatment. Filamentous algae densities were significantly different ($p<0.05$) in all three treatments (Table 3.4). Total phosphorus (TP) was measured pre-addition, and post-addition (days 0 and 14; Appendix C; Table C2). The nutrient addition increased the TP in both the Nutrient and Nutrient+Plant treatments to ~2 mg/L on day 0. After 14 day, the TP decreased back to its original levels.

3.4.2 Film trends in surface water and crushed glass

Surface water

Trends in surface waters were non-significant, no statistical approaches were fit and patterns were viewed qualitatively (Table 3.6; Figure 3.3 a, c, e). Films in surface waters, in all three treatments followed the same trend where film densities stayed around the same density (large error bars; Table 3.6; Figure 3.3 a, c, e). Film densities were the highest in both the Control (4±1 and, ranged from 3 to 6 films) and Nutrient (4±1, and ranged from 0 to 6 films) treatments, and lowest in the Nutrient+Plant (2±1 and, ranged from 0 to 4 films) treatment. Films were not detected in the surface waters of one replicate in the Control treatment (tank 12), in two of the replicates in the Nutrient

treatment (tanks 2 and 10), and one replicate (tank 3) in the Nutrient+Plant treatment over the course of the study. Some of the overall visual observations for films in surface waters included: found floating by themselves on the surface water throughout the study, few films attached to large fibre hetero-aggregates, films did not appear to form homo-aggregates with other films, and were found frozen in new ice as the surface water froze (Appendix C; Figures C12-C16). Film densities were negatively correlated with biofilm development (AFDW) in both the Control (r=-0.2) and Nutrient treatments (r=-0.2), and positively correlated in the Nutrient+Plant treatments in the surface water (r=0.3) though not statistically significant (Table 3.5).

Crushed glass substrate

Films in crush glass across all three treatments followed a decreasing trend over time all following one-phase decay trends (Table 3.6; Figure 3.3 b, d, f). The half-life calculated was a resuspension rate of films leaving the crushed glass and was the quickest in the Control treatment ($t_{1/2}$= 2 days; k=0.3), intermediate in the Nutrient treatment ($t_{1/2}$=14 days; k=0.05) and slowest in the Nutrient+Plant treatment ($t_{1/2}$=16 days; k=0.04; Table 3.6; Figure 3.3 b, d, f). In all three treatments, films had the greatest densities on Day 14 in the crushed glass (as films initially settled from the surface water quickly), and then crushed glass substrate counts declined exponentially, presumably due to resuspension (Figure 3.3 b, d, f). Films were not found in the crushed glass after day 14 for the Control treatment, day 35 for the Nutrient treatment and day 46 for the Nutrient+Plant treatment. Film/m^2 densities in the crushed glass were the highest on average in both the Nutrient (84±53, and ranged from 0 to 246 films) and Nutrient+Plant (84±53, and ranged from 0 to 281 films) treatments compared to the Control treatment (21±21, and ranged from 0 to 105 films). Films were not found in the crushed glass of one replicate in the Control treatment (tank 7), were detected in all replicates in both the Nutrient and Nutrient+Plant treatments throughout the study. Qualitative observations for films in the crushed glass included: spatial heterogeneity, large hetero-aggregates of films and fibres settled to the bottom of the tanks, films stuck in filamentous algae and films bound in the root system of cattails, and films settling by themselves on crushed glass (Figure C12-15). Densities of films were negatively correlated with biofilm

development (AFDW) in both the Control (r=-0.5) and Nutrient+Plant treatments (r=-0.6) though not statistically significant, and was positively correlated in the Nutrient treatment in the crushed glass (r=0.8) which was statistically significant (Table 3.5).

3.4.3 Fibres/m^2

Surface water

Unlike films, trends of fibres in surface waters were not consistent across the three treatments (Figure 3.4 a, c, e). The Control treatments fit an exponential one-phase decay trend where densities decreased over time (Table 3.6; Figures 3.4 a), and the Nutrient treatment fit a linear regression trend where low densities stayed about the same (large error bars; Table 3.6; Figures 3.4 c). No statistical approach was fit for the Nutrient+Plant treatment, as none considered here could explain the extremely high density on day 72 (Figure 3.4 e). The Control treatment had the fastest surface settling rate ($t_{1/2}$=6 days) followed by the Nutrient treatment which appeared to have a fibre resuspension rate ($t_{1/2}$=14 days; Figure 3.4 a, c). Densities of fibres were the highest on average in the Nutrient+Plant treatment (14±13 and ranged from 1 to 67 fibres) which was due to the one high density of 194 fibres sampled on day 55, which was the highest density of fibres detected in the surface water of all three treatments. The Control had the second highest fibre densities (2±1, and ranged from 0 to 5 fibres), followed by the Nutrient treatment (1±0, and ranged from 0 to 2 fibres), which had the lowest densities. Fibres were detected in the surface waters of all three replicate tanks in all three treatments. Qualitative observations for fibres in the surface water included: spatial heterogenous distribution, fibres formed large homo and hetero-aggregates with other fibres, films and filamentous algae. The aggregates were found either free floating on the surface water, or stuck on stake flags, and frozen within surface water ice layer (Appendix C; Figures C11-16). Densities of fibres in surface waters across all three treatments were significantly correlated with biofilm development (AFDW) in the surface water, negatively in both the Control (r=-0.8) and Nutrient treatments (r=-0.9) and positively in the Nutrient+Plant treatment (r=0.9; Table 3.5).

Crushed glass substrate

In all three treatments, fibres had the highest densities on Day 14 (as fibres settled from the surface water quickly initially), and then fibre densities either reached a threshold (Control and Nutrient treatments; no statistical approach fit; Figure 3.4 b, d), or continued to decrease over time (Nutrient+Plant treatment; one-phase decay trend; Figure 3.4 f). The half-life for all three treatments ranged from quickest in the Control treatment ($t_{1/2}$= 1 day), intermediate in the Nutrient treatment ($t_{1/2}$= 5 days) and slowest in the Nutrient+Plant treatment ($t_{1/2}$= 17 days; Table 3.6; Figure 3.4 b, d, f). Fibres were detected in the crushed glass at a 10,000-fold increase in the Control, 30,000-fold increase in the Nutrient and a 1,500-fold increase in the Nutrient+Plant treatment compared to the surface waters (Figure 3.4 a, b, c, d, e, f). Densities of fibres within the crushed glass were the highest on average in the Nutrient treatment (39,516±9,877, and ranged from 17,362 to 76,570 fibres), intermediate in the Nutrient+Plant treatment (20,680±8,953, and ranged from 2105 to 51315 fibres), and lowest in the Control treatment (17,040±3,006, and ranged from 10523 to 26973 fibres). The Control treatment had the quickest rate of fibre resuspension, ($t_{1/2}$=1 day; k=0.8), followed by the Nutrient treatment ($t_{1/2}$=5 days; k=0.1), and lastly the Nutrient+Plant treatment ($t_{1/2}$=17 days; k=0.04; Table 3.6; Figure 3.4 b, d, f). Qualitative observations for fibres in the glass substrate included: spatial heterogenous distribution, large homo-and hetero-aggregates that had settled and rest on the crushed glass, and clumps of fibres attached to cattail stems and roots (Appendix C; Figures C11-16). Densities of fibres in the glass substrate of all three treatments were non-significantly correlated with biofilm development (AFDW), positively in both the Control (r=0.3) and Nutrient treatments (r=0.6), and negatively correlated in the Nutrient+Plant treatment (r=-0.1; Table 3.5).

3.4.4 Ice formation

Overall trends

Total densities of microplastics (films and fibres) were not significantly affected by ice formation in the surface water ($p>0.05$; Table 3.7), yet in the glass substrate were significantly affected by ice formation ($p=0.004$; Table 3.7).

Film/m^2 trends

Film surface water densities were not significantly affected by ice formation ($p>0.05$; Table 3.7). In the surface water, films in all three treatments followed the same settling trend, as densities decreased after ice melted in the spring by 1.4 films/m^2 (Figure 3.5 a). However, there were no significant effect of treatment on the role of ice formation in the surface water ($p>0.05$; Table 3.8), indicating that losses were equal across all treatments.

Film densities in the glass substrate in all three treatments increased after ice off (Figure 3.5 b). Both the Control and Nutrient treatments had similar film densities in the glass substrate ($p=0.8$; Table 3.8). Films were not detected in the glass substrate in any treatment before ice on (day 72). The Control treatment had the highest density (234 films) of films deposited into the crushed glass after ice melt, followed by the Nutrient treatment (175 films), and lastly the Nutrient+Plant treatment (35 films). Films were detected in all three replicate tanks for all treatments in the spring after the ice melted (day 251). In the glass substrate, film densities were significantly affected by ice formation ($p<0.001$; Table 3.7). There was also a significant treatment effect on film densities in the glass substrate following ice off ($p=0.005$; Table 3.8), where the Nutrient+Plant treatment was significantly different from both the Control ($p=0.006$) and the Nutrient treatments ($p=0.012$; Table 3.8).

Fibres/m^2 trends

Fibres were not affected by ice formation in the surface water ($p>0.05$; Table 3.7), and there were no significant treatment effects on fibre densities after ice formation in the surface water ($p>0.05$; Table 3.8). Fibres were not affected by ice formation in the crushed glass ($p>0.05$; Table 3.7), yet there were significant treatment effects on fibre substrate densities after ice formation ($p<0.1$; Table 3.8).

3.4.5 Biofilm development

The AFDW (mg/L) for the three treatments displayed different trends over time (Figure 3.6). Mean AFDW in the Control treatment remained relatively stable over time,

with only a slight increase in early October, mean (±SE) AFDW was 253±114 mg/L, and ranged from 82 to 699 mg/L (Figure 3.6). Mean AFDW in the Nutrient treatment, increased from August 28, 2018 to September 12, 2018 following the addition of the nutrient addition, then declined to levels similar to the Control treatment, mean (±SE) AFDW was 366±172 mg/L, and ranged from 125 to 1037 mg/L (Figure 3.6). Mean AFDW in the Nutrient+Plant treatment had the greatest densities and highest variability, as AFDW densities increased from the start of the experiment to early October mean (±SE) AFDW was 1233±600 mg/L, and ranged from 461 to 3589 mg/L (Figure 3.6).

Densities of both films and fibres in the surface water followed the same trends with AFDW (though not statistically significant in films but were statistically significant in fibres $p<0.1$) as densities of films and fibres were negatively correlated with AFDW in both the Control and Nutrient treatments, yet positively correlated in the Nutrient+Plant treatment (Table 3.5; Figure 3.6). Densities of both films and fibres were both positively and negatively correlated with AFDW in the sediments (though not statistically significant; Table 3.5; Figure 3.6).

3.5 Discussion

Summary

There were treatment effects on both film and fibre behaviour during the open water season, and after ice formation. Further, both the Nutrient and Nutrient+Plant treatments had higher biofilm densities on average, which disproved my first hypothesis, where I assumed that the nutrients would only enhance biofilm development initially until they were used up (day14), yet biofilm densities in the Nutrient+Plant treatment kept increasing over time until October 2, then densities dropped October 8 likely due to cold water temperatures. In the surface water, films in all three treatments were at low densities throughout. In the glass substrate, films settled quickly, and then densities decreased until films were undetectable, suggesting resuspension back into the water column. Cattails within the Nutrient+Plant treatment appeared to hold onto films during

the open water season delaying resuspension which supported my second hypothesis, where I predicted cattails will enhance sedimentation/ settling of microplastics. Films in the glass substrate of the Nutrient+Plant treatment had the slowest resuspension rate ($t_{1/2}$=16 days) compared to both the Nutrient ($t_{1/2}$=14 days) and Control ($t_{1/2}$=2 days) treatments, which supported with my third hypothesis that both enhanced biofilm development due to the nutrient addition and cattails will delay resuspension of films in both the Nutrient and Nutrient+Plant treatments.

Fibres appeared to behave differently across treatments in the surface water yet were detected in low densities in all three treatments. The overall trend for glass substrate densities was a decrease with time, similar to films, though unlike films, fibres appeared to reach a threshold density in the glass substrate (Control and Nutrient treatments) or decrease over time (Nutrient+Plant treatment). Fibres had the highest glass substrate densities on Day 14 in all three treatments, and fibre resuspension half-lives were quickest in the Control ($t_{1/2}$= 1 day) treatment, intermediate in the Nutrient ($t_{1/2}$= 5 days) treatment, and slowest in the Nutrient+Plant ($t_{1/2}$= 17 days) treatment. Even though fibres appeared to resuspend from the glass substrate, they were not detected in the surface water, so either fibres are resuspending into the water column and do not reach the surface waters, or since fibres form large aggregates they are not resuspending hence the threshold reached in both the Control and Nutrient treatments, which supported my fourth hypothesis, that fibres will form large aggregates and not resuspend.

Ice formation enhanced film deposition in all three treatments, yet treatment had no effect on film behaviour in either the surface water or crushed glass, which supported with my fifth hypothesis that ice formation and subsequent melting will enhance the settling of films. Ice formation led to different treatment effects for fibres in both the surface water and within the crushed glass either delaying resuspension, or enhancing deposition.

Treatment effects on microplastic behaviours

Both the addition of nutrients and the presence of emergent macrophytes affected the behaviour of both films and fibres in the mesocosms. While there were no clear treatment effects on film behaviour in surface waters, the rate of both film and fiber resuspension from the substrates was delayed in the presence of nutrients, with or without plants present, relative to control tanks. Within the crushed glass substrate in both the Control and Nutrient treatments, fibres reached a density threshold during the open water season, and did not appear to resuspend. The Nutrient+Plant treatment appeared to delay film resuspension the longest.

The addition of nutrients likely influenced both film and fibre behaviour within the mesocosms as a result of enhanced biofilm development. Nutrients enhanced both filamentous algae colonization and biofilm development, which in turn likely played a role in delayed resuspension rates of microplastics in both the Nutrient and Nutrient+Plant treatments. Filamentous algae colonization was significantly different among tanks ($p<0.05$: Table 3.4) and densities were higher in both the Nutrient and Nutrient+Plant treatments relative to the Controls, forming a large mat along the crushed glass substrate. The filamentous algae mat would have impacted the vertical transport of films making it harder for microplastics to resuspend and re-enter the water column, hence their slower resuspension rates.

Cattails may have also influenced both film and fibre behaviours in the mesocosms. Cattails appeared to delay the resuspension of films, and make fibres disappear from the crushed glass. Cattails entrained both films and fibres within their sticky root systems, making it harder for the microplastics to re-enter the water column. Emergent macrophytes stabilize sediments, reduce sediment (in our case microplastic) resuspension and turbidity in aquatic ecosystems (Madsen et al. 2001). Plants (lettuce) are also able to uptake microplastics (polystyrene microbeads 0.2 µm in size) from their root systems and transport into plant tissues via intercellular spaces, and once in the vascular systems, transpiration transported microplastics from roots to stems and leaves (Li et al. 2019, 2020). The microbeads formed aggregates (chains and ball-like shapes) within the plant (Li et al. 2019, 2020).

The fact that cattails within our study entrained microplastics, provides potential evidence that the use of floating wetlands may work as a bioremediation technique where microplastics stick to cattail root systems thereby taking microplastics out of the system (United Nations Environment Programme 2017). Constructed wetlands may also be an important tool employed as a tertiary method in WWTP, where 100% of microplastics (Jönsson 2016), and >99.99% (this study) are removed from the surface water. Cattails already may play an important role in nutrient and pollutant removal, and can also be employed as a removal technique for microplastics in aquatic systems thereby reducing their bioavailability.

Aggregation behaviour of films and fibres

Aggregation behaviour occurred throughout the study. Fibres formed homo-aggregates, yet films did not form homo-aggregates. Both films and fibres formed large hetero-aggregates (up to 20 cm measured longest length from end to end) formed of fibres, films, filamentous algae and garbage floating within the mesocosms. These aggregates adhered to anything protruding out of the water (e.g., flags, cattails). Aggregation within this study likely occurred due to our experimental design (i.e., high number of particles dosed). Aggregation may not occur until a critical number of microplastics are added to a system, and it is currently unclear what the critical number is at this time. Aggregation behaviour of both films and fibres was similar to Chapter 2 (long-term study), except the for the size of the aggregates. In the long-term study, the aggregates were quite small and were basically fibre bundles. Microplastic aggregation behaviour within our study is consistent with that observed in other microplastic studies (Lagarde et al. 2016; Long et al. 2017; Alimi et al. 2018; Li et al. 2018; Michels et al. 2018; Cunha et al. 2019).

Microplastic particle collision needs to occur in order for aggregates to form (Michels et al. 2018). Within this study, wind was the likely collision force within our mesocosms. Our mesocosms were set-up in an open field (average wind speed summer of 2018: 20 km/hour and ranged from 0 to 49 km/hour), where it was often windy, and wind conditions created currents within the surface water pushing

microplastics together enabling them to form aggregates. Biofouling of films and fibre likely enhanced aggregation behaviour by making them sticky, which made them able to attach to each other and other suspended particles within the mesocosms. However, reflects natural processes that would occur and influence microplastic behaviour in natural environments.

Aggregation behaviour of microplastics is accelerated by biofilm formation (Michels et al. 2018), and which within this study likely impacted both and resuspension behaviour. Biofouled microplastics are more likely to form aggregates and as more microplastics are biofouled, the aggregate grows in size, increasing settling rates (Michels et al. 2018). Within this study, there was an extreme outlier sampled on day 55 in the surface water where an aggregate of 270 fibres were sampled in tank 11. This was highest density sampled within all treatments throughout the study, and can likely be explained as an aggregate of fibres that was sampled. Since the aggregate was sampled on the surface water, it was likely stuck to something in the surface water and was unable to settle. Aggregation behaviour of fibres may have also enhanced their resuspension behaviour after ice formation, as the larger aggregate broke apart due to ice formation defouling, and algal decay, altering the aggregates buoyancy leading to microplastic resuspension.

Ice formation and subsequent melting effects on microplastics

There were seasonal ice formation effects on both films and fibres, as well as treatment effects on the role of ice formation in the deposition of films in the glass substrate. Ice formation enhanced film settling/deposition (densities decreased in the surface water (not statistically significant) and films had the same behaviour in the long-term study (Chapter 2). Ice formation and subsequent melting enhanced film deposition in the glass substrate (statistically significant), yet films behaved differently in the long-term study as ice formation had no effect in the first winter, and enhanced resuspension (though not statistically significant) in the second winter. There were no statistically significant effects of ice formation in either the surface water or glass substrate and fibre behaviour following ice formation and subsequent melt in the spring. In the surface

water, fibres resuspended (though were not statistically significant) in both the Control and Nutrient+Plant treatments and fibres displayed the same resuspension behaviour in Chapter 2, yet the Nutrient treatment behaved differently as fibres settled. Ice formation enhanced resuspension of fibres from the glass substrate in both the Control and Nutrient treatments and fibres displayed the same resuspension behaviour in Chapter 2, yet

fibres in the Nutrient+Plant treatment settled.

Temperature and density effects of water in the mesocosms after ice formation and melting likely enhanced film deposition. As the water temperature increased and the density of water decreased in the spring, biofilm growth within the mesocosms increased (assuming biofilm growth occurs quickly in less than a week after ice off), which likely led to microplastic's biofouling and particles becoming neutrally and/or negatively buoyant, increasing settling rates. This may have led to lower densities of films (in all treatments) and fibres (Nutrient treatment only) in surface and higher densities in the crushed glass after ice formation. There were significant differences among treatments in glass substrate film deposition before versus after ice formation. The large mat of filamentous algae still remained from summer 2018 after the ice melted in the spring in both the Nutrient and Nutrient+Plant treatments which trapped the films that settled after ice formation and subsequent melting, therefore the two treatments (Nutrient and Nutrient+Plant) had lower densities of films in the glass substrate compared to the Control treatments which did not have a filamentous algae layer blocking film transport within the tanks. Cattails within the Nutrient+Plant treatment likely also entrained the films within their root systems, and also delayed resuspension (evidence during open water season Nutrient+Plant treatment had the longest resuspension half-life), which led to it having the lowest film densities within the crushed glass. The biofilm growth on cattail stems and roots (Pietrangelo et al. 2018) as well as dense filamentous algae growth that remained within mesocosm like a carpet even after senesce from cold winter temperatures were sticky, trapped fibres, likely altering resuspension. These processes i.e., biofilm development, aquatic macrophytes

(cattails), and filamentous algal growth in aquatic systems may all affect microplastic behaviour and their ultimate fate in the environment.

Temperature/density effects of the water, biofilm decay, ice crystal formation, and treatment effects likely led to fibre resuspension within the surface water (Control and Nutrient+Plant treatments) and in the glass substrate (Control and Nutrient treatments). Both biofilm decay and water density changes in the fall may have also enhanced the resuspension of fibres within the mesocosms. As water became cooler in the fall, biofilm on the microplastics likely started to decay. Water also became denser (colder water temperatures), compared to fibres which were now lighter than water, which may have also promoted fibre resuspension back into the water column. Treatment effects also likely affected fibre resuspension behaviour. The Control treatment did not have the initial nutrient addition which led to the large blanket of filamentous algae (lowest densities) forming within both the Nutrient and Nutrient+Plant treatments. In the spring the blanket of filamentous algae on the glass substrate/ water column was still visible in both the Nutrient and Nutrient+Plant treatments, so in the Control treatment there was essentially nothing “holding” fibres within the substrates after ice formation, therefore the fibres resuspended. Fibres also resuspended from the glass substrate in the Nutrient treatment, yet were not detected in the surface waters which was likely dur to the blanket of filamentous algae within the tanks, trapping fibres as they resuspended into the water column. Resuspension of fibres within the surface water of the Nutrient+Plant treatment was likely due to the senescence of cattails. Fibres already stuck on cattail stems near within the water column, were dislodged from cattails in the fall/ early spring as the cattails died, and were longer sticky as the biofilm on their surface decayed. Ice crystal formation may have also driven microplastic resuspension. Ice crystals could form around the fibres within the water column or crushed glass within the tanks as the temperature cooled. As the ice crystal formed around the fibre, it become positively buoyant and floated back to the surface water and froze. This behaviour could account for the decrease in densities in the crushed glass s in the spring. Fibre densities increased in the surface water in the spring, which may be the

result of the fibres frozen within the surface water, awaiting biofouling or aggregation before they can settle again.

To our knowledge, there is no literature to date on how ice formation and subsequent melting might affect microplastics densities. The three treatments within our study demonstrate how both films and fibres could behave with nutrient additions (TP ~2 mg/L), cattails, and within our Canadian climate (over wintering and subsequent freeze-thaw cycle). Films within low nutrient freshwater systems with limited plant growth will likely resuspend quicker compared to systems with higher nutrient levels, and aquatic macrophytes. Treatment did not appear to alter film behaviour (deposition) before versus after ice formation yet did alter fibre behaviour (both deposition and resuspension) in both the surface water and crushed glass. This behaviour provides insight on monitoring best practices, and mass balance within freshwater systems, which should take into account recent ice formation. With decreasing ice cover on lakes due to climate change, there is a higher potential for sediments (and microplastics within them) to resuspend by 30% (Niemistö and Horppila 2007). This will decrease the amount of time microplastics have to bind to the sediments, therefore increasing their potential to resuspend in the spring (Kleeberg et al. 2013; Zhang et al. 2020), prolonging microplastic bioavailability within the water column and complicating the notion that sediments are the ultimate sink for microplastics.

Implications of my study

Films displayed cyclical behaviour during the open water season in the surface water, which was likely driven by biofilm development. Both film and fibre behaviour were affected by the treatments (cattails and nutrient addition). Consistency in our experimental observations suggests that behavioural patterns were likely driven by the nutrient addition which enhanced biofilm development, and filamentous algae growth. Cattails also influenced both film and fibre behaviour delaying film resuspension from the crushed glass, and entraining films and fibres taking them out of the system. The use of cattails as a bioremediation technique as floating wetlands, where microplastics stick to cattail root systems thereby taking microplastics out of the system (United

Nations Environment Programme 2017). Cattails already may play an important role in nutrient and pollutant removal, and can also be employed as a removal technique for microplastics in aquatic systems thereby reducing their bioavailability. Constructed wetlands may also be an important tool employed as a tertiary method in WWTP, where if given the appropriate amount of time, >99.99% (this study) up to 100% (Jönsson 2016), are removed from the surface water. Fibres formed large hetero-aggregates with a few films, which again has the potential to be used as a removal technique in WWTP (Zhang and Chen 2020; Wang et al. 2021).

Ice formation influenced both film and fibre behaviour, which was likely caused by ice crystal formation, and the changing temperature (density) of the water causing both biofilm decay and changes in the particle's buoyancy. Ice formation enhanced settling/deposition of films in both the surface water and crushed glass layer. Fibres behaved differently in each treatment following ice formation in both the surface water and sediments. It appears as though fibres are more sensitive to treatment effects (i.e., nutrient and aquatic plants) compared to films following ice formation.

There are still significant gaps in freshwater microplastic research in particular how microplastics behave in the water column when exposed to nutrient additions, cattails, and as the aquatic systems they enter freeze due to winter ice formation in our Canadian climate. This study has begun to advance our understanding of these processes. Water quality (nutrients) likely enhance microplastic settling via biofilm and filamentous algal growth, and cattails entrain and trap microplastics delaying or even preventing resuspension back into the water column, which ultimately affects microplastic fate.

3.6 References

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., and Tufenkji, N. 2018. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. **52**(4): 1704–1724.

Anderson, J.C., Park, B.J., and Palace, V.P. 2016. Microplastics in aquatic environments: implications for Canadian ecosystems. Environ. Pollut. **218**: 269–280.

Anderson, P.J., Warrack, S., Langen, V., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastic contamination in Lake Winnipeg, Canada. Environ. Pollut. **225**: 223–231.

Andrady, A.L. 2011. Microplastics in the marine environment. Mar. Pollut. Bull. **62**(8): 1596–1605.

Ballent, A., Corcoran, P.L., Madden, O., Helm, P.A., and Longstaffe, F.J. 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. Mar. Pollut. Bull. **110**(1): 383-395.

Barnes, D.K.A., Galgani, F., Thompson, R.C., and Barlaz, M. 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. **364**(1526): 1985–1998.

Baxter, L.R., Sibley, P.K., Solomon, K.R., and Hanson, M.L. 2013. Interactions between atrazine and phosphorus in aquatic systems: effects on phytoplankton and periphyton. Chemosphere **90**(3): 1069–1076.

Bhattacharya, P., Lin, S., Turner, J.P., and Ke, P.C. 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C **114**(39): 16556–16561.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., and Thompson, R. 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. **45**(21): 9175–9179.

Camenen, B. 2007. Simple and general formula for the settling velocity of particles. J. Hydraul. Eng. **133**(2): 229–233.

Campbell, S.H., Williamson, P.R., and Hall, B.D. 2017. Microplastics in the gastrointestinal tracts of fish and the water from an urban prairie creek. Facets **2**(1): 395–409.

Cardinal, P. 2013. Assessing nutrient and pharmaceutical removal efficiency from wastewater using shallow wetland treatment mesocosms. Masters thesis submitted to the University of Manitoba. pp. 1-229.

Cooksey, K.E., and Wigglesworth-Cooksey, B. 1995. Adhesion of bacteria and diatoms to surfaces in the sea: a review. Aquat. Microb. Ecol. **9**(1): 87–96.

Corcoran, P.L., Norris, T., Ceccanese, T., Walzak, M.J., Helm, P.A., and Marvin, C.H. 2015. Hidden plastics of Lake Ontario, Canada and their potential preservation in the sediment record. Environ. Pollut. **204**: 17–25.

da Costa, J.P., Duarte, A.C., and Rocha-Santos, T.A.P. 2017. Microplastics – occurrence, fate and behaviour in the environment. Compr. Anal. Chem. **75**: 1–24.

Cunha, C., Faria, M., Nogueira, N., Ferreira, A., and Cordeiro, N. 2019. Marine vs freshwater microalgae exopolymers as biosolutions to microplastics pollution. Environ. Pollut. **249**: 372–380.

Dietrich, W.E. 1982. Settling velocity of natural particles. Water Resour. Res. **18**(6): 1615–1626.

Dris, R., Gasperi, J., Saad, M., Mirande, C., and Tassin, B. 2016. Synthetic fibers in atmospheric fallout: a source of microplastics in the environment? Mar. Pollut. Bull. **104**(1–2): 290–293.

Environment and Climate Change Canada. 2019. Economic study of the Canadian plastic industry, markets and waste: summary report to Environment and Climate Change Canada. pp. 1-43.

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., and

Amato, S. 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. **77**(1–2): 177–182.

Fang, W., Hu, J.Y., and Ong, S.L. 2009. Influence of phosphorus on biofilm formation in model drinking water distribution systems. J. Appl. Microbiol. **106**(4): 1328–1335.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., and Boldgiv, B. 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. **85**(1): 156–163.

Geilfus, N., Munson, K.M., Sousa, J., Germanov, Y., Bhugaloo, S., Babb, D., and Wang, F. 2019. Distribution and impacts of microplastic incorporation within sea ice. Mar. Pollut. Bull. **145**(June): 463–473.

Georg Mehlhart, M.B. 2012. Study on land-sourced litter(LSL) in marine environments:review of sources and literature in the context of initiative of Declaration of the Global Plastics Associations for Solutions on marine Litter. Öko-Institut eV, Darmstadt/freibg. **49**(0): 30–40.

Goss, H., Jaskiel, J., and Rotjan, R. 2018. Thalassia testudinum as a potential vector for incorporating microplastics into benthic marine food webs. Mar. Pollut. Bull. **135**(May): 1085–1089.

Govender, J., Naidoo, T., Rajkaran, A., Cebekhulu, S., Bhugeloo, A., and Naidoo, S. 2020. Towards characterising microplastic abundance, typology and retention in mangrove-dominated estuaries. Water (Switzerland). **12**(10): 1-24.

Helm, P.A. 2020. Occurrence, sources, transport, and fate of microplastics in the Great Lakes--St. Lawrence river basin. *In* Contaminants of the Great Lakes. *Edited by* J. Crossman and C. Weisener. Springer International Publishing, Cham. pp. 15–47.

Humeniuk, B.W., Wong, C.S., and Hanson, M.L. 2019. Crushed glass as a constructed wetland substrate: invertebrate community responses to simulated wastewater inputs. Proc. Manitoba’s Undergrad. Sci. Eng. Res. **5**(1): 14–27.

Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan,

R., and Law, K.L. 2015. the Ocean. **347**(6223): 3–6.

Jönsson, R., 2016. Mikroplast i dagvatten och spillvatten - Avskiljning i dagvattendammar och anlagda våtmarker (Master Thesis). The Department of Earth Science, Uppsala University. Available from: http://www.diva-portal.org/smash/get/diva2:1049924/FULLTEXT01.pdf.

Kaiser, D., Kowalski, N., and Waniek, J.J. 2017. Effects of biofouling on the sinking behavior of microplastics. Environ. Res. Lett. **12**(12):1-10.

Kalčíková, G. 2020. Aquatic vascular plants – A forgotten piece of nature in microplastic research. Environ. Pollut. **262:** 1-3.

Khatmullina, L., and Isachenko, I. 2017. Settling velocity of microplastic particles of regular shapes. Mar. Pollut. Bull. **114**(2): 871–880.

Kleeberg, A., Freidank, A., and Jöhnk, K. 2013. Effects of ice cover on sediment resuspension and phosphorus entrainment in shallow lakes: combining in situ experiments and wind-wave modeling. Limnol. Oceanogr. **58**(5): 1819–1833.

Kooi, M., Van Nes, E.H., Scheffer, M., and Koelmans, A.A. 2017. Ups and downs in the ocean: effects of biofouling on vertical transport of microplastics. Environ. Sci. Technol. **51**(14): 7963–7971.

Lagarde, F., Olivier, O., Zanella, M., Daniel, P., Hiard, S., and Caruso, A. 2016. Microplastic interactions with freshwater microalgae: hetero-aggregation and changes in plastic density appear strongly dependent on polymer type. Environ. Pollut. **215**: 331–339.

Lambert, S., Sinclair, C., and Boxall, A. 2014. Occurrence, degradation and effect of polymer-based materials in the environment. Rev. Environ. Contam. Toxicol. **227**: 1–53.

Li, L., Yang, J., Zhou, Q., Peijnenburg, W.J.G.M., and Luo, Y. 2020. Uptake of microplastics and their effects on plants. Handb. Environ. Chem. **95**(June): 279–298.

Li, L., Zhou, Q., Yin, N., Tu, C., and Luo, Y. 2019. Uptake and accumulation of microplastics in an edible plant. Kexue Tongbao/Chinese Sci. Bull. **64**(9): 928–934.

Li, S., Liu, H., Gao, R., Abdurahman, A., Dai, J., and Zeng, F. 2018. Aggregation kinetics of microplastics in aquatic environment: complex roles of electrolytes, pH, and natural organic matter. Environ. Pollut. **237**: 126–132.

Lobson, C. 2018. Aquatic insects as a vector for antibiotic resistant gene-bearing bacteria. Masters thesis submitted to the University of Manitoba. pp 1-114.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., and Soudant, P. 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. **175**: 39–46.

Long, M., Paul-Pont, I., Hégaret, H., Moriceau, B., Lambert, C., Huvet, A., and Soudant, P. 2017. Interactions between polystyrene microplastics and marine phytoplankton lead to species-specific hetero-aggregation. Environ. Pollut. **228**: 454–463.

Madsen, J.D., Chambers, P.A., James, W.F., Koch, E.W., and Westlake, D.F. 2001. The interaction between water movement, sediment dynamics and submersed macrophytes. Hydrobiologia **444**: 71–84.

Magnusson, K., Eliasson, K., Fråne, A., Haikonen, K., Hultén, J., Olshammar, M., Stadmark, J., and Voisin, A. 2016. Swedish sources and pathways for microplastics to the marine environment. a review of existing data. IVL Sven. miljöinstitutet (C 183): 1–89. Available from www.ivl.se.

Masura, J., Baker, J., Foster, G., and Arthur, C. 2015. Laboratory Methods for the Analysis of Microplastics in the Marine Environment. NOAA Mar. Debris Progr. **Technical**(July): 1-29.

Van Melkebeke, M., Janssen, C., and De Meester, S. 2020. Characteristics and sinking behavior of typical microplastics including the potential effect of biofouling: implications for remediation. Environ. Sci. Technol. **54**(14): 8668–8680.

Michels, J., Stippkugel, A., Lenz, M., Wirtz, K., and Engel, A. 2018. Rapid aggregation

of biofilm-covered microplastics with marine biogenic particles. Proc. R. Soc. B Biol. Sci. **285**(1885):1-9.

Nguyen, T.H., Tang, F.H.M., and Maggi, F. 2020. Sinking of microbial-associated microplastics in natural waters. PLoS One **15**(2): 1–20.

Niemistö, J.P., and Horppila, J. 2007. The contribution of ice cover to sediment resuspension in a shallow temperate lake: possible effects of climate change on internal nutrient loading. J. Environ. Qual. **36**(5): 1318–1323.

Pichel, W.G., Churnside, J.H., Veenstra, T.S., Foley, D.G., Friedman, K.S., Brainard, R.E., Nicoll, J.B., Zheng, Q., and Clemente-Colón, P. 2007. Marine debris collects within the North Pacific subtropical convergence zone. Mar. Pollut. Bull. **54**(8): 1207–1211.

Pietrangelo, L., Bucci, A., Maiuro, L., Bulgarelli, D., and Naclerio, G. 2018. Unraveling the composition of the root-associated bacterial microbiota of Phragmites australis and Typha latifolia. Front. Microbiol. **9**(AUG): 1–13.

Pruter, A.T. 1987. Sources, quantities and distribution of persistent plastics in the marine environment. Mar. Pollut. Bull. **18**(6 SUPPL. B): 305–310.

Randell, M. 2019. Chitobiase as a surrogate measure of aquatic invertebrate biomass and secondary production in an environmental effects monitoring context. Masters thesis submitted to the University of Manitoba. pp. 1-139.

Rillig, M.C. 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. **46**(12): 6453–6454.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., Frond, H. De, Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., and Hung, C. 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. **38**(4): 703–711.

Rummel, C.D., Jahnke, A., Gorokhova, E., Kühnel, D., and Schmitt-Jansen, M. 2017. Impacts of biofilm formation on the fate and potential effects of microplastic in the aquatic environment. Environ. Sci. Technol. Lett. **4**(7): 258–267.

Solomon, K.R., Smith, K., and Stephenson, G.L. 1982. Depth integrating samplers for use in limnocorrals. Hydrobiologia **94**(1): 71–75.

United Nations Environment Programme. 2017. UN Environment promotes environmentally sound practices globally and in its own activities . Our distribution policy aims to reduce UN Environment ' s carbon footprint . *In* Frontiers. pp 1-92.

Vanderpont, A.K. 2018. Otoliths as indicators of trace element exposure in freshwater fish: a mesocosm experiment with manganese and an examination of hydro-impoundment on otolith trace element signatures. Masters thesis submitted to the University of Manitoba. pp. 1-360.

Vermaire, J.C., Pomeroy, C., Herczegh, S.M., and Haggart, O. 2017. Microplastic abundance and distribution in the open water and sediment of the Ottawa River, Canada, and its tributaries. Facets **2**: 301–314.

Villeneuve, A., Montuelle, B., Pesce, S., and Bouchez, A. 2013. Environmental river biofilms as biological indicators of the impact of chemical contaminants. *In* Encyclopedia of Aquatic Ecotoxicology. *Edited by* J.-F. Férard and C. Blaise. Springer Netherlands, Dordrecht. pp. 443–456.

Waldschläger, K., and Schüttrumpf, H. 2019. Effects of particle properties on the settling and rise velocities of microplastics in freshwater under laboratory conditions. Environ. Sci. Technol. **53**: 1958–1966.

Wang, X., Bolan, N., Tsang, D.C.W., Sarkar, B., Bradney, L., and Li, Y. 2021. A review of microplastics aggregation in aquatic environment: Influence factors, analytical methods, and environmental implications. J. Hazard. Mater. **402**(March 2020): 123496.

Warrack, S., Challis, J.K., Hanson, M.L., and Rennie, M.D. 2017. Microplastics flowing into Lake Winnipeg: densities, sources, flux, and fish exposures. Proc. Manitoba's

Undergrad. Sci. Eng. Res. **3**: 5–15. University of Manitoba Libraries.

Wright, S.L., Thompson, .C., and Galloway, T.S. 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. **178**: 483–492.

Ye, S., and Andrady, A.L. 1991. Fouling of floating plastic debris under Biscayne Bay exposure conditions. **22**(12).

Zhang, Y., Liang, J., Zeng, G., Tang, W., Lu, Y., Luo, Y., Xing, W., Tang, N., Ye, S., Li, X., and Huang, W. 2020. How climate change and eutrophication interact with microplastic pollution and sediment resuspension in shallow lakes: a review. Sci. Total Environ. **705**: 135979.

Zhang, Z., and Chen, Y. 2020. Effects of microplastics on wastewater and sewage sludge treatment and their removal: A review. Chem. Eng. J. **382**(July 2019): 122955.

Zhiyao, S., Tingting, W., Fumin, X., and Ruijie, L. 2008. A simple formula for predicting settling velocity of sediment particles. Water Sci. Eng. **1**(1): 37–43.

3.7 Tables

Table 3.1. Microplastic type, shape, colour, size, polymer, source, photo, number added, and density for microplastics added to mesocosm tanks in both the long-term and short-term study.

Morphology	Shape	Colour	Size (mm)	Polymer	Source	Photo	Number added	MP/L	MP/m^2
Film	Square	blue, yellow, orange, pink, green	<5	polyvinyl chloride	Post-it tags		31,977	4	602
Fibres	Line	orange	<5	thermoplastic polyester	Fleece fabric		8,575	3	488
						Total	40,522	7	1,090

Table 3.2. Kinetics of microplastics using two statical approaches (linear regression and exponential one-phase decay), to calculate: equation of line, half-life, and slope.

Kinetics	**Statistical Approach**	**Equation of line (y=mx+b)**	**Half-life ($t_{1/2}$)**	**Slope**
Zero order	Linear regression	$[A]=-kt+[A]_0$	$t_{1/2}= [A]_0/2*k$	-k
1st order	Exponential one-phase decay	$[A]=[A]_0*e^{-kt}$	$t_{1/2}=Ln(2)/k$	-k

Table 3.3. Mean (±SE), and range of water quality parameters for the three treatments: Control, Nutrient and Nutrient+Plant (n=3) over a 72-day period at the Prairie Wetland Research Facility (PWRF) in the short-term study.

Water quality parameter	Treatment	Mean (±SE)	Minimum	Maximum
Temperature (℃)	Control	14.9±0.8	2.5	24.8
	Nutrient	15.3±0.8	3.2	24.9
	Nutrient+Plant	15.4±0.8	3.5	25
pH	Control	9.7±0.02	9.5	10.1
	Nutrient	9.3±0.03	8.6	9.9
	Nutrient+Plant	9.1±0.03	8.4	9.6
Dissolved Oxygen (mg/L)	Control	10.2±0.3	5.1	14.4
	Nutrient	10.2±0.4	1.4	18
	Nutrient+Plant	9.3±0.4	1.3	14.6
Chlorophyll-a (µg/L)	Control	15.3±0.7	5.4	24.3
	Nutrient	17.6±3.7	3.4	136.6
	Nutrient+Plant	10.4±1.7	2.3	67.8
PAR (µmol/m^2/s)	Control	600±0	400	800
	Nutrient	700±100	400	900
	Nutrient+Plant	500±100	200	900

Table 3.3. Continued.

Water quality parameter	Treatment	Mean (±SE)	Minimum	Maximum
Depth (cm)	Control	42±1	35	48
	Nutrient	43±1	36	48
	Nutrient+Plant	42±1	36	48
General Hardness (mg/L)	Control	143±17	47	193
	Nutrient	183±18	73	233
	Nutrient+Plant	157±18	53	200
Alkalinity (mg/L)	Control	66.3±8	23	103
	Nutrient	87±15	33	167
	Nutrient+Plant	65±7	30	93
Conductivity (uS/cm)	Control	546±6	430	616
	Nutrient	512±5	428	592
	Nutrient+Plant	539±8	405	651
Filamentous Algae	Control	1.02±0.01	1	1.2
	Nutrient	1.4±0.1	1	2.8
	Nutrient+Plant	1.9±0.1	1	2.7

Table 3.3. Continued.

Water quality parameter	Treatment	Mean (±SE)	Minimum	Maximum
AFDW (mg/L)	Control	239±94	82	699
	Nutrient	343±142	125	1037
	Nutrient+Plant	1092±510	383	3589

Table 3.4. Two-way ANOVA mixed-effects model (REML; fixed effect= treatment, and random effect=tank), for filamentous algae in nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Replicate filamentous algae assessment values (n=3) were taken for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made using a scale of 1 to 3 (1= no algae present, 2= distinct algal masses visible, 3= algal colonization), to approximate algal growth or productivity (Baxter et al. 2013). Measurements were made weekly from July 19, 2018 to October 25, 2018, and after ice melted on April 18, 2019.

	p-value	**Statistically Significant**	**F**	**DFn**	**DFd**	**Post hoc**	**Treatments**	**p-value**	**Statistically Significant**	**q**	**DF**
Filamentous Algae	p=0.03	Yes	6.62	2	6	Tukey	Control versus Nutrient	p=0.009	Yes	4.85	16
							Control versus Nutrient+Plant	p<0.0001	Yes	8.81	16
							Nutrient versus Nutrient+Plant	p=0.002	Yes	5.78	16

Table 3.5. Comparison between transformed (ln(x+1)) densities of microplastics (films/m^2 and fibres/m^2) in the surface water or sediments and transformed (ln(x+1)) AFDW across all time periods (days 14, 29, 44, 49 and 55) within each treatment (n=3 replicates) using a correlation matrix. Correlation was considered statistically significant when $p<0.1$.

Treatment	Microplastic Type	Location	Pearson r	p-value	Statistically Significant
Control	Films/m^2	Surface Water	-0.2	0.8	No
	Films/m^2	Crushed glass	-0.5	0.3	No
	Fibres/m^2	Surface Water	-0.8	0.1	Yes
	Fibres/m^2	Crushed glass	0.3	0.6	No
Nutrient	Films/m^2	Surface Water	-0.2	0.8	No
	Films/m^2	Crushed glass	0.8	0.08	Yes
	Fibres/m^2	Surface Water	-0.9	0.03	Yes
	Fibres/m^2	Crushed glass	0.6	0.2	No
Nutrient+Plant	Films/m^2	Surface Water	0.3	0.6	No
	Films/m^2	Crushed glass	-0.6	0.3	No
	Fibres/m^2	Surface Water	0.9	0.03	Yes
	Fibres/m^2	Crushed glass	-0.1	0.9	No

Table 3.6. Summary of statistical approach, microplastic behaviour (resuspension or settling), half-life ($t_{1/2}$), equation of line, slope, R^2, and ice off pattern (resuspension or settling) of both films and fibres in nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Microplastic densities were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant).

Microplastic type	**Surface Water or Glass Substrate**	**Treatment**	**Statistical Approach**	**Resuspension or settling**	**R^2**	**Equation of Line**	**Slope (k)**	**$t_{1/2}$ (days)**	**Ice off pattern**
Films	Surface Water	Control	No fit	Settling					Settling
Films	Surface Water	Nutrient	No fit	Settling					Settling
Films	Surface Water	Nutrient+ Plant	No fit	Settling					Settling
Films	Glass substrate	Control	One-phase decay	Resuspension	0.6	$y=7236*e^{-0.3x}$	0.3	2	Settling
Films	Glass substrate	Nutrient	One-phase decay	Resuspension	0.2	$y=504.5*e^{-0.05x}$	0.05	14	Settling
Films	Glass substrate	Nutrient+ Plant	One-phase decay	Resuspension	0.7	$y=582.2*e^{-0.04x}-37.9$	0.04	16	Settling
Fibres	Surface Water	Control	One-phase decay	Settling	0.7	$y=26.7*e^{-0.1x}+0.5$	0.1	6	Resuspen-sion
Fibres	Surface Water	Nutrient	Linear	Resuspension	0.2	$y=0.02x+0.2$	0.02	14	Settling
Fibres	Surface Water	Nutrient+ Plant	No fit	Settling					Resuspen-sion

Table 3.6. Continued.

Microplastic type	Surface Water or Glass Substrate	Treatment	Statistical Approach	Resuspension or settling	R^2	Equation of Line	Slope (k)	$t_{1/2}$ (days)	Ice off pattern
Fibres	Glass substrate	Control	No fit	Settling	0.2	$y=577560451*e^{-0.8x}+14556$	0.8	1	Resuspension
Fibres	Glass substrate	Nutrient	One-phase decay	Settling	0.2	$y= 282413* e^{-0.1x}+29154$	0.1	5	Resuspension
Fibres	Glass substrate	Nutrient+ Plant	One-phase decay	Resuspension	0.5	$y=90070*e^{-0.04x}$	0.04	17	Settling

Table 3.7. Comparison of densities of films/m^2 and fibres/m^2 before versus after ice-off winter 2018-2019 (before= last sampling day before freeze-up, and after= first sampling date after ice melt), using a paired t-test, across all treatments. Data were transformed (ln(x+1)), and variation within the treatment was considered statistically significant when $p<0.05$. All treatments were combined.

Microplastic Type	**Location**	**p-value**	**One or two tailed**	**Statistically Significant**	**t**	**DF**
Films/m^2 and Fibres/m^2	Surface Water	p=0.99	Two-tailed	No	0.01	17
Films/m^2 and Fibres/m^2	Crushed glass	p=0.004	Two-tailed	Yes	3.28	17
Films/m^2	Surface Water	p=0.62	Two-tailed	No	2.163	8
Films/m^2	Crushed glass	p<0.0001	Two-tailed	Yes	15.46	8
Fibres/m^2	Surface Water	p=0.25	Two-tailed	No	1.242	8
Fibres/m^2	Crushed glass	p=0.44	Two-tailed	No	0.82	8

Table 3.8 Comparison of treatments and microplastic densities of films/m^2 and fibres/m^2 before minus after ice-off winter 2018-2019 (before= last sampling day before freeze-up, and after= first sampling date after ice melt), using a one-way ANOVA, and Tukey post hoc test. Data was transformed (ln(x+1)), and variation within the treatment was considered statistically significant when $p \leq 0.1$.

Microplastic Type	Location	Statistical Test	p-value	Statistically Significant	F (DFn, DFd)	Treatment	P-value	q, DF
Films/m^2	Surface Water	One-way ANOVA	p=0.67	No	0.43 (2, 6)			
Films/m^2	Crushed glass	One-way ANOVA	p=0.005	Yes	14.75 (2, 6)	Control vs Nutrient	p=0.8	0.21, 6
						Control vs Nutrient+Plant	p=0.006	7.1, 6
						Nutrient vs Nutrient+Plant	p=0.012	6.14, 6
Fibres/m^2	Surface Water	One-way ANOVA	p=0.56	No	0.63 (2, 6)			
Fibres/m^2	Crushed glass	One-way ANOVA	p=0.10	Yes	3.45 (2,6)	Control vs Nutrient	p=0.6	1.51, 6
						Control vs Nutrient+Plant	p=0.3	2.20, 6
						Nutrient vs Nutrient+Plant	p=0.08	3.71, 6

3.8 Figures

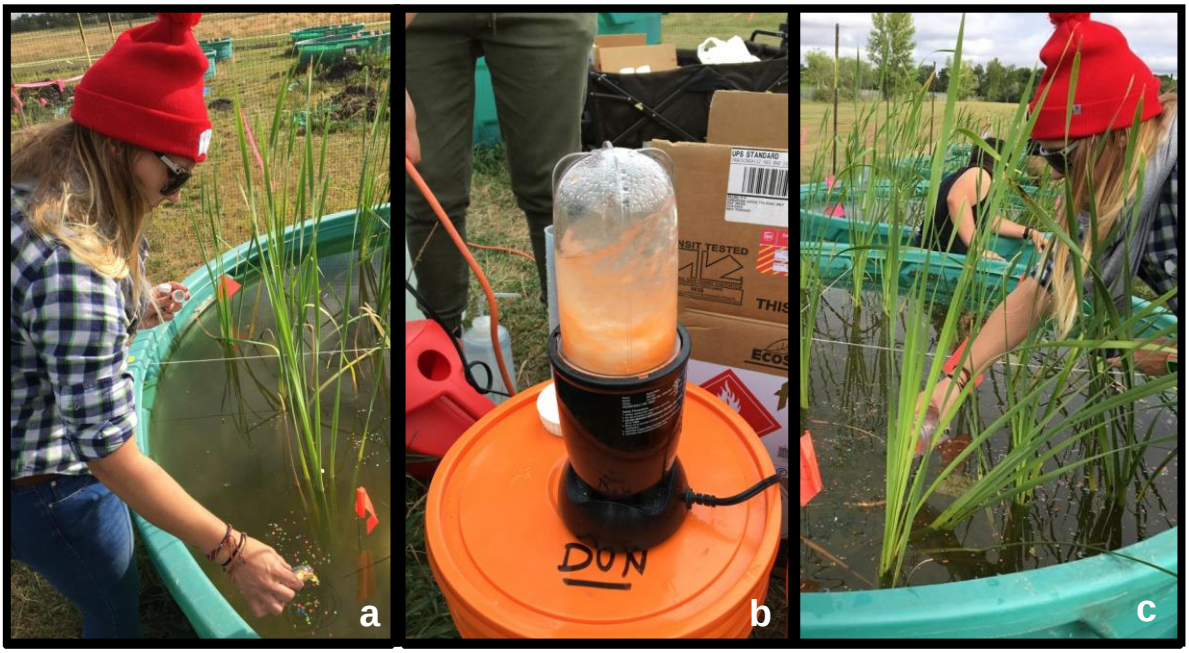


Figure 3.1. Short-term 2018 mesocosm study dosing on August 14, 2018. (a) Films sprinkled into quadrant. (b) Fibres were added to 250 ml of Milli-Q and blended in a magic bullet to ensure even dispersion in the tanks. (c) Fibres being dispersed in a zigzag pattern.

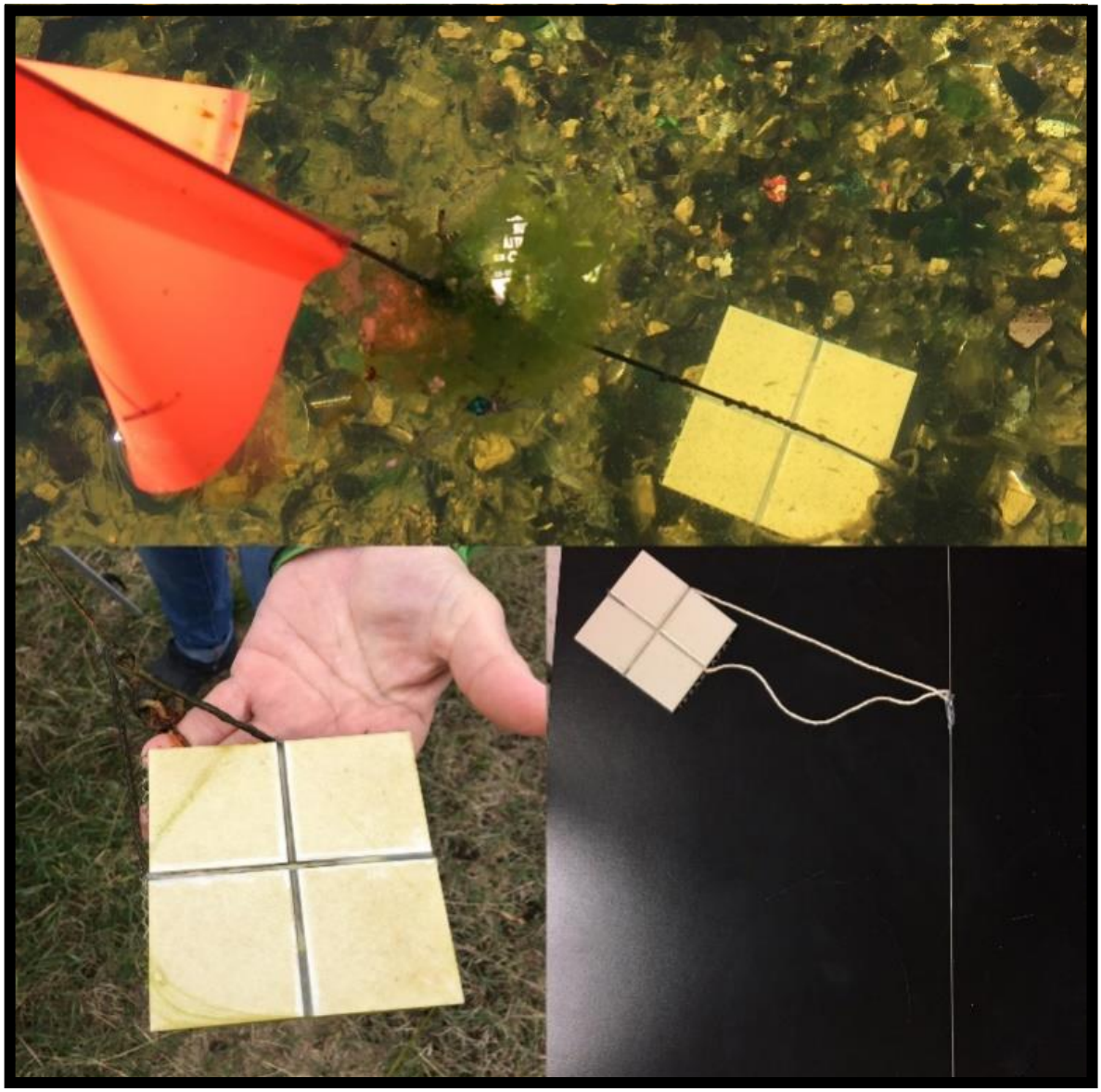


Figure 3.2 Ceramic tiles used in the short-term study. The string on the tiles were glued to a flag and deployed on the bottom of the tank.

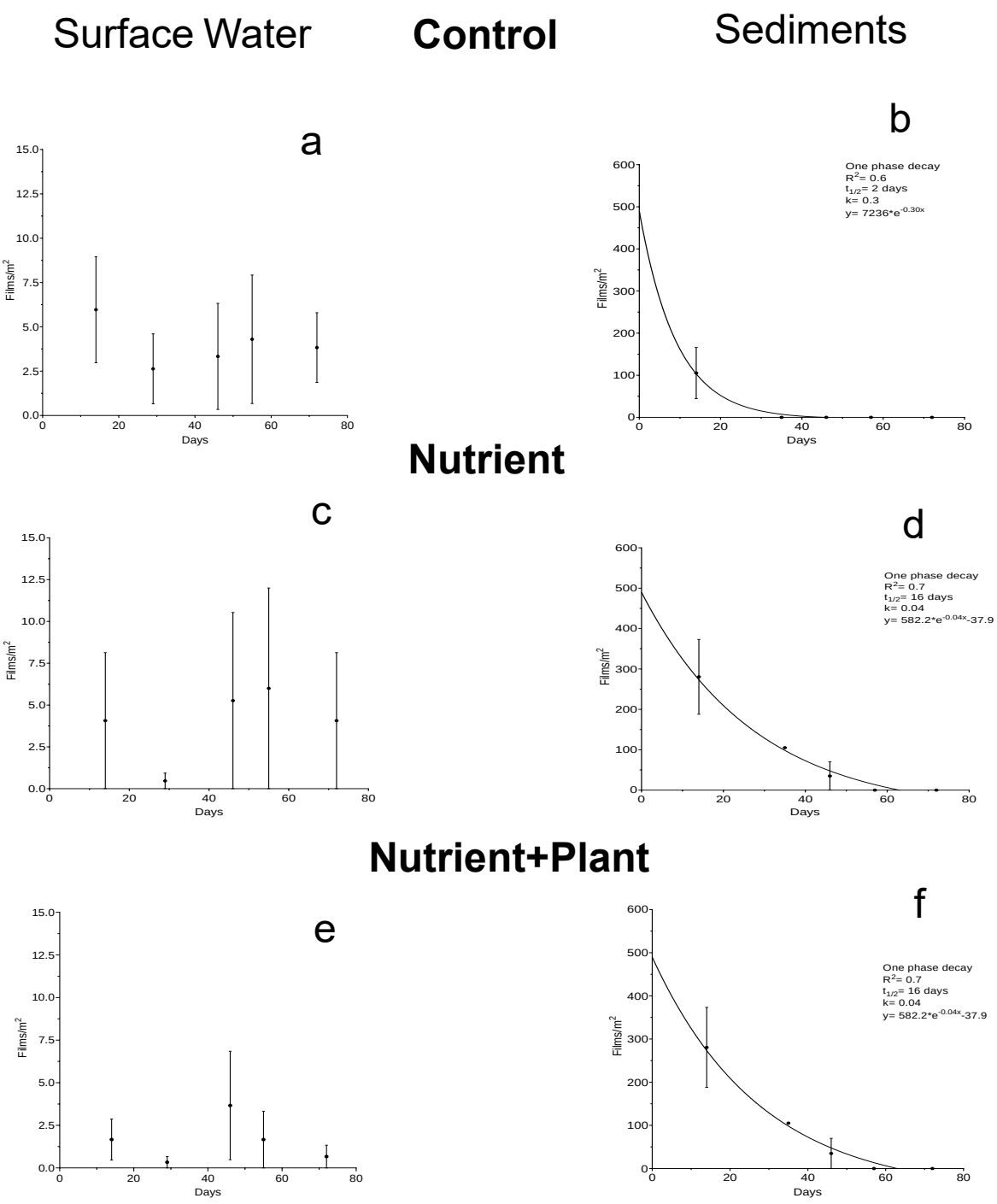


Figure 3.3. Overall trend of mean (±SE) films/m^2 of nine mesocosms at the Prairie Wetland Research Facility (PWRF). Film/m^2 densities were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Film/m^2 densities were measured biweekly in the surface water (a) Control, (c) Nutrient, (e) Nutrient+Plant and in the sediments (b) Control, (d) Nutrient, (f) Nutrient+Plant (from August 28, 2018 to October 25, 2018 (days 14-72). The initial density of films/m^2 added to the mesocosm was ~490.

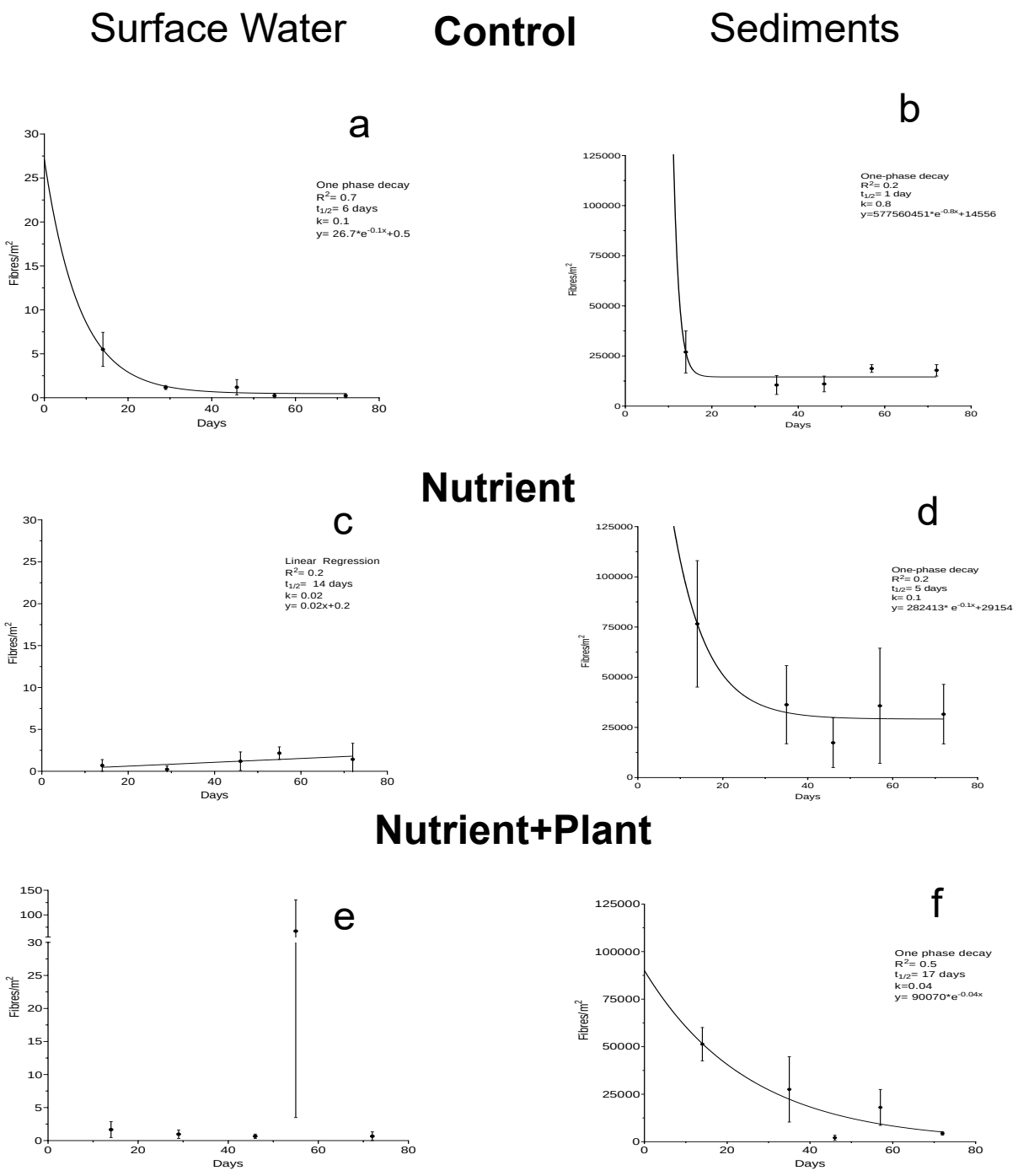


Figure 3.4. Overall trend of mean (±SE) fibres/m^2 of nine mesocosms at the Prairie Wetland Research Facility (PWRF). Fibre/m^2 densities were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Fibre/m^2 densities were measured biweekly in the surface water (a) Control, (c) Nutrient, (e) Nutrient+Plant and in the sediments (b) Control, (d) Nutrient, (f) Nutrient+Plant (from August 28, 2018 to October 25, 2018 (days 14-72). The initial density of fibres/m^2 added to the mesocosms was ~1200.

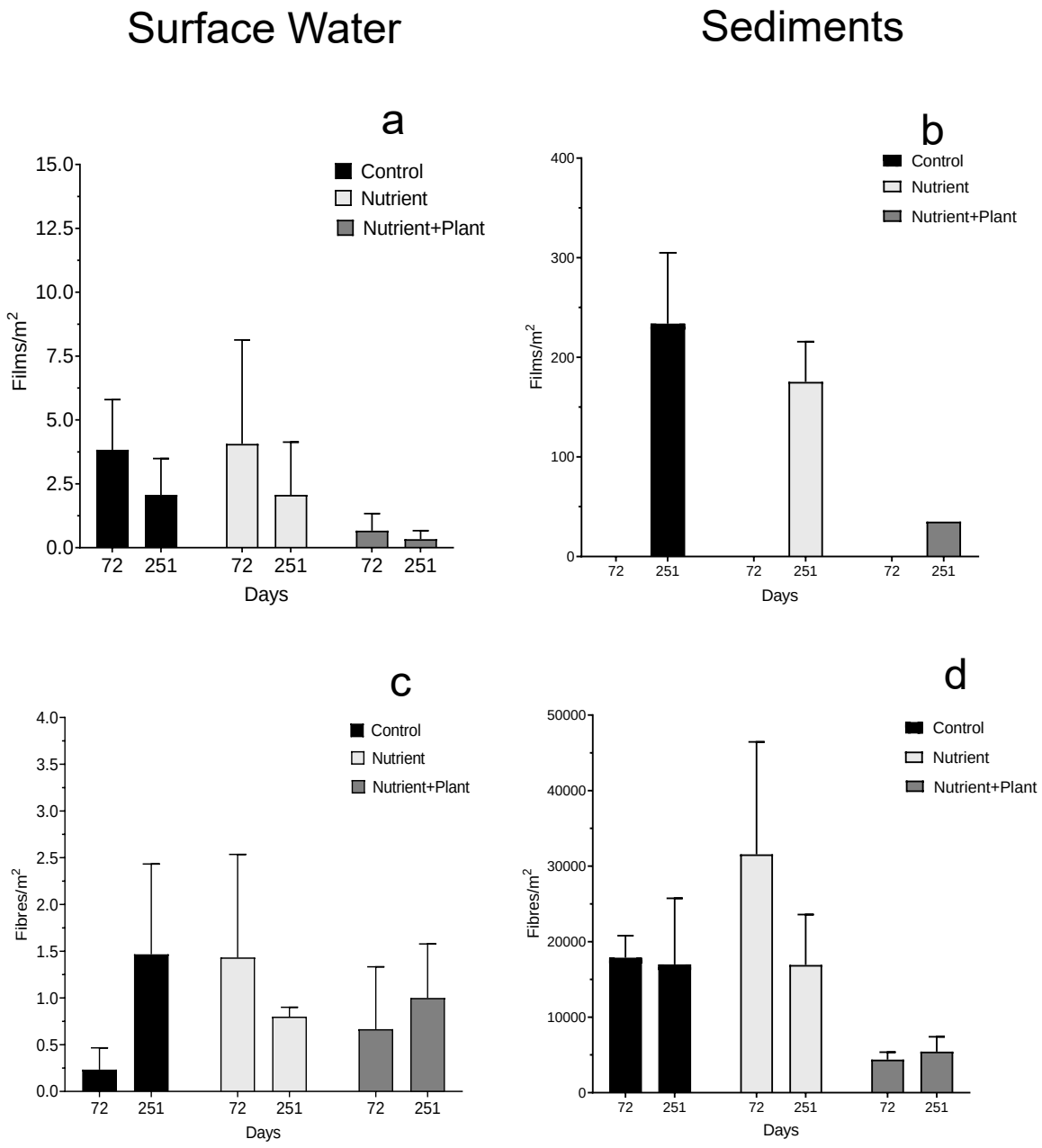


Figure 3.5. Plot of mean (±SE) microplastic/m^2 (a) surface water films/m^2, (b) sediments films/m^2, (c) surface water fibres/m^2, (d) sediments fibres/m^2 of nine mesocosms at the Prairie Wetland Research Facility (PWRF) before (day 72) and after (day 251) ice off. Microplastic/m^2 densities were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). The period of ice cover on the tanks is from days 73-250. The box plot shows the range of the density of microplastics detected, and the line in middle of box plot is the mean.

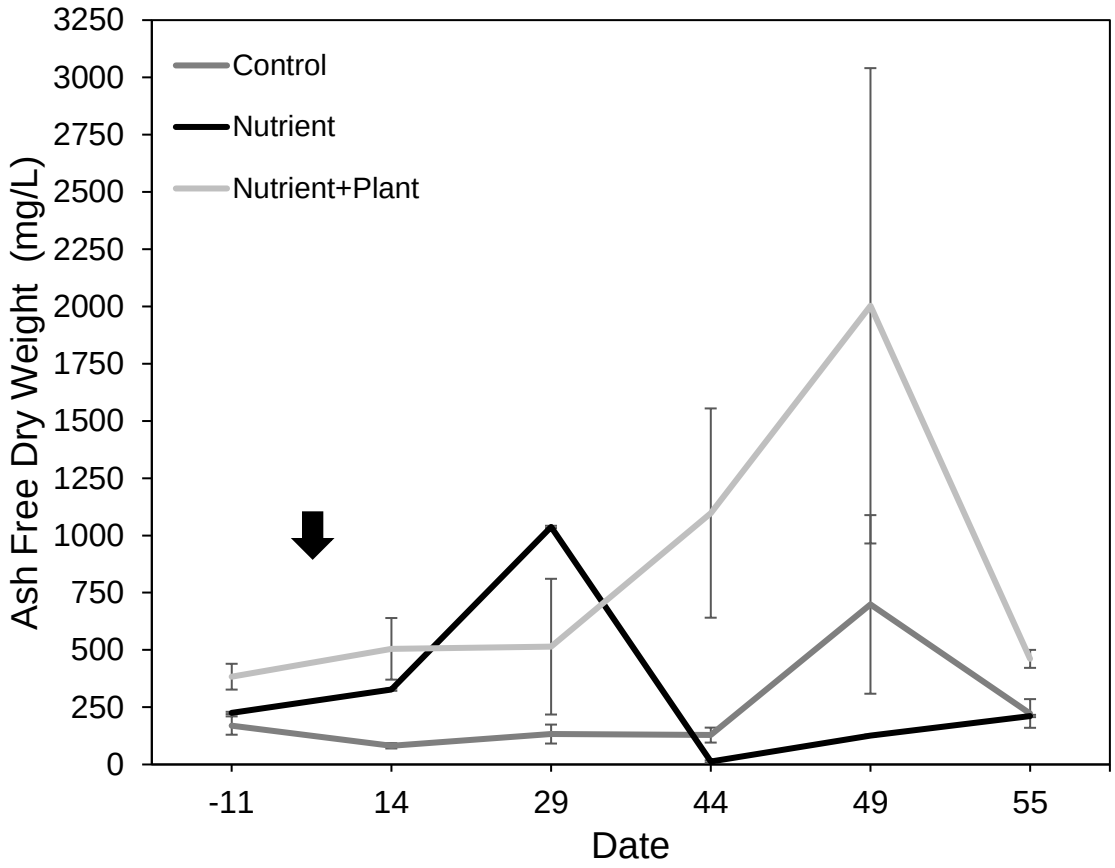


Figure 3.6. Mean±SE ash free dry weight (AFDW) densities (mg/L) of biofilm growth on tiles in nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. AFDW values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Biofilm densities were measured biweekly from August 3, 2018 (day -11) to October 8, 2018 (day -55) by scraping biofilm from ceramic tiles and ashed in a muffle furnace. Black arrow indicates synthetic wastewater additions to both the Nutrient and Nutrient+Plant treatments (excluding Controls).

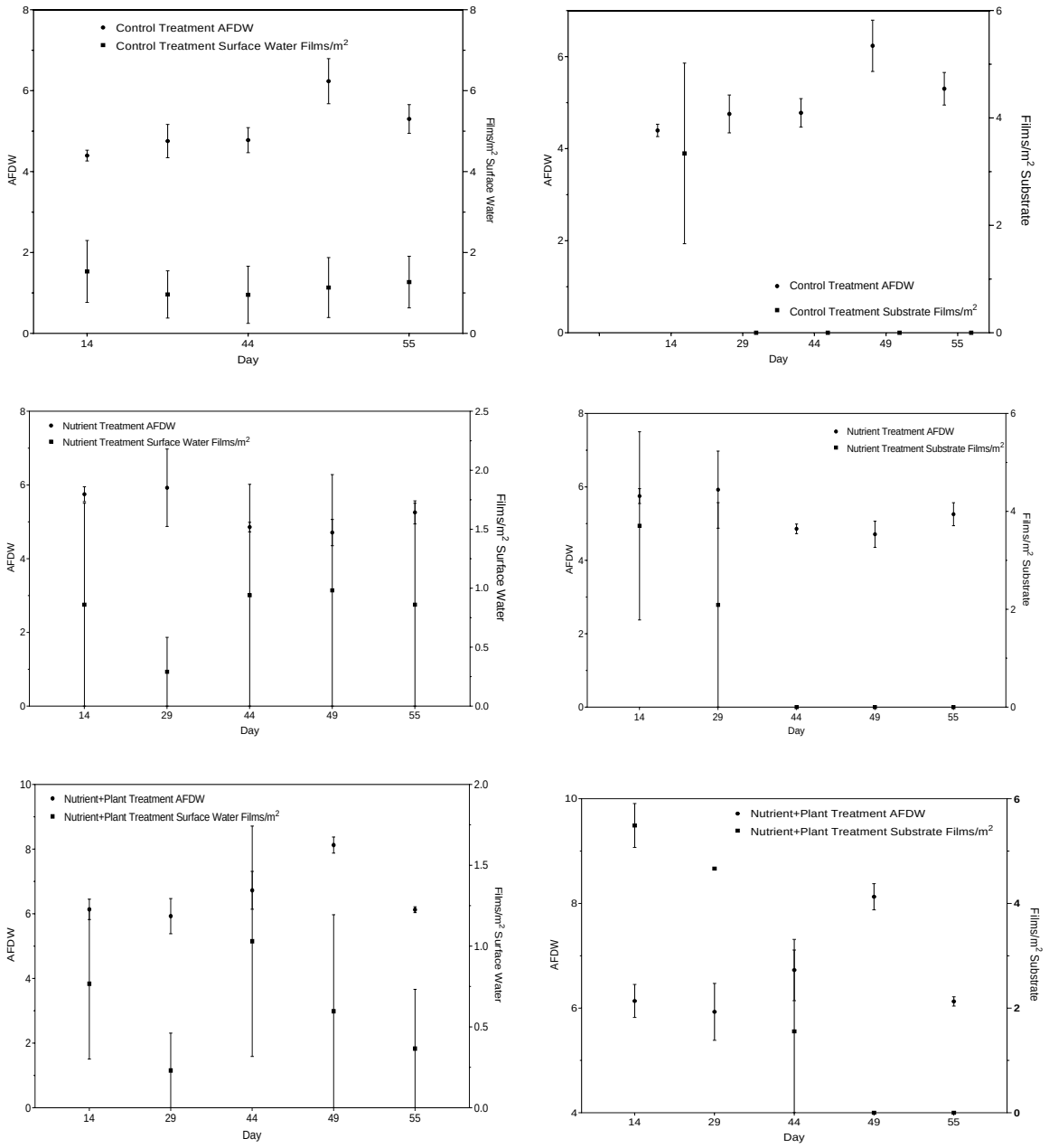


Figure 3.7. Films/m^2 densities (second y-axis; shape= squares) and biofilm densities as AFDW (first y-axis; shape= circles) from August 28, 2018, to October 8, 2018 plotted on a single figure (a) Control treatment films/m^2 in surface water, (b) Control treatment films/m^2 in substrate, (c) Nutrient treatment films/m^2 in surface water, (d) Nutrient treatment films/m^2 in the substrate, (e) Nutrient+Plant treatment films/m^2 in surface water, and (f) Nutrient+Plant treatment films/m^2 in substrate.

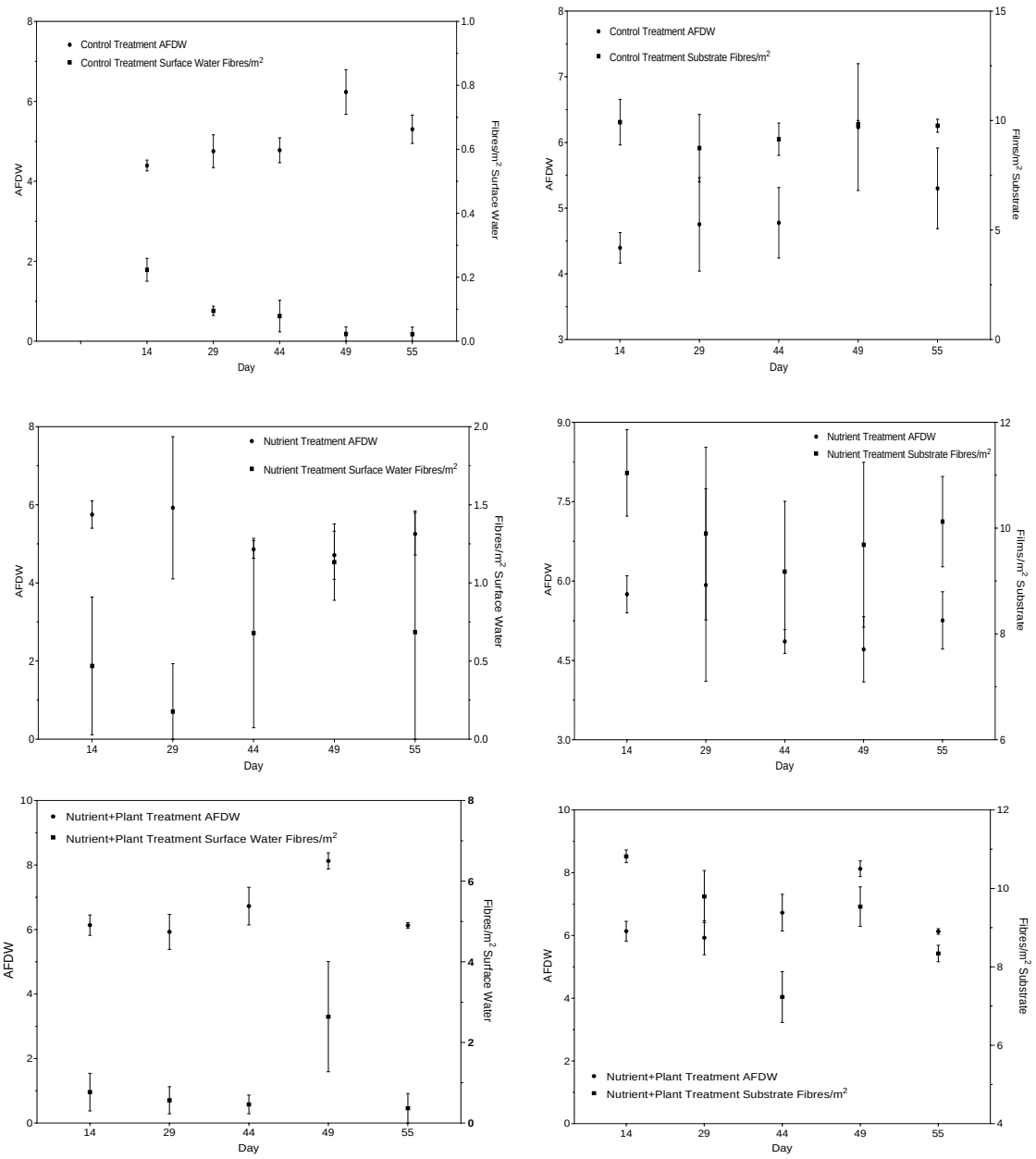


Figure 3.8. Fibres/m^2 densities (second y-axis; shape= squares) and biofilm densities as AFDW (first y-axis; shape= circles) from August 28, 2018, to October 8, 2018 plotted on a single figure (a) Control treatment fibres/m^2 in surface water, (b) Control treatment fibres/m^2 in substrate, (c) Nutrient treatment fibres/m^2 in surface water, (d) Nutrient treatment fibres/m^2 in the substrate, (e) Nutrient+Plant treatment fibres/m^2 in surface water, and (f) Nutrient+Plant treatment fibres/m^2 in substrate.

Chapter 4: Synthesis of the two mesocosm studies

4.1 General discussion

This thesis sought to better understand the long-term fate and behaviour of microplastics in freshwater systems, and the potential impact of nutrients, cattails, and overwintering on both settling and resuspension. The main findings in our long-term 622-day study (Chapter 2) were that all microplastic types decreased in surface waters over time, yet not all microplastics (foams and fragments) settled to the sediments. Biofilm growth was correlated with decreased densities of microplastics in the surface waters, and higher microplastic densities in the sediments. Of all the parameters measured, biofilm was most likely driver of microplastic settling and deposition. All microplastics displayed seasonal patterns in the surface water during the open water season, as surface densities generally decreased from spring to fall. Within the sediments, both films and fibres displayed seasonal patterns during the open water season, as microplastic densities generally increased as deposition occurred again in the sediments from spring to fall until just before ice on in late fall, when densities suddenly declined. Some, but not all microplastics displayed aggregation behaviour i.e., foams, microbeads and fibres formed unique homo and hetero-aggregates, while fragments and films did not. The mesocosms completely froze twice over two separate winters, and ice formation appeared to enhanced settling/deposition of films, and also enhanced the resuspension of fibres in both the surface water and sediments.

The main findings in our short-term 251-day study (Chapter 3) were that both a nutrient addition and the presence of emergent macrophytes (cattails) both had significant behavioural effects on films and fibres during the open water season, and after the ice melted in the spring. Film densities decreased rapidly before first sampling date i.e., 14 days, and densities remained relatively low and stable in the surface water over time. In the glass substrate, films settled rapidly initially, and then substrate densities decreased until they could not be detected, presumably resuspending back into the water column.

Fibres decreased over time in the surface waters of both the Control and Nutrient+Plant treatments, yet behaved differently in the Nutrient treatment as densities appeared to increase slightly during the open water season. Fibres in the glass substrate in both the Control and Nutrient treatments followed the overall trend of decreasing and then levelling off during the open water season, yet the Nutrient+Plant densities decreased over time. Based on correlations between biofilm (AFDW) and microplastic densities in the surface waters/crushed glass, my results strongly suggest that biofilm development was again likely the driver of microplastic settling and resuspension behaviour. Fibres hetero-aggregated, and formed large floating mats with films, other fibres, and filamentous algae. The mesocosms over wintered only once, and ice formation enhanced settling/deposition of films in all three treatments, yet had different effects on fibres in each treatment in both the surface water and crushed glass. The cattails appeared to enhance deposition of microplastics and hold onto microplastics as they became stuck in their sticky root system, therefore the microplastics were no longer moving freely within the mesocosm which further delayed their resuspension.

Not all microplastics settled

Chapter 2 found that all of the microplastic types (foams, films, fragments, microbeads, and fibres) decreased in the surface waters over time, but not all settled to the sediments (foams and fragments did not) within our 622-day time frame. I relied solely on natural processes occurring within the tanks (e.g., biofouling, wind, aggregation) to enable microplastic settling. This gives a more realistic timeline of evaluating settling and resuspension behaviour of microplastics (e.g., > 622 days) compared to other studies which force particles into the water column by artificial means (e.g., sonification or surfactant; Eitzen and Ruhl 2020). A major finding of my work is that microplastics (foams and fragments in particular) may persist on water surfaces and/or in the water column longer than previously thought. Other studies conducted in motionless water have found foamed polystyrene to have a settling velocity of 28 cm/s and polyetheylene fragments had a settling velocity of 5 cm/s (Waldschläger and Schüttrumpf 2019) which differs from my study. Further, my study indicates that factors

such as water quality (e.g., nutrients), emergent macrophytes, weathering, and climate likely play a role in both the settling and resuspension of microplastics. Future studies might focus on how long it takes different microplastic shapes and polymer types to settle under “natural processes” i.e., not using surfactants and other physical methods to force buoyant microplastics into the water column of freshwater systems, as well as the mechanisms that enhance settling e.g., biofilm development.

Microplastic seasonality

Chapter 2 revealed that microplastics exhibited seasonality during the open water season in the surface water as densities decreased from spring to fall. In the sediments, both films and fibres displayed seasonality during the open water season as densities increased (settled) from spring to fall. Consistency in our experimental observations suggests that seasonal patterns are likely driven by biofilm development as biofilm densities were correlated with microplastic settling. The short-term study of Chapter 3 was only 251 days, and that time frame was not long enough to observe seasonal patterns.

My findings suggest that microplastic behaviour may be distinct over particular seasons or seasonal events, and that tracking and prediction of microplastic behaviour in the water column may be possible using biofilm development as an indictor. As biofilm densities reach a specific threshold density on a microplastic (this density will likely be unique and specific to the polymer type), it may encourage microplastics settling. In the future, more research is needed on the impact of biofilm development on microplastic settling, resuspension, and biofilm species preference for microplastic polymers. To date, there is no other existing research on how seasonality may affect microplastic behaviour, and how different climates (larger temperature ranges across the seasons) might affect microplastic behaviours, and ultimately how long they will persist in the surface water/water column.

Ice formation

Ice formation and subsequent melting affected the behaviour of films and fibres in both the long and short-term studies. The fibres and films behaved similarly after ice

melted in the spring in both the long-term and Control treatment of the short-term study. Ice formation affected film and fibre behaviour differently, suggesting ice formation may affect different microplastic types uniquely. Complex microplastic behaviours driven by ice formation were likely caused by ice crystal formation, and the changing temperature (density) of the water causing both biofilm decay and changes in the particle's buoyancy. To my knowledge, there is no other existing literature to date on how ice formation and subsequent melting would affect microplastic fate and behaviour, as well as resuspension of microplastics under "natural" freshwater conditions, as microplastic resuspension has only been observed with laboratory experiments (Möhlenkamp et al. 2018).

Microplastic behaviour following ice formation and its subsequent melting provides insight on monitoring best practices, which should occur both before and after ice melts, and how a recent ice melt should be taken into account while calculating microplastic mass balance within freshwater systems, in particular those that freeze to the sediment/substrate layer. It should be noted that the mesocosms froze entirely to the sediment/substrate layer, which may not be the same as in all lakes, especially deeper ones, where a portion of the water column does not freeze. In these types of lakes microplastics will likely behave differently within the unfrozen water and sediments than within my two studies. Microplastics within unfrozen lake water and sediments during winter can have complex behaviours and movement patterns as they are not stuck within frozen ice. With decreasing ice cover on lakes due to climate change (increased open water season), there is a higher potential for sediments (and microplastics within them) to resuspend (Niemistö and Horppila 2007). This will decrease the amount of time microplastics have to bind to the sediments, therefore increasing their likelihood to resuspend in the spring (Kleeberg et al. 2013; Zhang et al. 2020), prolonging microplastic bioavailability within the water column and complicating the notion that sediments are the ultimate sink for microplastics. Future work should focus on how ice crystals freeze around microplastics and how this affects their behaviour in the water column.

Aggregation

Some (but not all) microplastic types formed hetero-aggregates (microbeads, foams, fibres in the long-term study, and both films and fibres in the short-term study). In both studies, films did not appear to form homo-aggregates (aggregates with other films). Within the long-tern study, aggregates were smaller in size, compared to the short-term study where aggregates were up to 20 cm in size. Aggregation behaviour can alter microplastic settling rates, either increasing or decreasing settling rates depending on the microplastic's buoyancy and biofilm species that develop on particular microplastics (Long et al. 2015). The buoyancy of the aggregate will affect its specific location in the water column which will in turn affect weathering processes (via photo-degradation; mechanical degradation, and biological degradation) degradation rates of the microplastics (Alimi et al. 2018), and ultimately their fate in aquatic ecosystems (Long et al. 2015).

Aggregation behaviour of microplastics could be used as a removal technique in WWTP (Zhang and Chen 2020; Wang et al. 2021). WWTP's could enhance favourable aggregation conditions (e.g., stimulate bacteria/algal growth, movement of water enabling contact between microplastics). Once aggregates begin to form, the skimming process could then remove the aggregates from the surface water. This would remove microplastics during the wastewater treatment process and therefore microplastics will not be released back into aquatic ecosystems. More research should be conducted in the future on favourable aggregation conditions for different microplastics (sources, polymers, sizes). This information could be used to create individualized action plans for WWTPs, as microplastic pollution is likely unique for each municipality (based on sources), and a one-size-fits-all approach will not capture all microplastics entering the WWTP.

Effects of emergent plants on microplastic behaviour

In the short-term study of Chapter 3, the presence of emergent cattails appeared to have affected the behaviour of both films and fibres in the mesocosms. The cattails seemed to delay film resuspension from the crushed glass by entraining microplastics

within its root system. Cattails entraining microplastics within our study provides evidence that the use of floating wetlands may work as a bioremediation technique where microplastics stick to cattail root systems thereby taking microplastics out of the system (United Nations Environment Programme 2017). Cattails already play an important role in nutrient and pollutant removal, and can also be employed as a removal technique for microplastics in aquatic systems thereby reducing their bioavailability in freshwater ecosystems. Future work should focus on how emergent plants affect microplastic behaviour, and the potential for cattails as a removal technique of microplastics in freshwater aquatic ecosystems.

Constructed wetlands may also be an important tool employed as a tertiary method in WWTP. Jönsson (2016) found that constructed wetlands can remove 100% of microplastics (<300 µm; 3.5-86 days). The long-term study found that small mesocosm wetlands can remove >99.9% of films in 622 days, and 100% of fibres by day 377 from the surface waters. The short-term study found that constructed wetlands can remove >99.9% of both films and fibres by day 14 from the surface waters.

Limitations of the research

There were some issues that arose during the long-term study during the experimental design. When I was dosing fibres, I pulled pre-cut and weighed fibres apart by hand. There was likely some human error and not all fibres were separated individually. A solution to combat this issue was employed in short-term study, where I blended the fibres with water, so fibres were separated individually. Another issue was that I required a second dose of microplastics within both studies. In both studies it did not seem like the initial amount of some microplastics added were enough, so I quickly decided to add more, which made the results (in particular, surface water densities) in the first year of the long-term study difficult to interpret. In retrospect, I should have set-up another control tank for both studies, and dosed them with the initial amount of microplastics to see if this would be enough for me to detect them using my sampling methods. . In the long-term study I dosed one month after the initial dosing, and in the short-term study, I dosed one week after the initial dosing. To provide a clearer understanding of when the second dosing occurred, it is clearly marked on the figures in

Chapter 2, yet not in Chapter 3, as I dosed again before I sampled. Within both studies, I relied on natural processes within the mesocosms to enable settling. This created some issues as some of the microplastics blew out of the tanks when there were strong winds, especially foams, which were initially quite hydrophobic and were repelled by water.

There were also some issues that arose during the both studies during microplastic sampling. During the take-down of our study, many microplastics were found under the lip of the mesocosm, that had been lost as water levels rose and fell. This would have led to some mass balance error. I did not have a solution for this, and I did not account for microplastics lost under the lip of the mesocosm in any of our calculations. I assumed when sampling, that microplastics were homogenously spread throughout the mesocosm, and over time microplastics settled to the sediments and stayed there. When I was sampling, I was getting a snapshot of which microplastics were left in the surface water or substrates at that specific time point. Yet microplastics formed aggregates, were stuck on plants, resuspended and were heterogeneously distributed throughout the tanks, and therefore this snapshot may not have provided all of the information needed to understand the complexities of microplastic behaviour. In retrospect, a solution to this issue would have been to sample microplastics at different depths in the water column, which would have provided additional insight on resuspension behaviour, and how long the different microplastics persist in the water column.

Edge effects of the mesocosms also likely created issues when sampling. Microplastics were often found pressed against the sides of the tanks due to surface water tension. It was difficult to sample the microplastics pressed against the sides of the tanks as our apparatus could not get right up against the side of the tank. Again, this likely led to sampling error, which was not accounted for when calculating microplastic densities in either studies.

Summary and future research recommendations

While significant gaps exist in freshwater microplastic research with regards to how microplastics behave in the water column throughout distinct seasons (including ice formation) especially within our Canadian climate, my research has begun to advance our understanding of these processes. Sediments may not be the ultimate sink for microplastics as previously thought, as their behaviour and processes that occur within the water column are far more complex (aggregation, biofouling, ice crystal formation, water temperature-density effects) and factors of the water body (i.e., climate and water quality) will also affect both behaviour and their ultimate fate. Future research should focus on microplastic behaviour during distinct seasons, resuspension, biofilm species preference for microplastics, climate effects on microplastic behaviour, ice crystal formation and microplastic behaviour, and floating wetlands using cattails as a bioremediation technique.

4.2 References

Alimi, O.S., Farner Budarz, J., Hernandez, L.M., and Tufenkji, N. 2018. Microplastics and nanoplastics in aquatic environments: aggregation, deposition, and enhanced contaminant transport. Environ. Sci. Technol. **52**(4): 1704–1724.

Eitzen, L., and Ruhl, A.S. 2020. ParticlesSize and pre-treatment effects on polystyrene microplastic settlement in water: implications for environmental behavior and Ecotoxicological Tests. Water (Switzerland) **12**(3436): 1–12.

Jönsson, R., 2016. Mikroplast i dagvatten och spillvatten - Avskiljning i dagvattendammar och anlagda våtmarker (Master Thesis). The Department of Earth Science, Uppsala University. Available from: http://www.diva-portal.org/smash/get/diva2:1049924/FULLTEXT01.pdf.

Kleeberg, A., Freidank, A., and Jöhnk, K. 2013. Effects of ice cover on sediment resuspension and phosphorus entrainment in shallow lakes: combining in situ experiments and wind-wave modeling. Limnol. Oceanogr. **58**(5): 1819–1833.

Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., and Soudant, P. 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. **175**: 39–46.

Möhlenkamp, P., Purser, A., and Thomsen, L. 2018. Plastic microbeads from cosmetic products: An experimental study of their hydrodynamic behaviour, vertical transport and resuspension in phytoplankton and sediment aggregates. Elementa. **6:** 1-16.

Niemistö, J.P., and Horppila, J. 2007. The contribution of ice cover to sediment resuspension in a shallow temperate lake: possible effects of climate change on internal nutrient loading. J. Environ. Qual. **36**(5): 1318–1323.

United Nations Environment Programme. 2017. UN Environment promotes environmentally sound practices globally and in its own activities Our distribution policy aims to reduce UN Environment's carbon footprint. *In* Frontiers. pp 1-92.

Waldschläger, K., and Schüttrumpf, H. 2019. Effects of particle properties on the settling

and rise velocities of microplastics in freshwater under laboratory conditions. Environ. Sci. Technol. **53**(4): 1958–1966.

Wang, X., Bolan, N., Tsang, D.C.W., Sarkar, B., Bradney, L., and Li, Y. 2021. A review of microplastics aggregation in aquatic environment: influence factors, analytical methods, and environmental implications. J. Hazard. Mater. **402**(March 2020): 123496.

Zhang, Y., Liang, J., Zeng, G., Tang, W., Lu, Y., Luo, Y., Xing, W., Tang, N., Ye, S., Li, X., and Huang, W. 2020. How climate change and eutrophication interact with microplastic pollution and sediment resuspension in shallow lakes: a review. Sci. Total Environ. **705**: 135979.

Zhang, Z., and Chen, Y. 2020. Effects of microplastics on wastewater and sewage sludge treatment and their removal: a review. Chem. Eng. J. **382**(July 2019): 122955.

Appendix A: Introduction

Tables

Table A1 Types of plastic, common abbreviations, densities, and common product types typically found within aquatic environments (adapted from Andrady, 2011; Hildalgo-Ruz et al. 2012; Anderson et al. 2016; Costa et al. 2017).

Plastic Type	Abbreviation	Density (g/cm^3)	Use
Polyethylene	PE	0.91-0.96	Grocery bags, plastic bottles, microbeads
Low-density polyethylene	LDPE, LLDPE	0.91-0.93	Packaging, drinking straws, floor tiles
High-density polyethylene	HDPE	0.94	Milk containers, laundry detergent bottle
Polypropylene	PP	0.83-0.85	Bottle caps, rope, packaging (takeout containers), utensils
Polystyrene	PS	1.04-1.1	CD cases, red “solo” cup
Foamed polystyrene	PS	0.023-0.045	Foamed packaging, takeout containers,
Poly(vinyl chloride)	PVC	1.16-1.58	Pipes, flooring
Cellulose acetate	CA	1.3	Cigarette filters, makeup remover cloths
Thermoplastic polyester	PET, PES	1.24-2.3	Bottles, packaging, textiles, building and construction
Polyamides (nylons)	PA	1.15	Netting, textiles, rope, toothbrush bristles, fishing line

Table A2. Sources and transportation pathways of microplastics that enter aquatic environments (adapted from Duis and Coors 2016).

Source	Example of microplastic	Reference
Primary microplastics		
Personal care products	Microbead used as an exfoliant/abrasive	Lassen et al. 2015
Medical applications	Microbead used as a carrier that delivers active pharmaceutical agent	Sundt et al. 2014; Lassen et al. 2015
Industrial abrasives	Microbeads/ pellets used as an exfoliant/abrasive	Sundt et al. 2014
Pre-production plastics	Pellets melted down to create other plastics	Moore 2008; Andrady 2011; Derraik 2002
Secondary microplastics		
Littering/ dumping	Larger macroplastics degrade into films, fragments, foams	Pruter 1987; Barnes et al. 2009; Mehlhart 2012
Recycling Plant	Accidental losses of larger macroplastics degrade into films, fragments, foams, foams	Pruter 1987; Barnes et al. 2009; Lambert et al. 2014; Jambeck et al. 2015
Landfill	Accidental dumping, microplastics leach into ground water	Rillig 2012; Jambeck et al. 2015
Plastic mulch, soil additive	Foams in soil, films from mulch as it degrades	Lambert et al. 2014; Rillig et al. 2012
Synthetic textiles	Fibres shed as clothing washed	Browne et al. 2011
Hygiene products	Microfibres shed from tampons, pads	Duis and Coors 2016
Fishing gear	Nets	

Table A2. Continued.

Source	**Example of microplastic**	**Reference**
	Secondary microplastics	
Shipping	Lost cargo, dumping of waste	do Sul and Costa 2007; Andrady 2011; Pruter 1987; Lambert 2014
Pathways	Example	Reference
Weather phenomena	Heavy rainfall, tsunami, flooding	Thompson et al. 2005; Lambert et al. 2014
Storm water runoff	water moving over land	Rillig 2012; Wagner et al. 2014; Dris et al. 2015;
Wind	Blow plastics into nearby waterways	Pruter 1987; Barnes et al. 2009; Mehlhart 2012
Wastewater effluent	effluent from wastewater treatment plants released into water ways	Dris et a., 2015; Carr et al. 2016; Mason et al. 2016;
Aerial Deposition	Fibres released from air as dust particles	Sundt et al. 2014; Rillig 2012
Sludge	Applied to agricultural fields as fertilizer	Habib 1998; Zubris and Richards 2005; Eriksen et al. 2013
Sewer overflow	Flooding	Lambert et al. 2014
Groundwater	Seepage from source to another body of water	Mintenig et al. 2019

Table A3. Densities of microplastics (mp) found in surface water, and sediments of Canadian freshwater systems.

Water body type	Sample type	Surface water	Density	Reference
Lake	Surface water	Lake Winnipeg	193,420 mp/km^2	Anderson et al. 2017
Lake	Surface water	Lake Superior	5,391 mp/km^2	Eriksen et al. 2013
Lake	Surface water	Lake Erie	105,503 mp/km^2	Eriksen et al. 2013
Lake	Surface water	Lake Huron	2,779 mp/km^2	Eriksen et al. 2013
River	Surface water	Red River	800,000 mp/km^2	Warrack et al. 2017
River	Surface water	Assiniboine River	1,200,000 mp/km^2	Warrack et al. 2017
River	Surface water	Nelson River	114,000 mp/km^2	Warrack et al. 2017
Lake	Sediment	Lake Ontario	980 mp/kg	Ballent et al. 2016
River	Sediment	Ottawa River	0.22 mp/g	Vermaire et al. 2017
River	Sediment	Lake Ontario tributaries	610 mp/kg	Ballent et al. 2016
River	Sediment	St. Lawrence River	13,832 mp/m^2	Castañeda et al. 2014

Figures

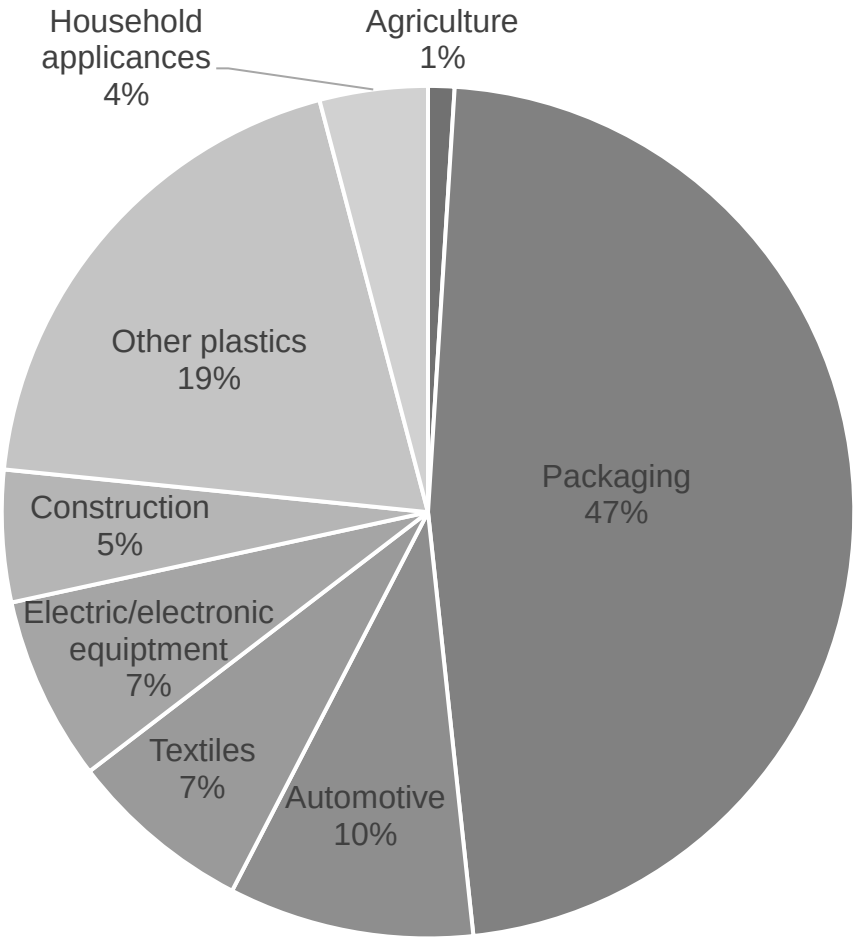


Figure A1. Percentages of plastic waste generated in Canada (Environment and Climate Change Canada 2019).

Appendix B: Long-term Study

Tables

Table B1. Formulas and calculations used in data analyses (h=height, l=length, w=width, r=radius).

Long-term/ Short-term Study	Surface Water	Formula	
Mesocosm diameter (m)	2.7		
Mesocosm radius (m)	1.35		
Height of mesocosm (h)	0.72		
SA of mesocosm (m^2)	17.56	$2*pi*r*h+2*pi*r^2$	
Topsoil height per tank	0.23		
Water sampled in mesocosm	0.49	h-soil height	
Mesocosm water volume (m^3)	2.81	$pi*r^2*h$	
Mesocosm water volume (L)	2805.52	Mesocosm water volume (m^3)*1000	
Circular sampler diameter (m)	0.085		
Circular sampler radius (m)	0.043	Circular sampler diameter/2	
Total SA of sampler rectangle (m^2)	0.70	2(l*w)+ 2(l*h)+2(w*h)	h=0.0425; w=0.085; l=2.7
SA of sampler sampling (m^2)	1.39	Total SA of sampler rectangle/2	
distance sampled (m)	2.7		

Table B1. Continued.

Long-term Study	Surface Water	Formula
Volume sampled (m^3)	0.020	l*w*h
Volume sampled (L)	19.51	Volume sampled (m^3) * 1000
Volume sampled (L) using circular sampler	5.32	0.0887 L; 0.09m sampler length; 2.7/0.09=30; 30*0.0887*2
Fraction of water sampled	0.2%	Volume sampled using circular sampler
Multiplying factor to convert # of MPs to whole mesocosm (L)	527.16	Mesocosm water volume (L)/ Volume sampled (L) using circular sampler
Long-term Study	**Sediments**	**Formula**
Sediment Box length (m)	0.12	
Sediment Box width (m)	0.08	
Sediment Box height (m)	0.05	
Volume of Sediment Box (m^3)	0.00048	l*w*h
SA of sediment sample (m^2)	0.039	2*(wl+hl+hw)
Fraction of sediment sampled	17.04%	SA of sediment sample/SA of mesocosm

Table B1. Continued.

Short-term Study	Sediments	Formula
Sediment Ring Diameter (m)	0.11	
Sediment Ring Radius (m)	0.055	
Sediment Ring Height (m)	0.03	
Volume of water sampled	1L	All counts are/L
Area of sediment sample (m^2)	0.0095	$PI*r^2$
Fraction of sediment sampled	0.35%	Area of sediment sample (m^2)/SA of mesocosm (m^2)

Table B2. Mean filamentous algae assessment of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Filamentous algae assessment values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a scale of 1 to 3 (1= no algae present, 2= distinct algal masses visible, 3= algal colonization), to approximate algal growth or productivity (Baxter et al. 2013).

Date	Filamentous Algae
2017-06-12	1
2017-06-22	1
2017-06-29	1
2017-07-06	1
2017-07-13	1
2017-07-20	1
2017-07-27	1
2017-08-02	2
2017-08-11	2
2017-08-17	2
2017-08-24	1
2017-08-31	1
2017-09-06	1
2017-09-13	1
2017-09-20	1
2017-10-13	1
2018-05-14	1
2018-05-26	1
2018-05-31	1
2018-06-07	1
2018-06-16	1

Table B2. Continued.

Date	Filamentous Algae
2018-06-27	1
2018-07-04	1
2018-07-12	1
2018-07-18	1
2018-07-27	1
2018-08-03	1
2018-08-10	1
2018-08-13	1
2018-08-21	1
2018-08-29	2
2018-09-06	1
2018-09-13	1
2018-09-18	1
2018-09-21	1
2018-09-28	1
2018-10-05	1
2018-10-08	1
2018-10-10	1
2019-04-18	1

Figures

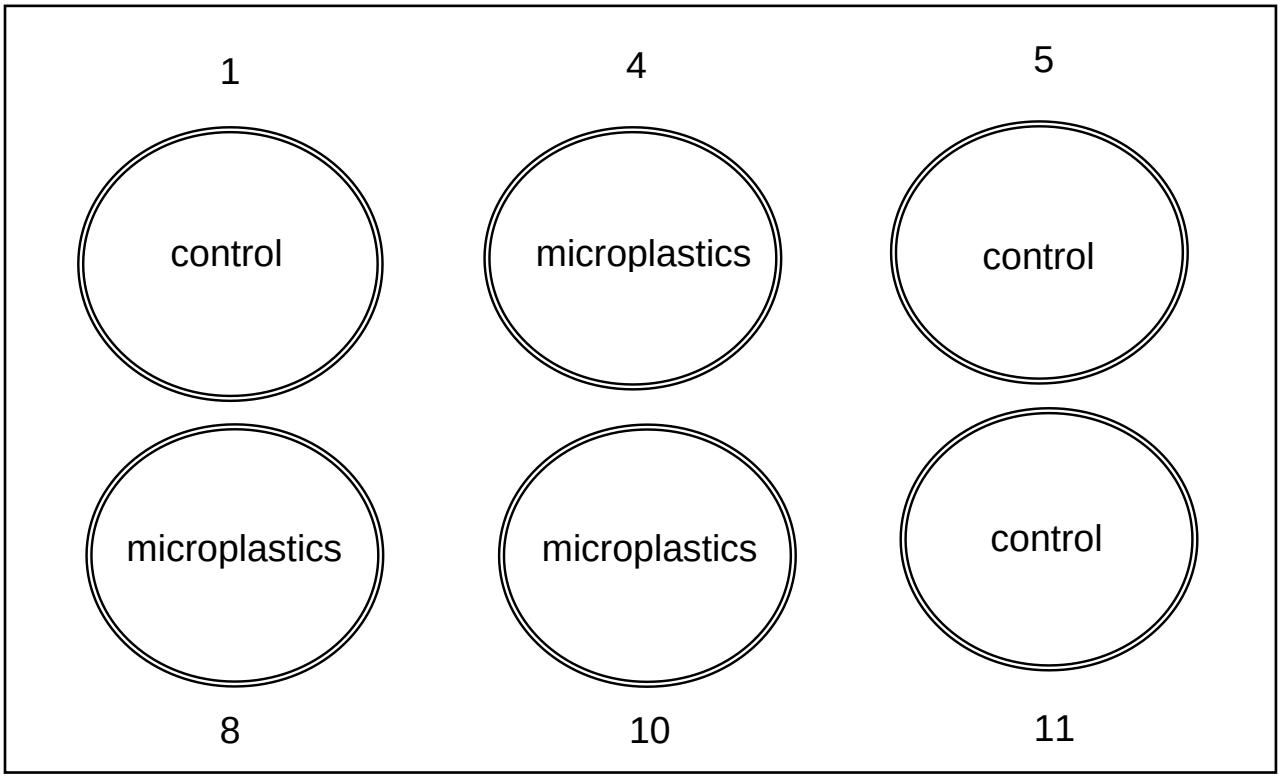


Figure B1. Experimental design of long-term study. Layout of randomly assigned treatments of six mesocosms. The treatments consist of control tanks 1, 5, 11 (sediment substrate, no microplastics), and microplastic tanks 4, 8, 10 (sediment substrate, and microplastics). A total of five microplastic morphologies (foams, films, fragments, microbeads, and fibres) were added to the microplastic treatment tanks.

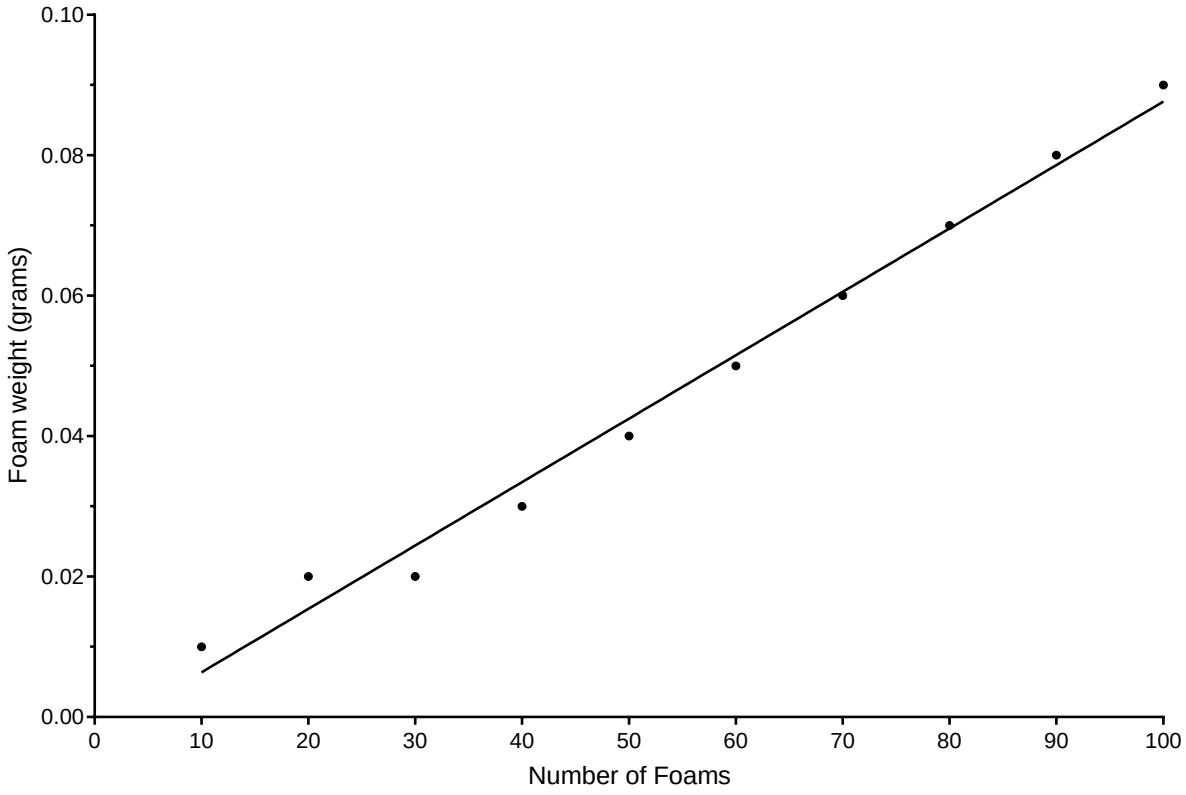


Figure B2. Density curve created for foams by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 foams on an analytical balance. The average weight of one foam was ~0.001 grams.

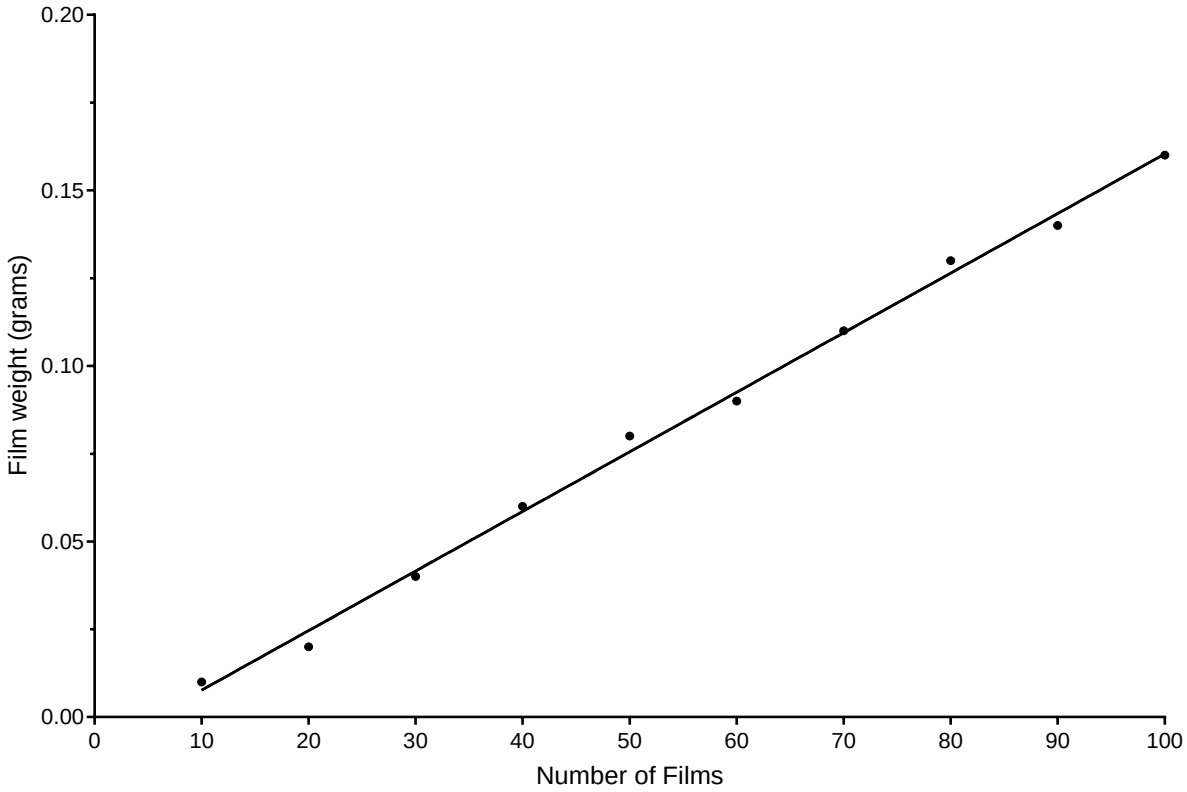


Figure B3. Density curve created for films by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 films on an analytical balance. The average weight of one film was ~0.0017 grams.

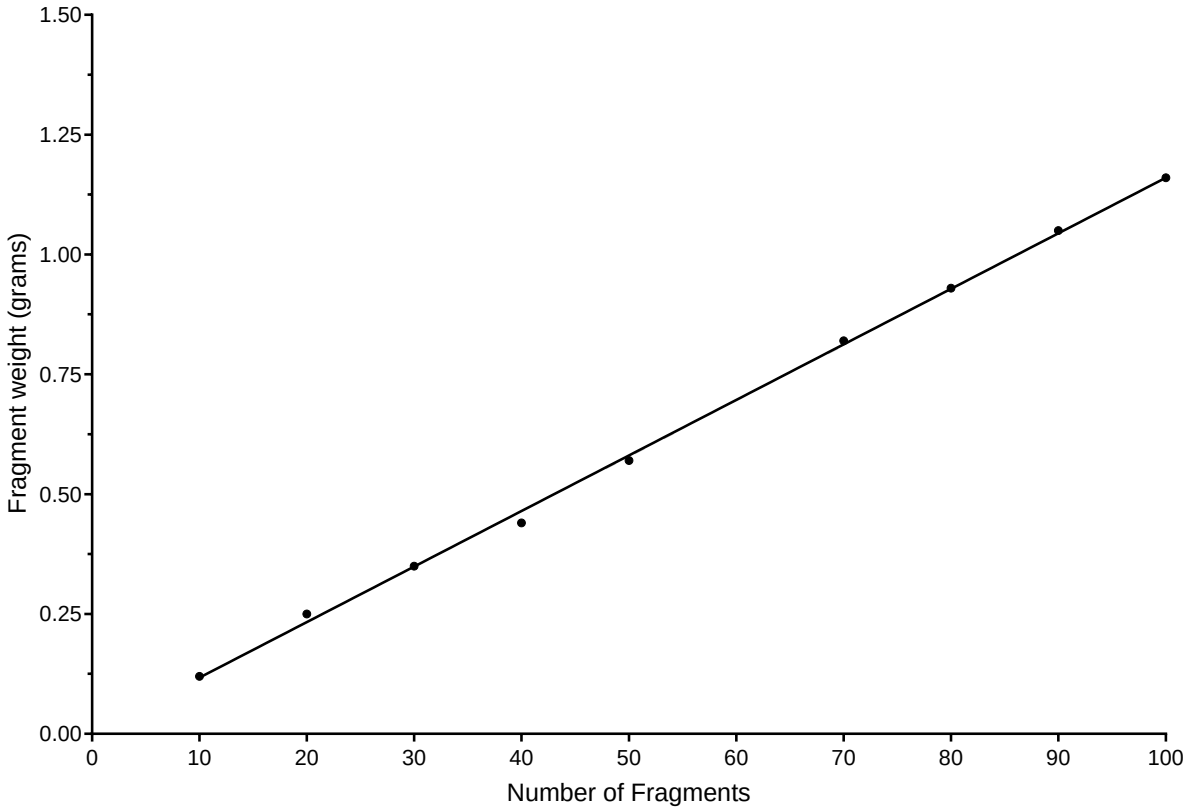


Figure B4. Density curve created for fragments by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 fragments on an analytical balance. The average weight of one fragment was ~0.012 grams.

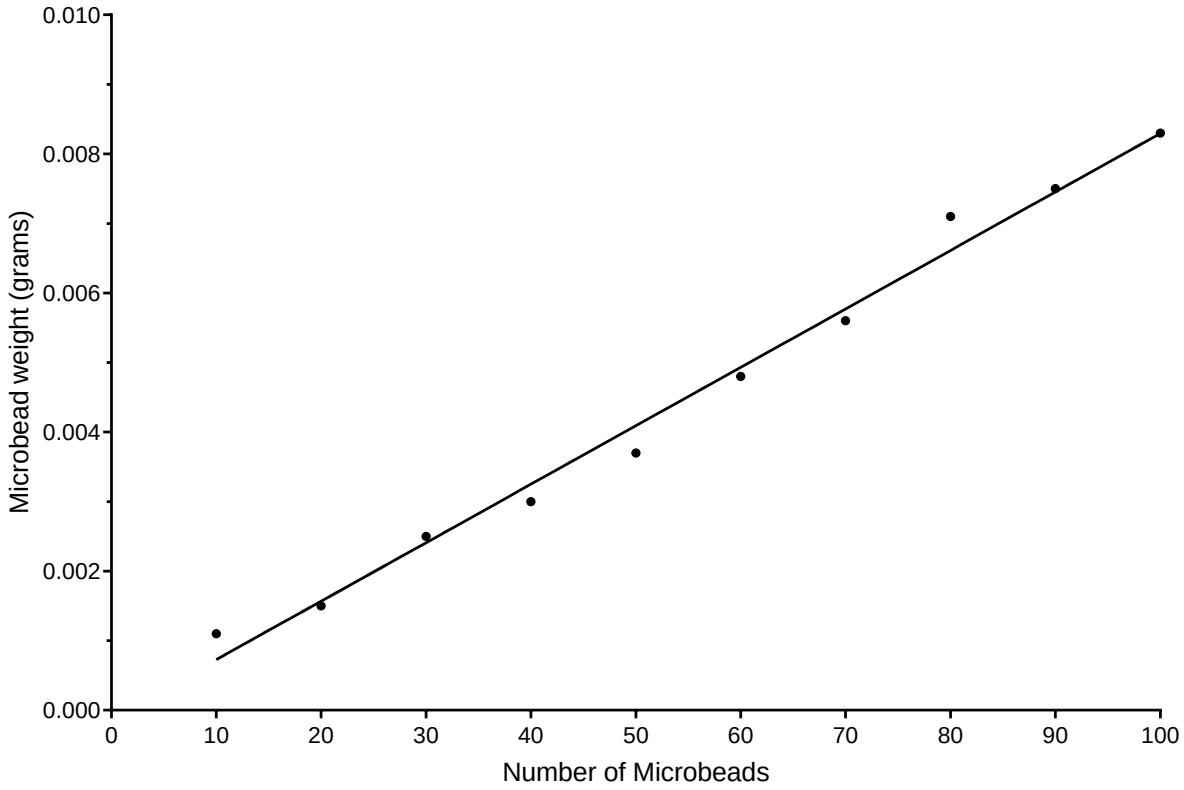


Figure B5. Density curve created for microbeads by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 microbeads on an analytical balance. The average weight of one microbead was ~0.000083 grams.

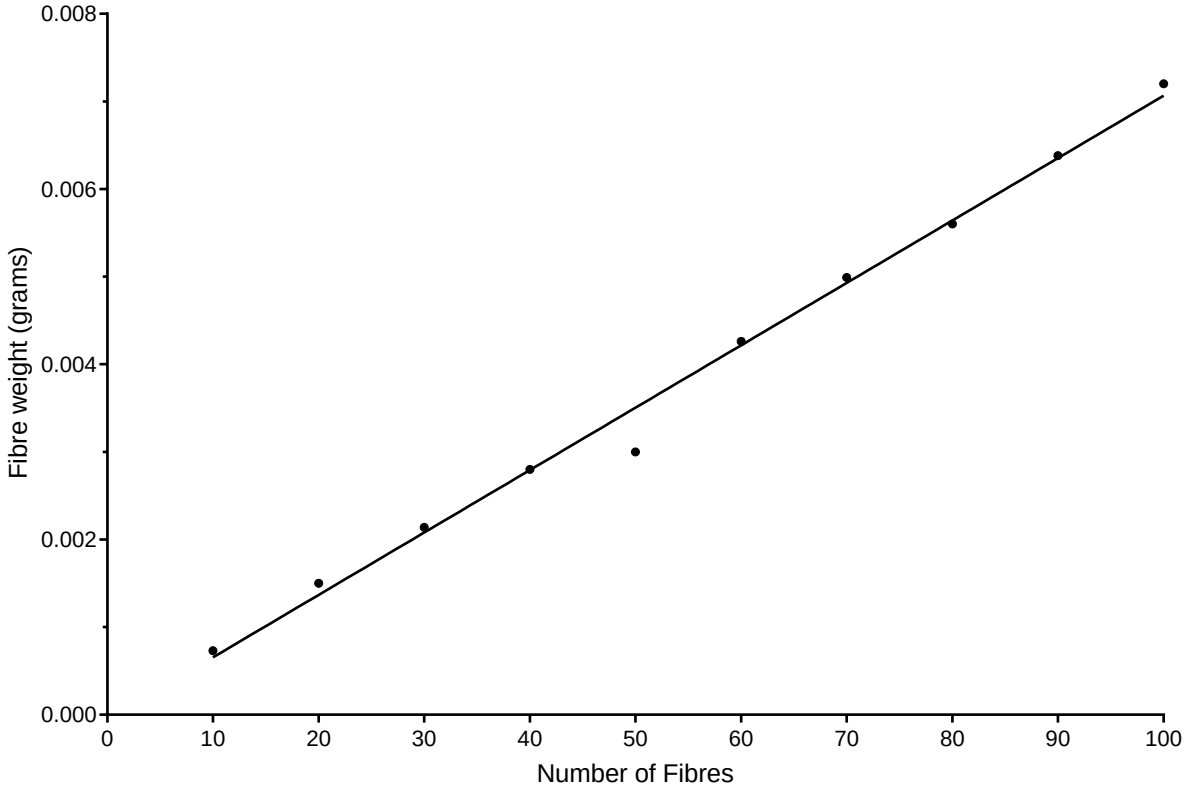


Figure B6. Density curve created for fibres by weighing a range of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 fibres on an analytical balance. The average weight of one fibre was ~0.000071 grams.

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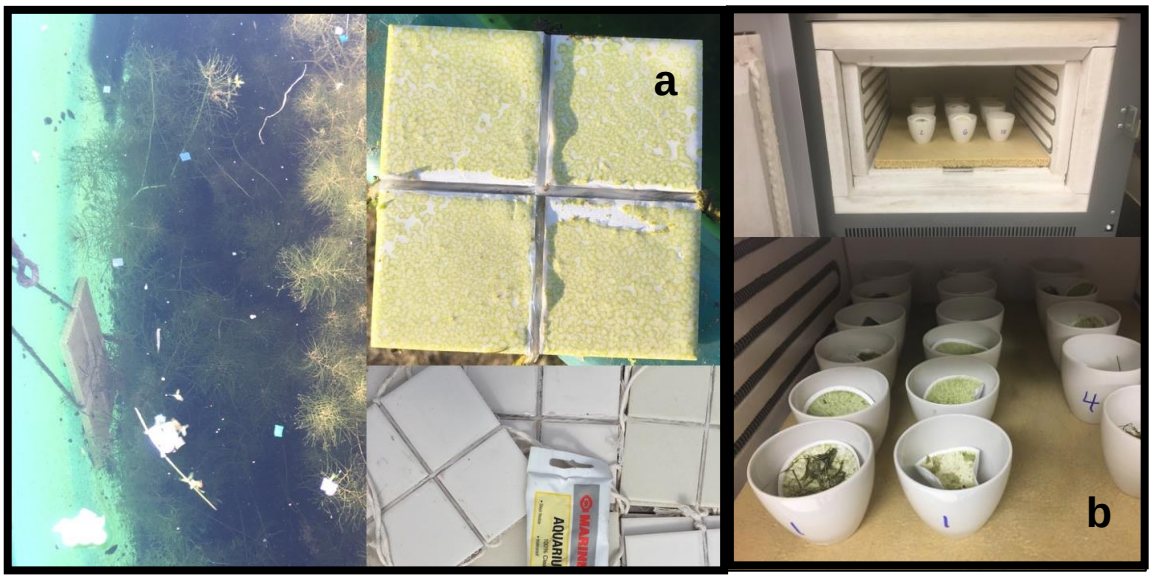


Figure B7. (a) Tile deployed off side of mesocosm, tile with biofilm growth, and tiles being made. (b) Muffle furnace with filtered biofilm samples in crucibles.

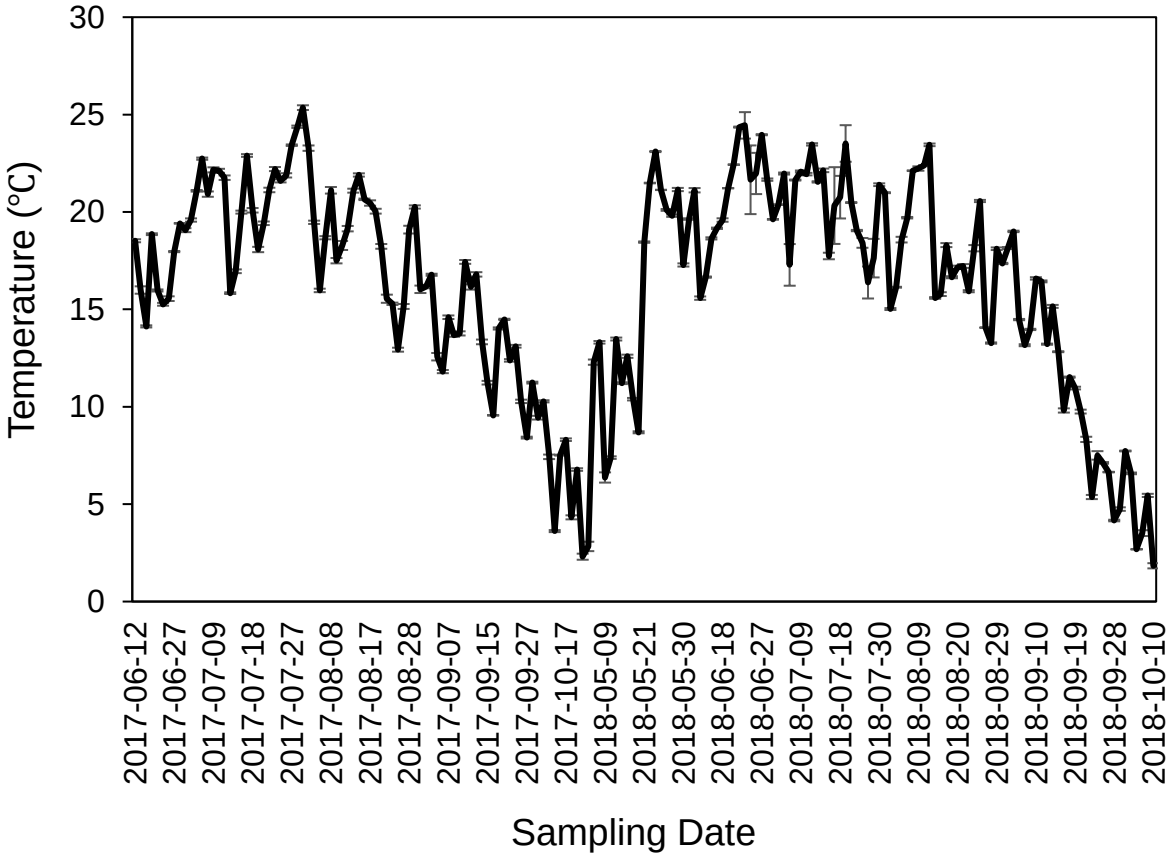


Figure B8. Mean±SE temperature (°C) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Temperature values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

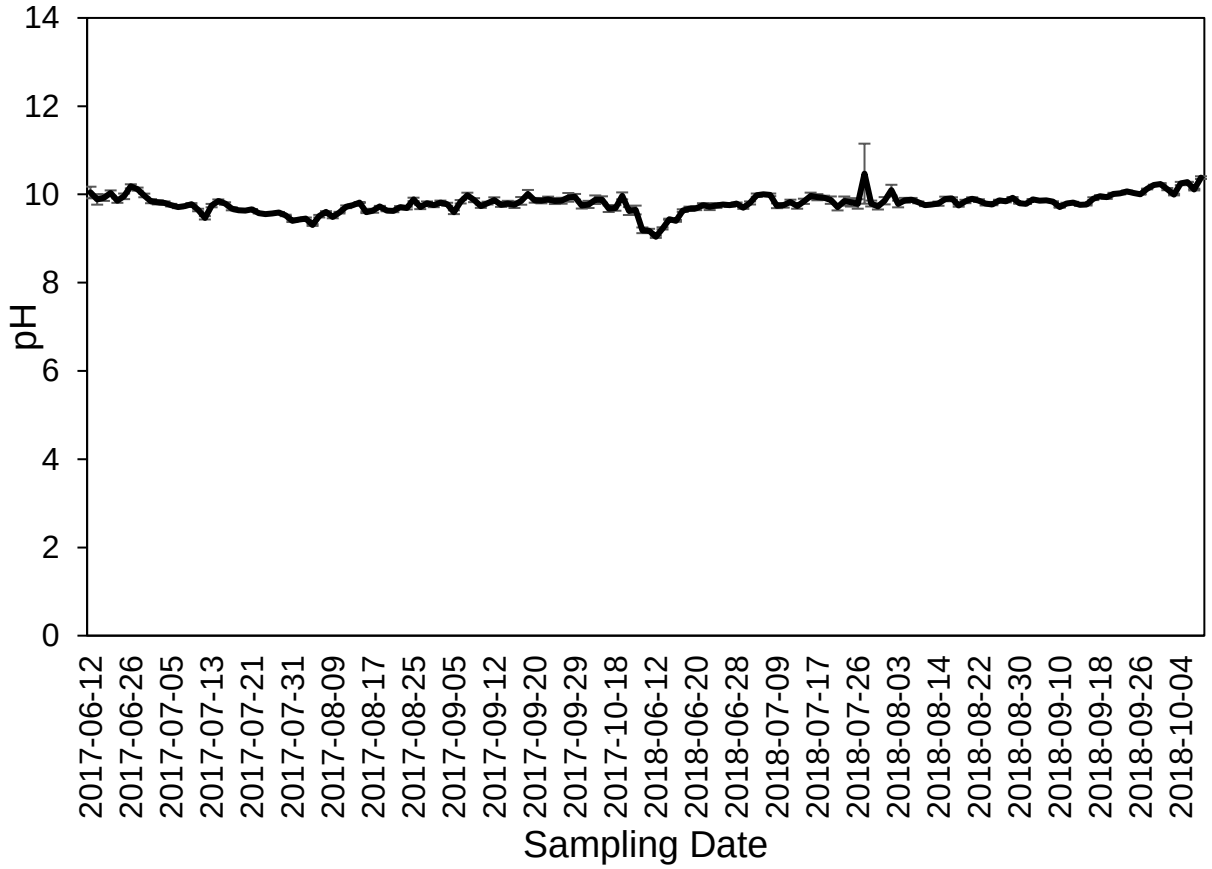


Figure B9. Mean±SE pH of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. pH values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

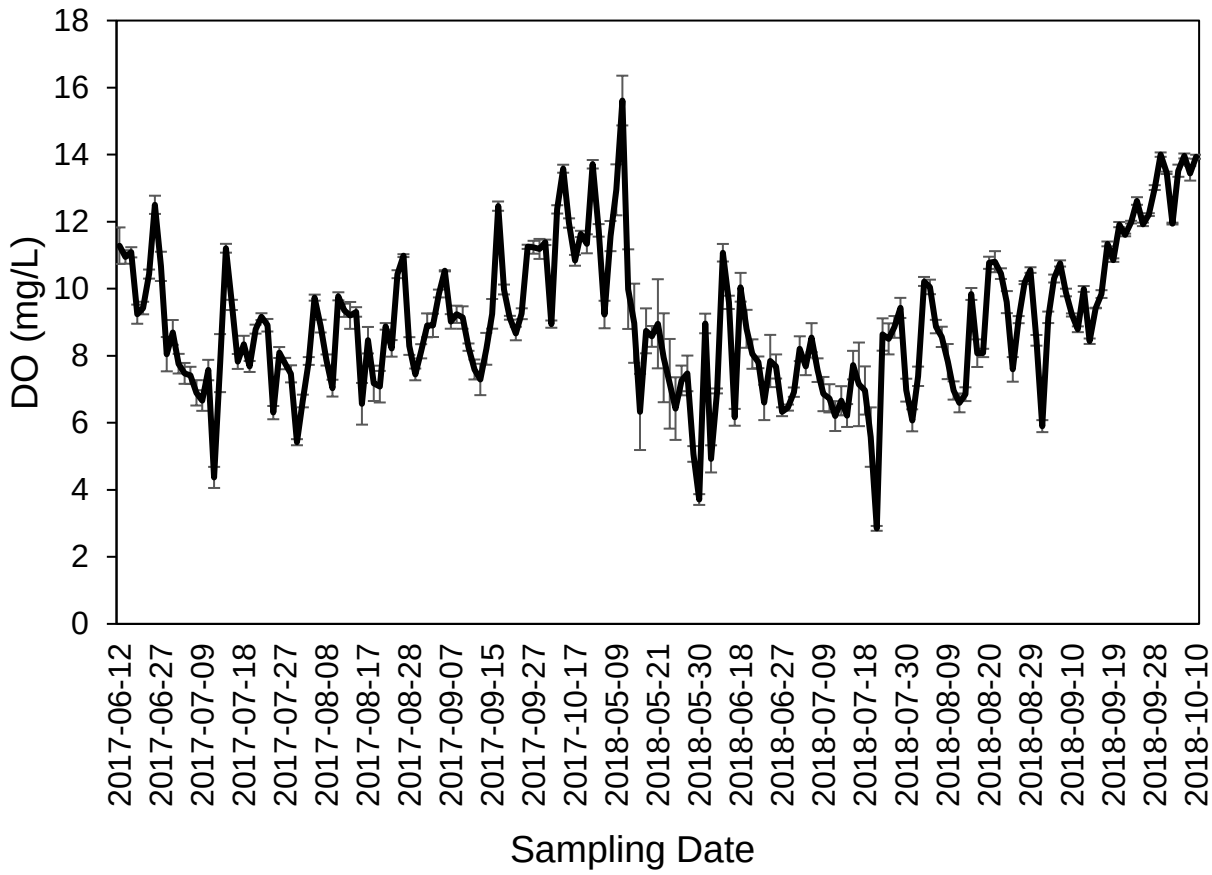


Figure B10. Mean±SE dissolved oxygen (DO) concentration (mg/L) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. DO values were averaged across replicates (n=3) for each for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

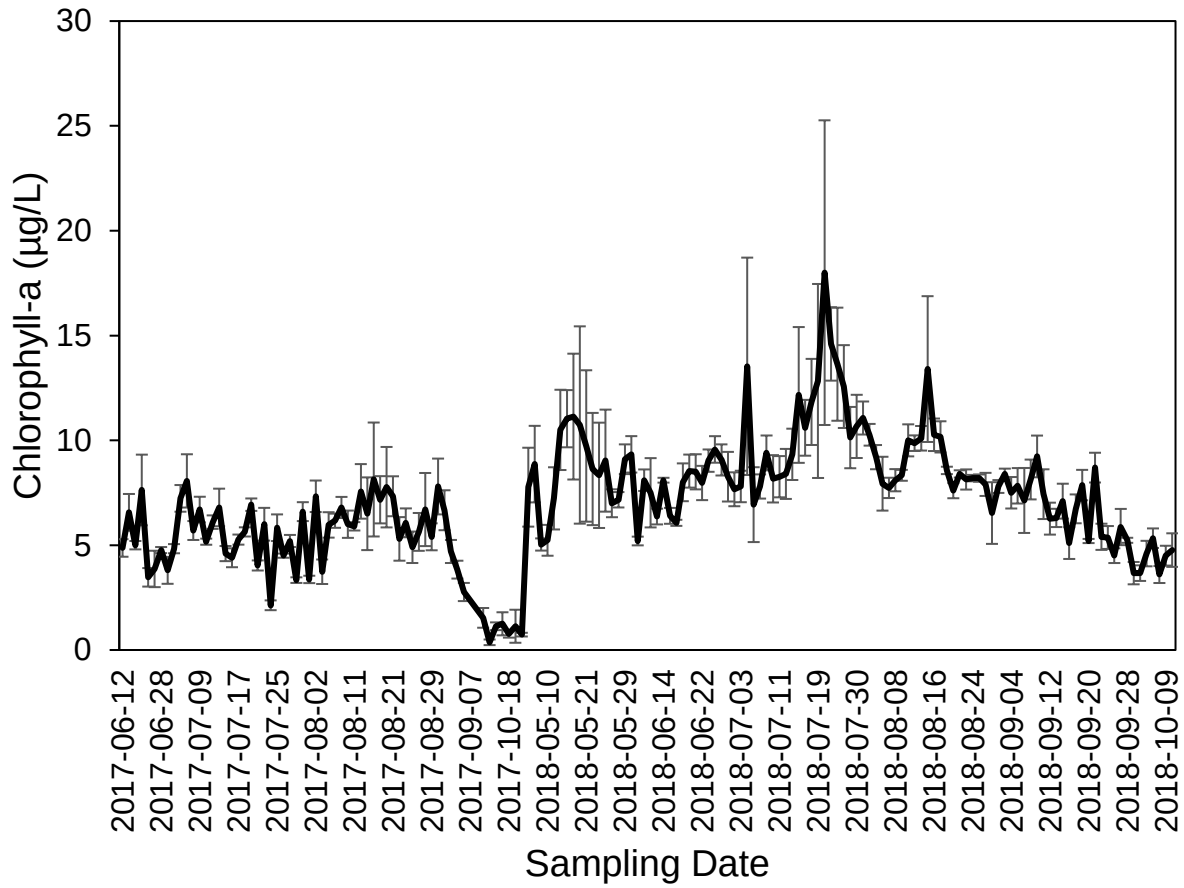


Figure B11. Mean±SE chlorophyll-a concentration (µg/L) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Chlorophyll-a values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

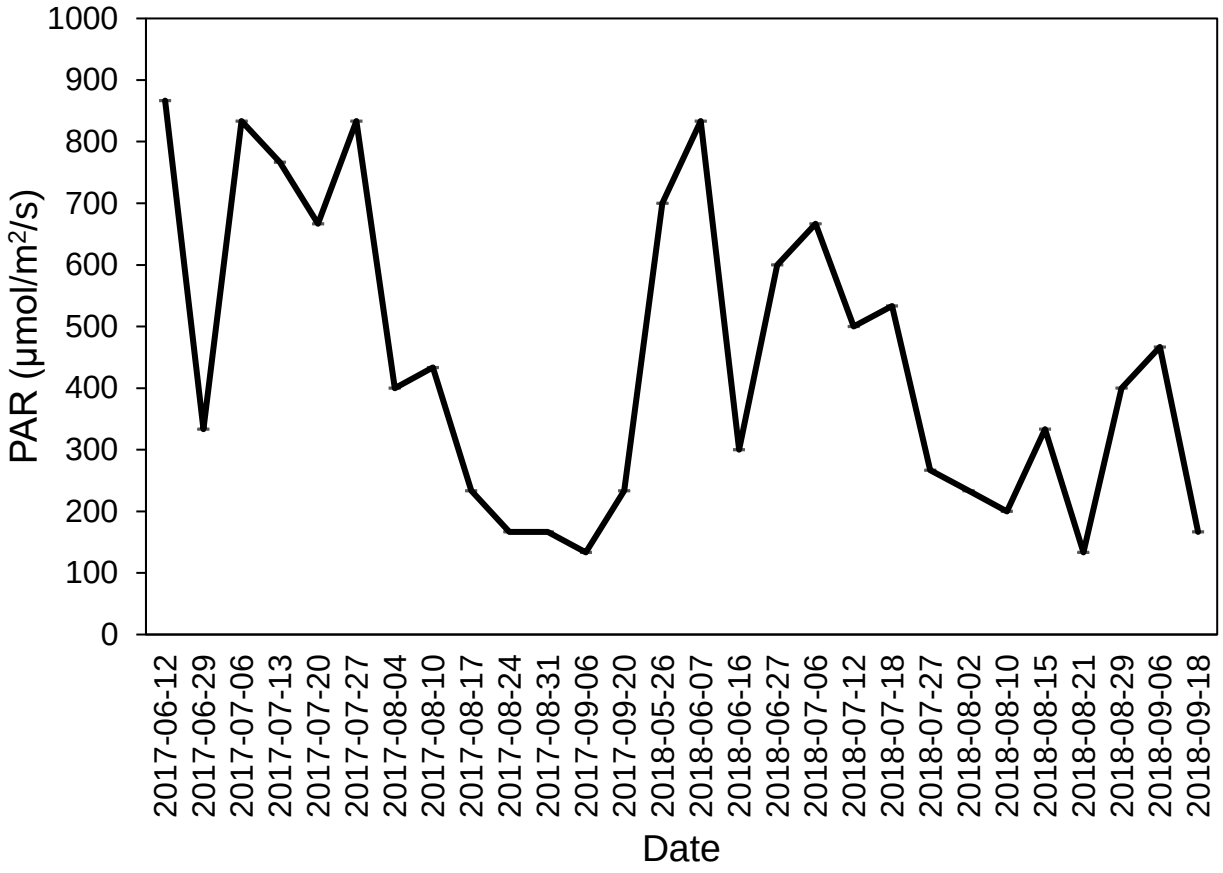


Figure B12. Mean±SE photosynthetically active radiation (PAR; µmol/m^2/s) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. PAR values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

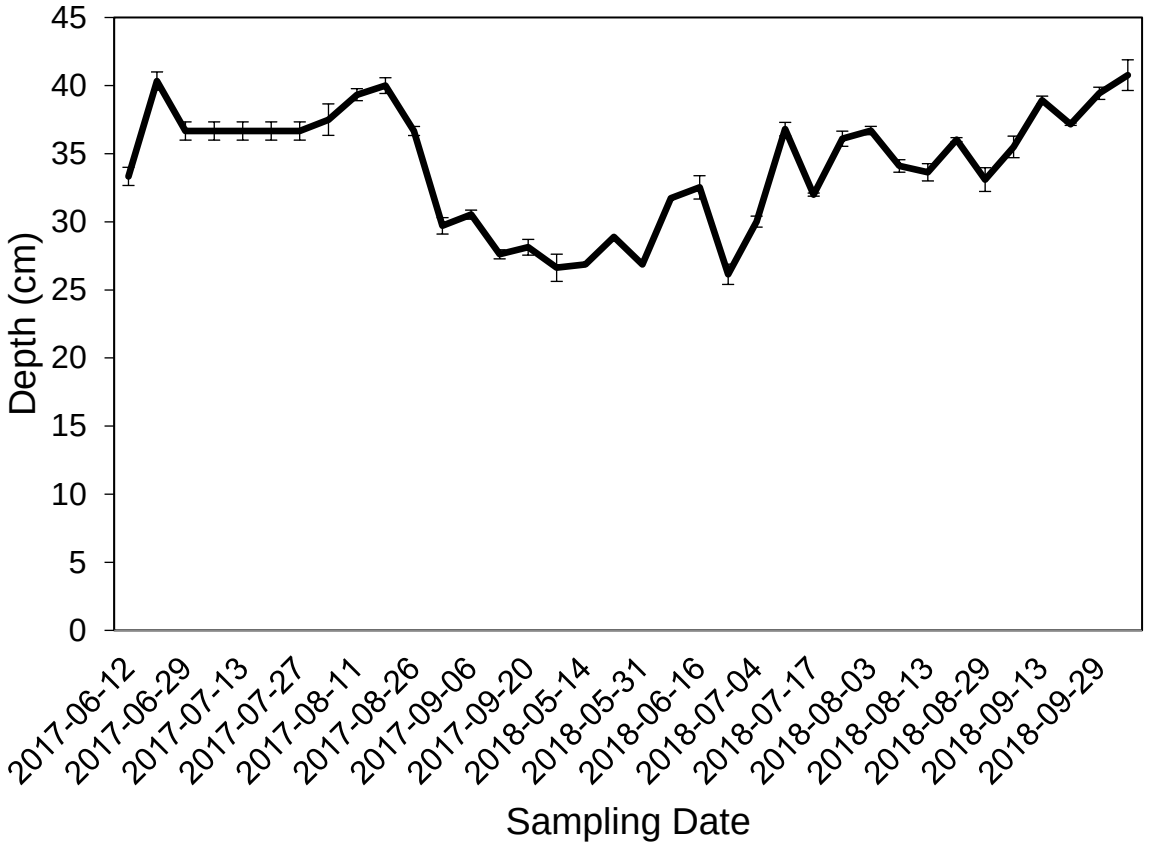


Figure B13. Mean±SE depths (cm) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Depth values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

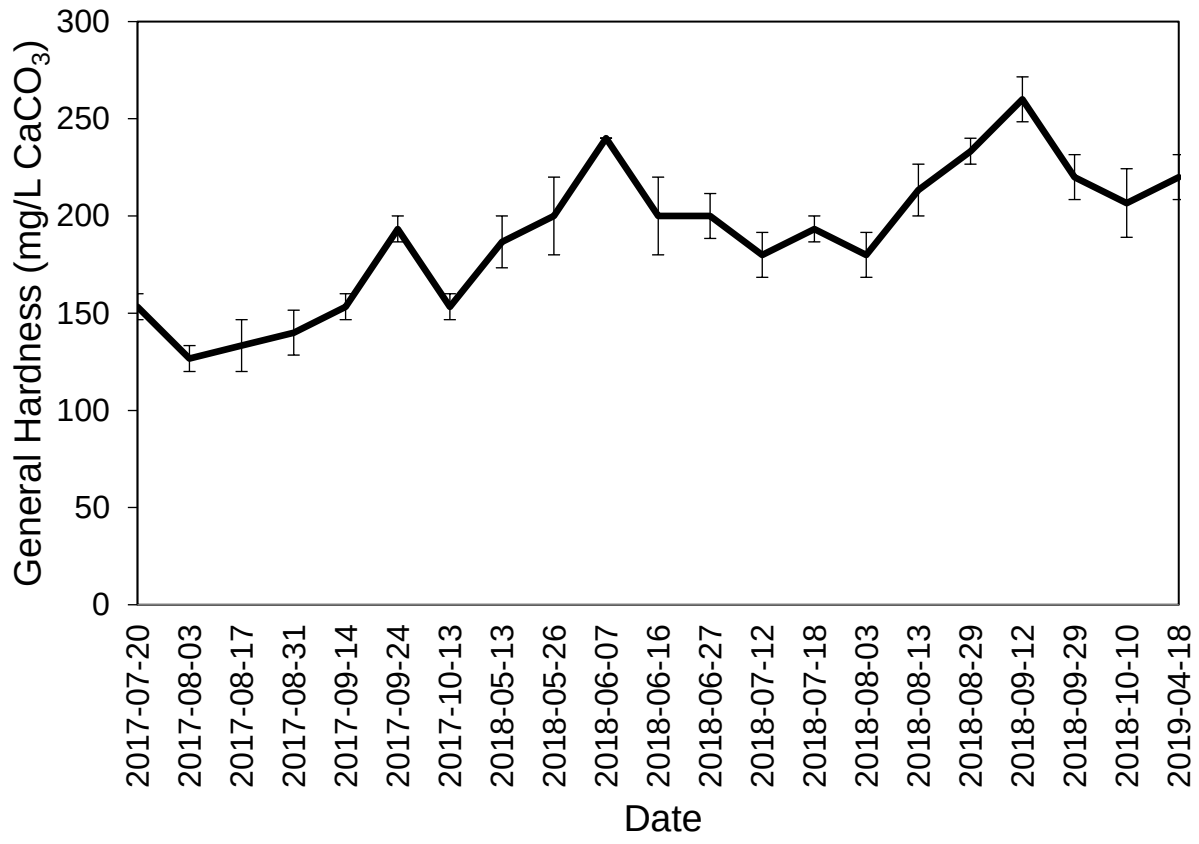


Figure B14. Mean±SE general hardness ($CaCO_3$) concentration (mg/L) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. General hardness values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

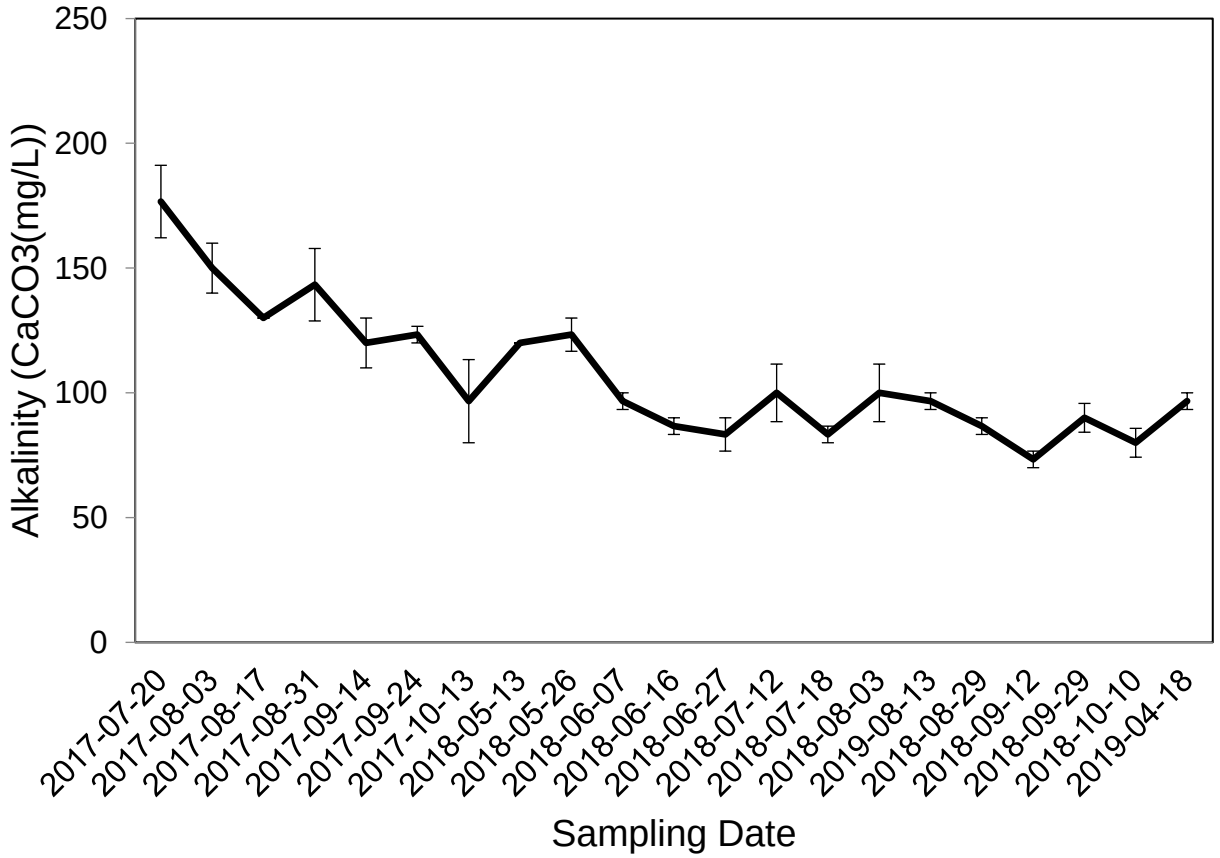


Figure B15. Mean±SE alkalinity ($CaCO_3$) concentration (mg/L) of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Alkalinity values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

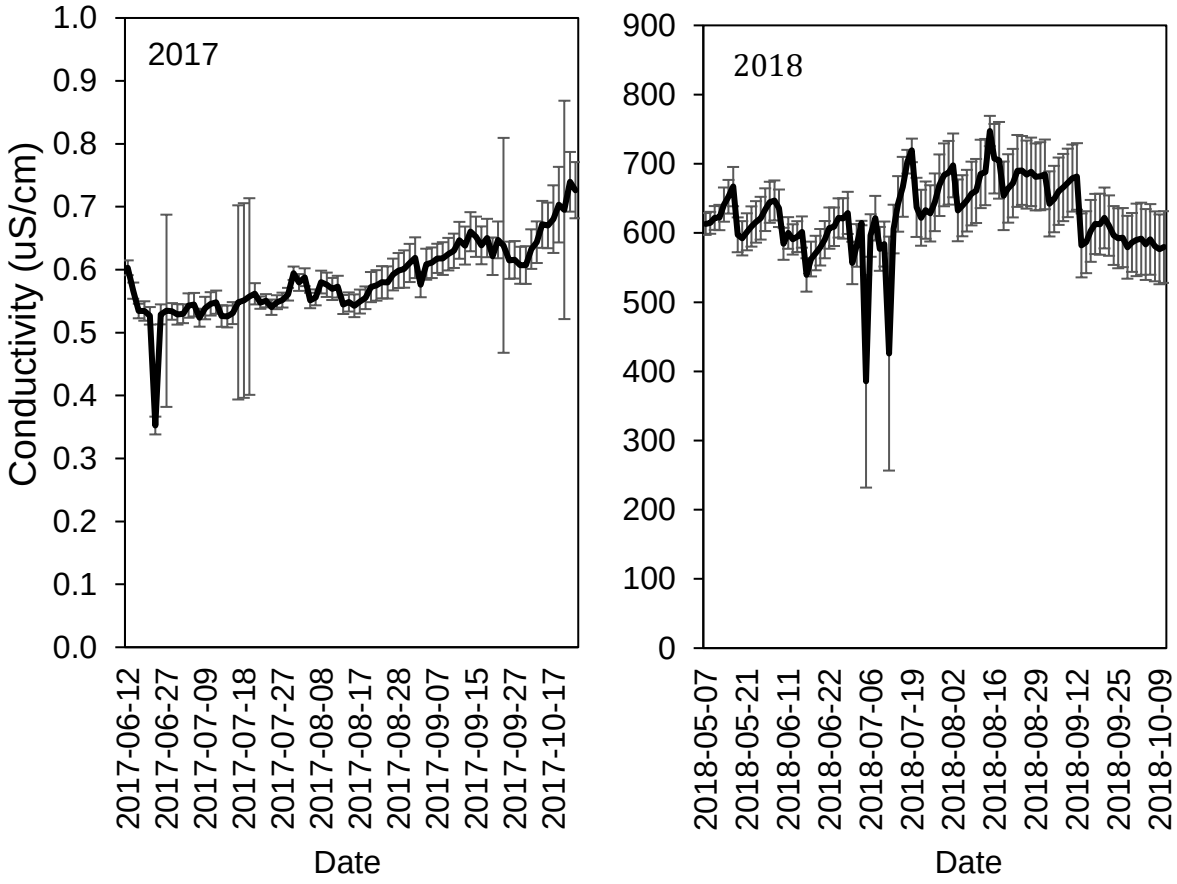


Figure B16. Mean conductivity concentration (uS/cm) in 2017 and 2018 of three mesocosms at the Prairie Wetland Research Facility (PWRF) in the long-term study. Conductivity values were averaged across replicates (n=3) for the microplastic treatment. Measurements were made using a YSI 6600 V2 Sonde every weekday morning from June 12, 2017 to October 10, 2018.

Figure B17. Foam behaviour (a) aggregation, (b) foams found outside of a tank (c) pressed against side of tank, (d) weathering and biofilm development on foams.

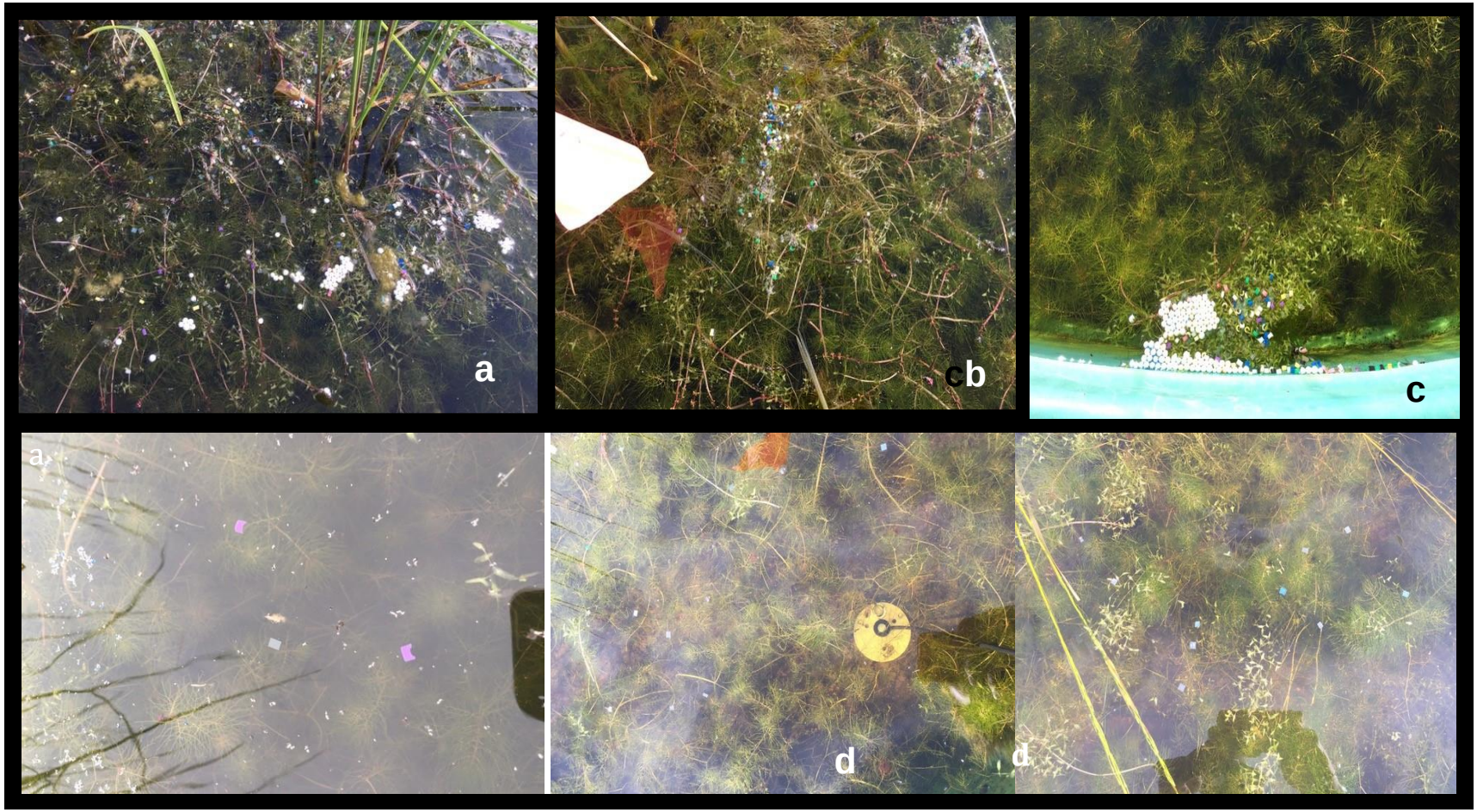


Figure B18. Fragment behaviour (a) aggregation with filamentous algae, (b) attached to emergent aquatic plants (c) pressed against side of tank, (d) fragments below surface water attached to submergent aquatic plants.

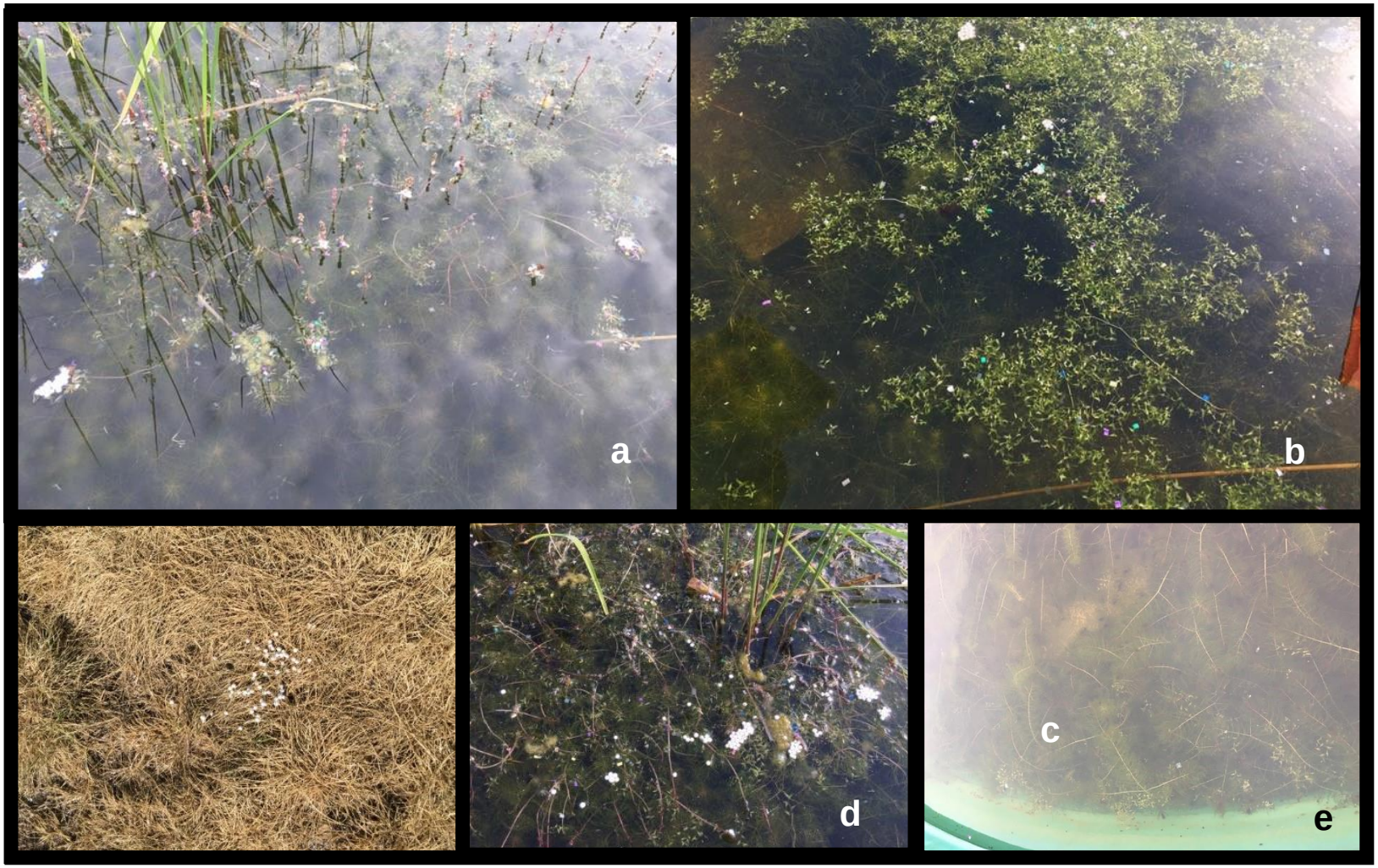


Figure B19. Film behaviour (a) aggregation with filamentous algae, (b) films floating by themselves in surface water, (c) films found outside of tank, (d) films attached to aquatic plants, and (e) weathering (visible colour change) of films.

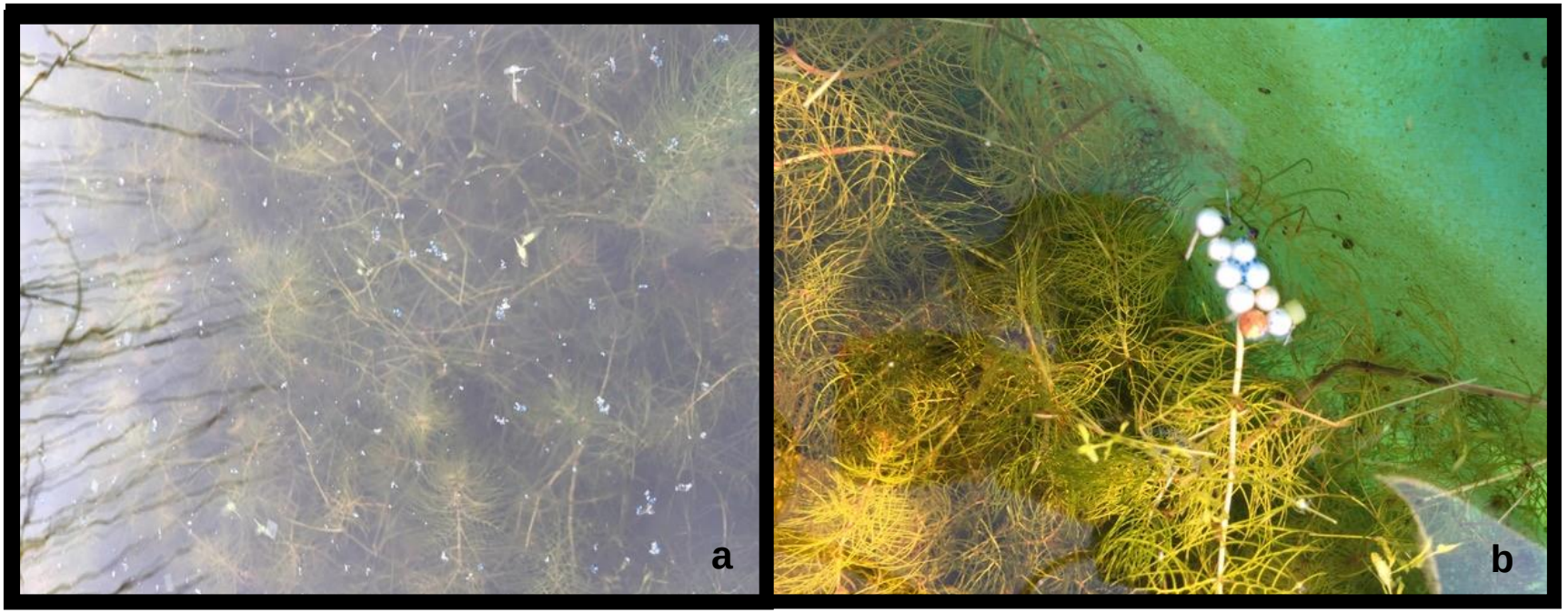


Figure B20. Microbead behaviour (a) homo-aggregation on surface water, and (b) hetero-aggregation with foams.

Appendix C: Short-term study

Tables

Table C1. Mean filamentous algae assessment of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Filamentous algae assessment values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made using a scale of 1 to 3 (1= no algae present, 2= distinct algal masses visible, 3= algal colonization), to approximate algal growth or productivity (Baxter et al. 2013). Measurements were made weekly from July 19, 2018 to October 25, 2018, and after ice melted on April 18, 2019.

Date	Control	Nutrient	Nutrient+Plant
2018-07-19	1	1	2
2018-07-27	1	1	1
2018-08-03	1	1	1
2018-08-10	1	1	2
2018-08-13	1	1	1
2018-08-14	1	1	1
2018-08-21	1	3	3
2018-08-29	1	1	2
2018-09-07	1	1	2
2018-09-14	1	1	2
2018-09-18	1	1	3
2018-09-28	1	1	2
2018-10-05	1	1	3
2018-10-08	1	2	3
2018-10-10	1	2	2
2018-10-25	1	1	2
2019-04-18	1	1	2

Table C2. Total phosphorus (TP; mg/L), of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. TP was measured using the integrative technique, samples were collected three times, pre-addition on August 14, 2018 (day 0), post-addition on August 14, 2018 (day 0), and on August 28, 2018 (day 14).

Tank	Treatment	Day 0 pre-addition TP (mg/L)	Day 0 post-addition TP (mg/L)	Day 14 TP (mg/L)
2	Nutrient	0.641	2.121	0.66
3	Nutrient+Plant	0.62	2.05	0.589
4	Nutrient+Plant	0.695	2.105	0.623
6	Control	0.553	0.553	0.688
7	Control	0.226	0.226	0.345
8	Nutrient	0.392	1.842	0.32
10	Nutrient	0.468	1.948	0.352
11	Nutrient+Plant	0.49	1.98	0.615
12	Control	0.587	0.587	0.65

Figures

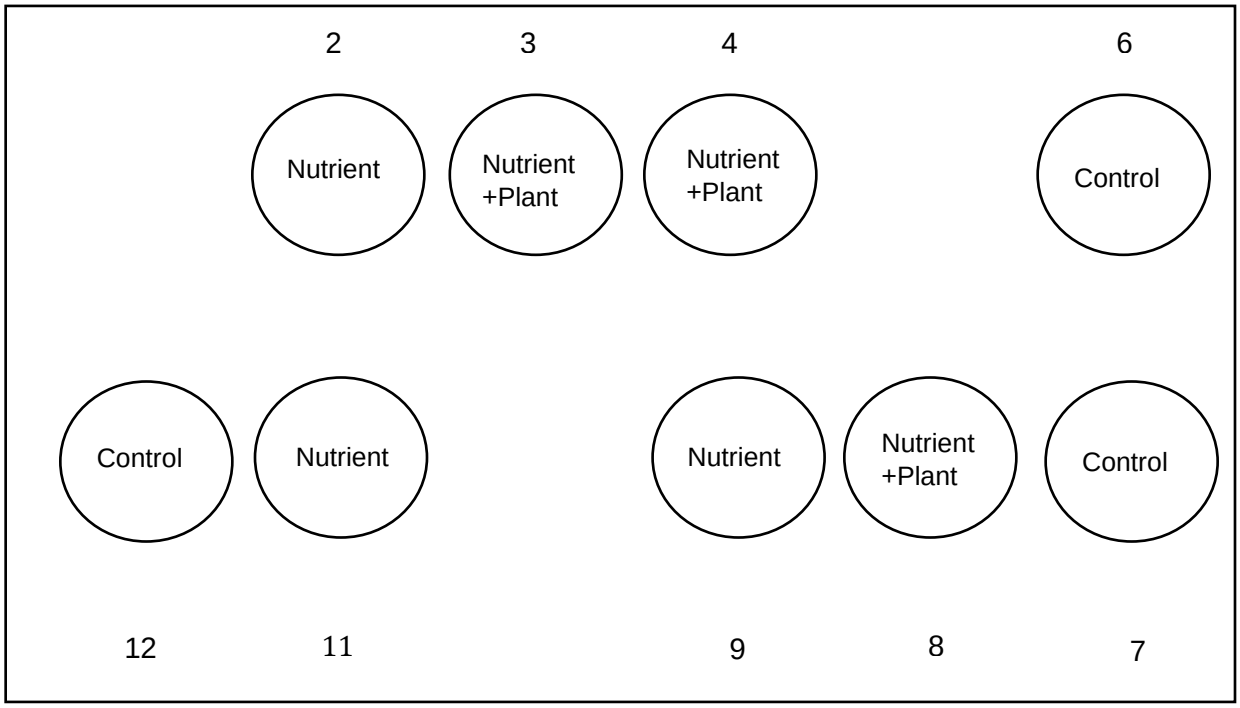


Figure C1. Experimental design of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. The treatments consist of: Control (microplastics only), Nutrient (synthetic wastewater addition, and microplastics), and Nutrient+Plant (synthetic wastewater addition, cattails, and microplastics).

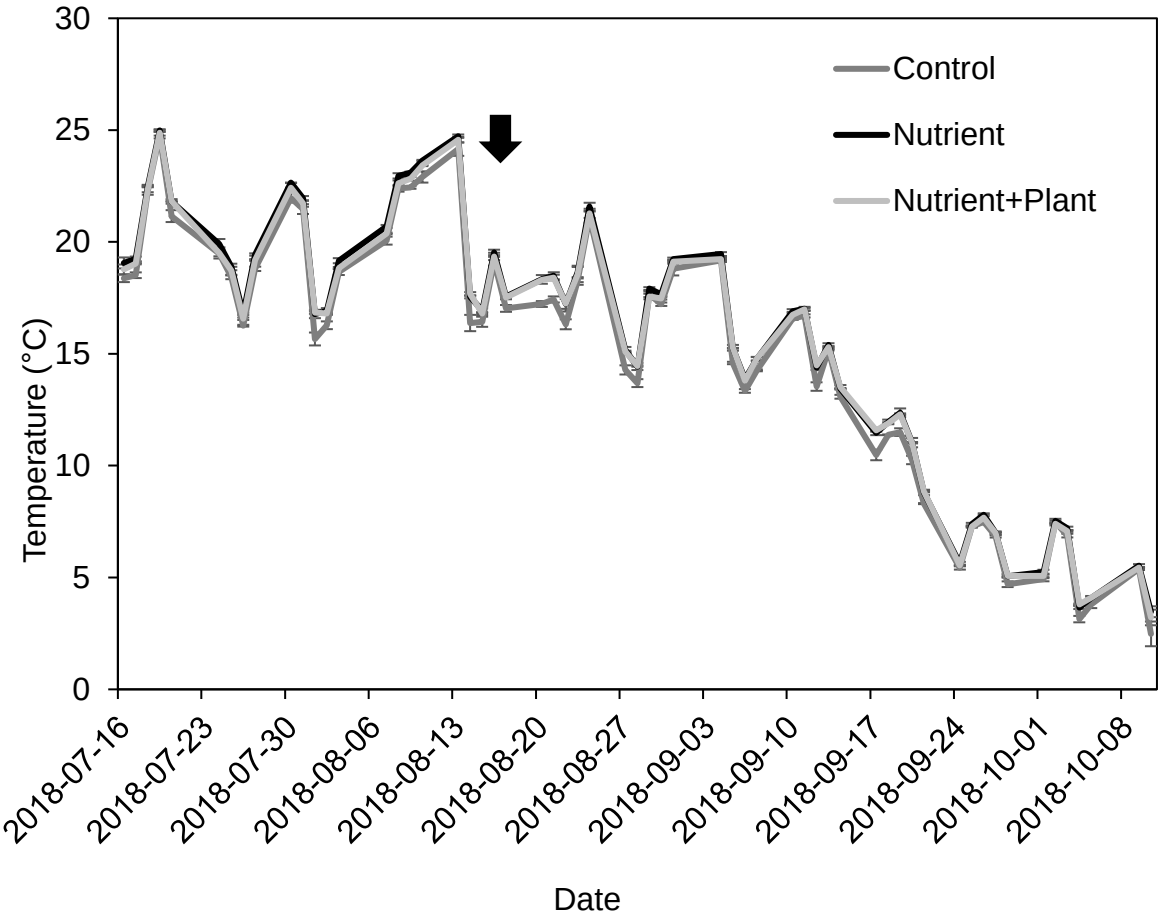


Figure C2. Mean±SE temperature (°C) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Temperature values were averaged across treatment replicates (n=3) for each of the three treatments (Control, Nutrient, and Nutrient+Plant). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

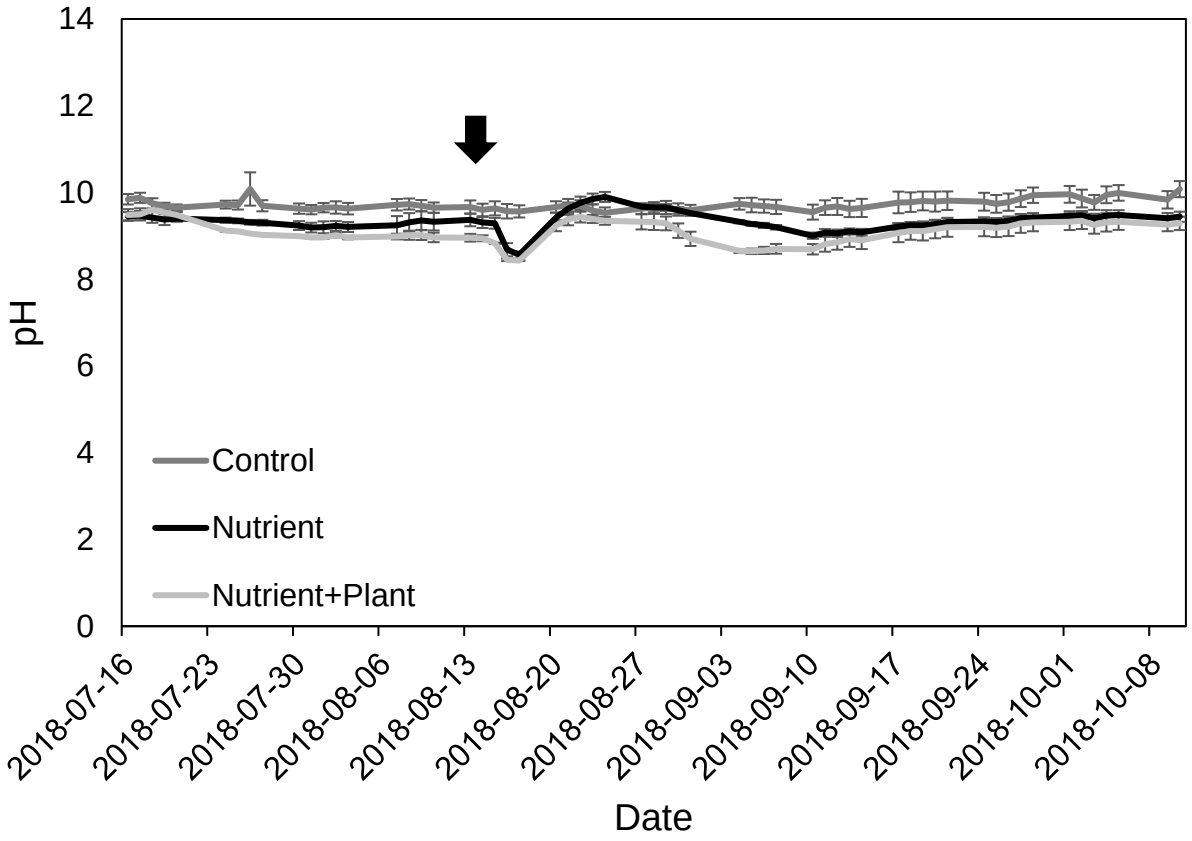


Figure C3. Mean±SE pH of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. pH values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

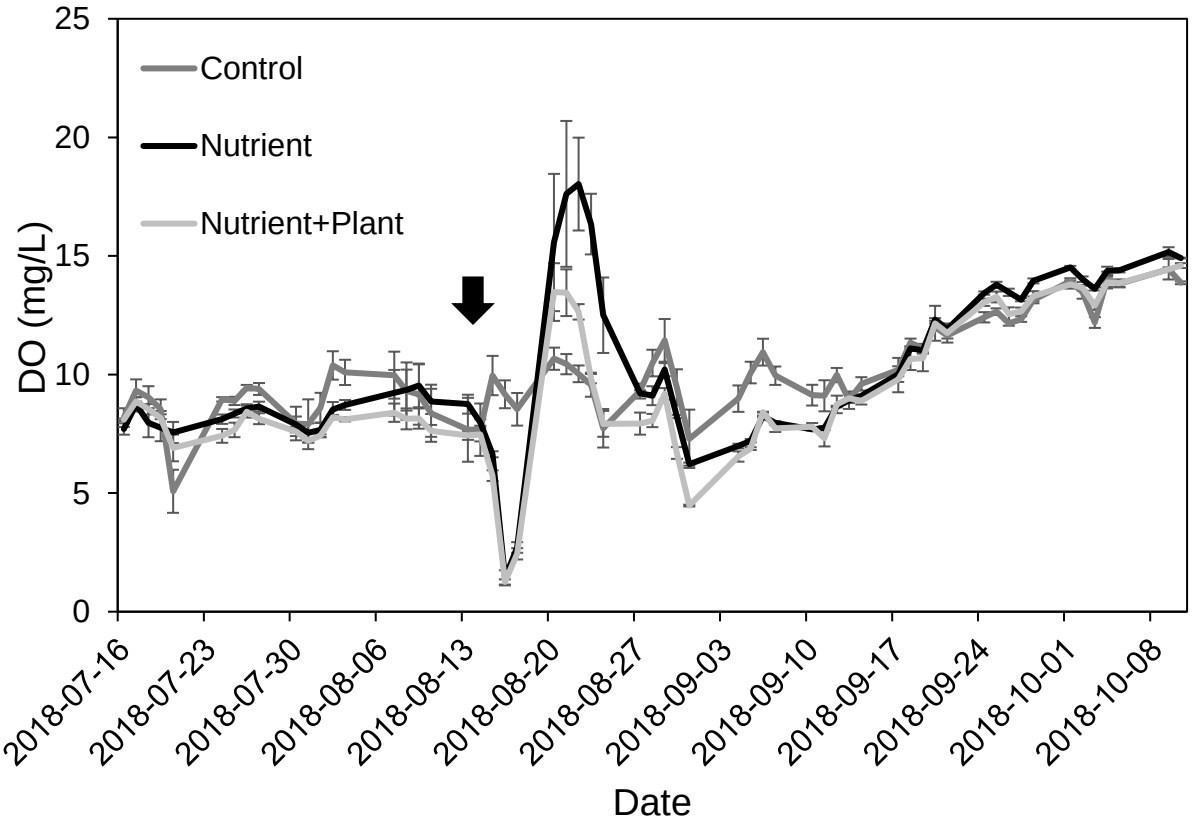


Figure C4. Mean±SE dissolved oxygen (DO) concentration (mg/L) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Dissolved oxygen values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient, and Nutrient+Plant). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

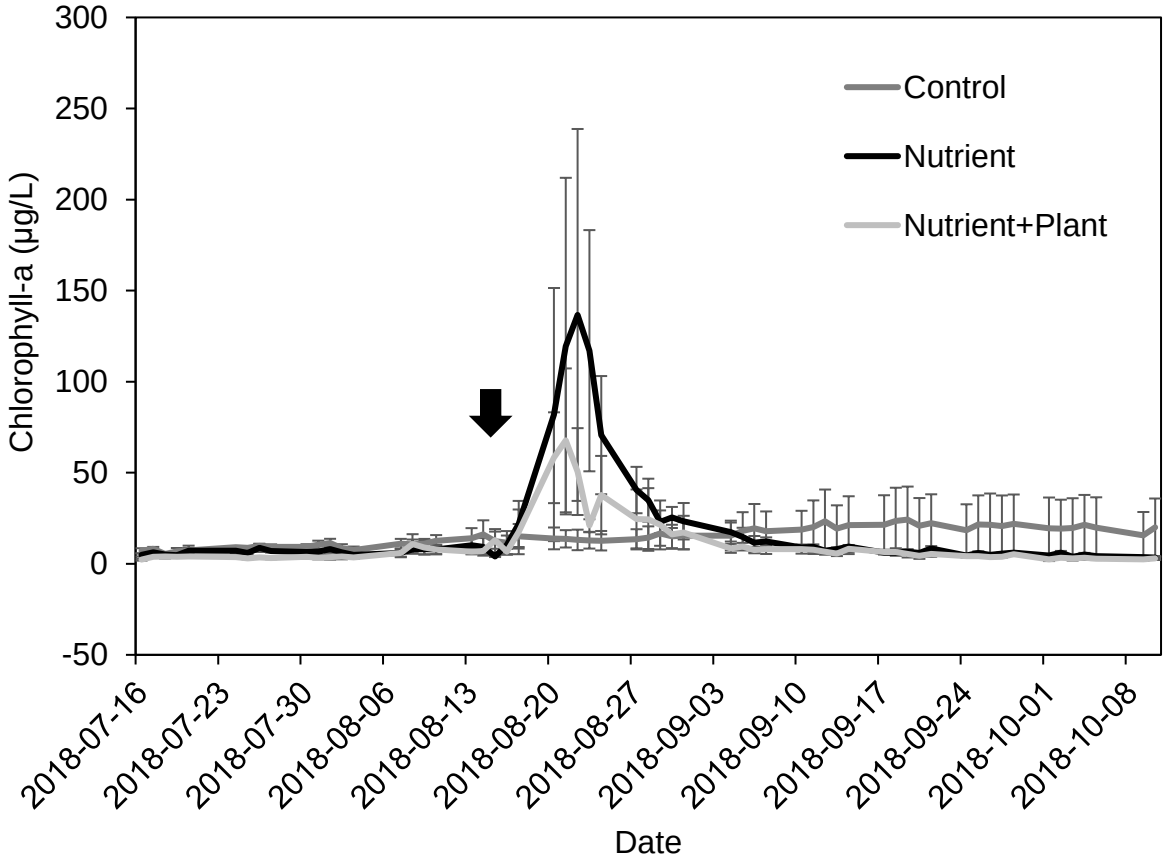


Figure C5. Mean±SE chlorophyll-a concentration (µg/L) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Chlorophyll values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

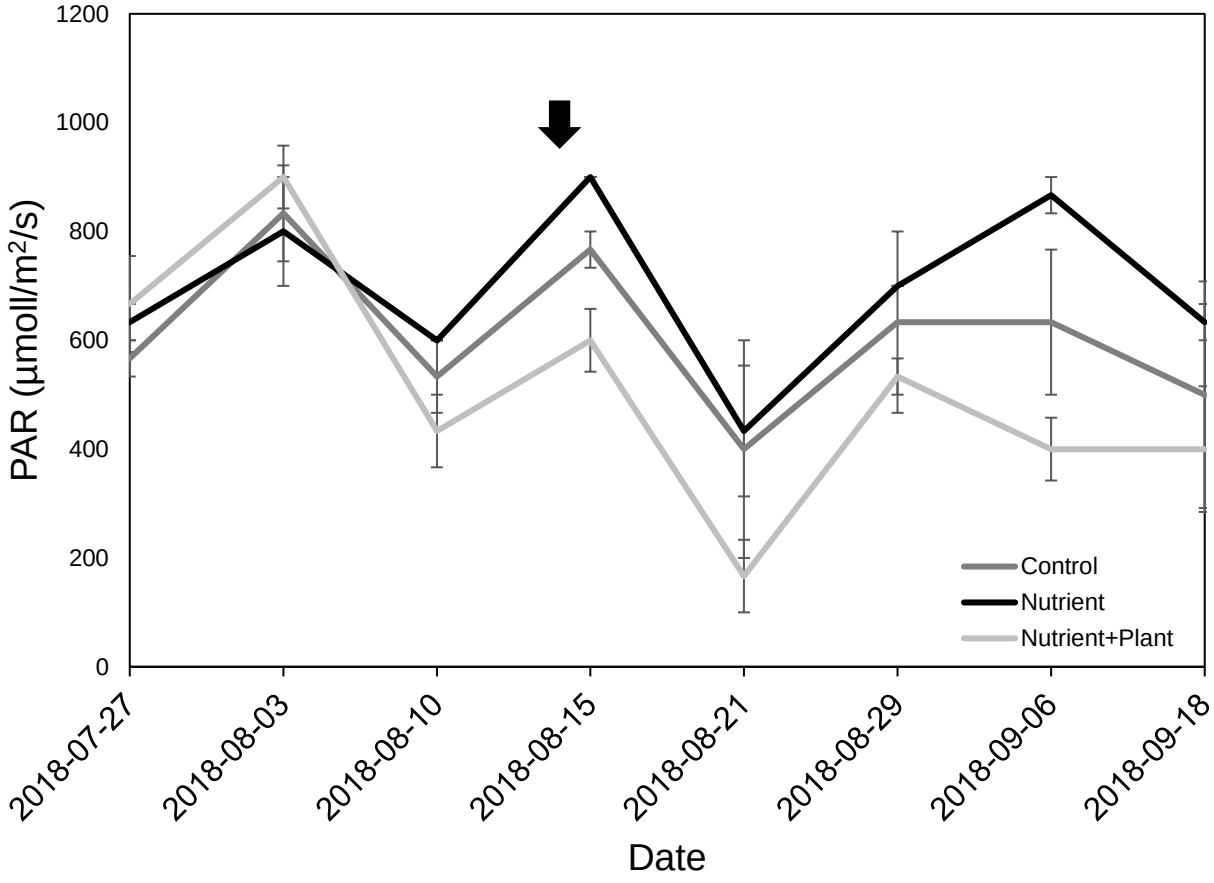


Figure C6. Mean±SE photosynthetically active radiation (PAR; µmol/m^2/s) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. PAR values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made with an Apogee MQ-200 quantum sensor weekly between 11:45 am and to 1:00 pm from July 27, 2018 to September 18, 2018 on sunny days. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

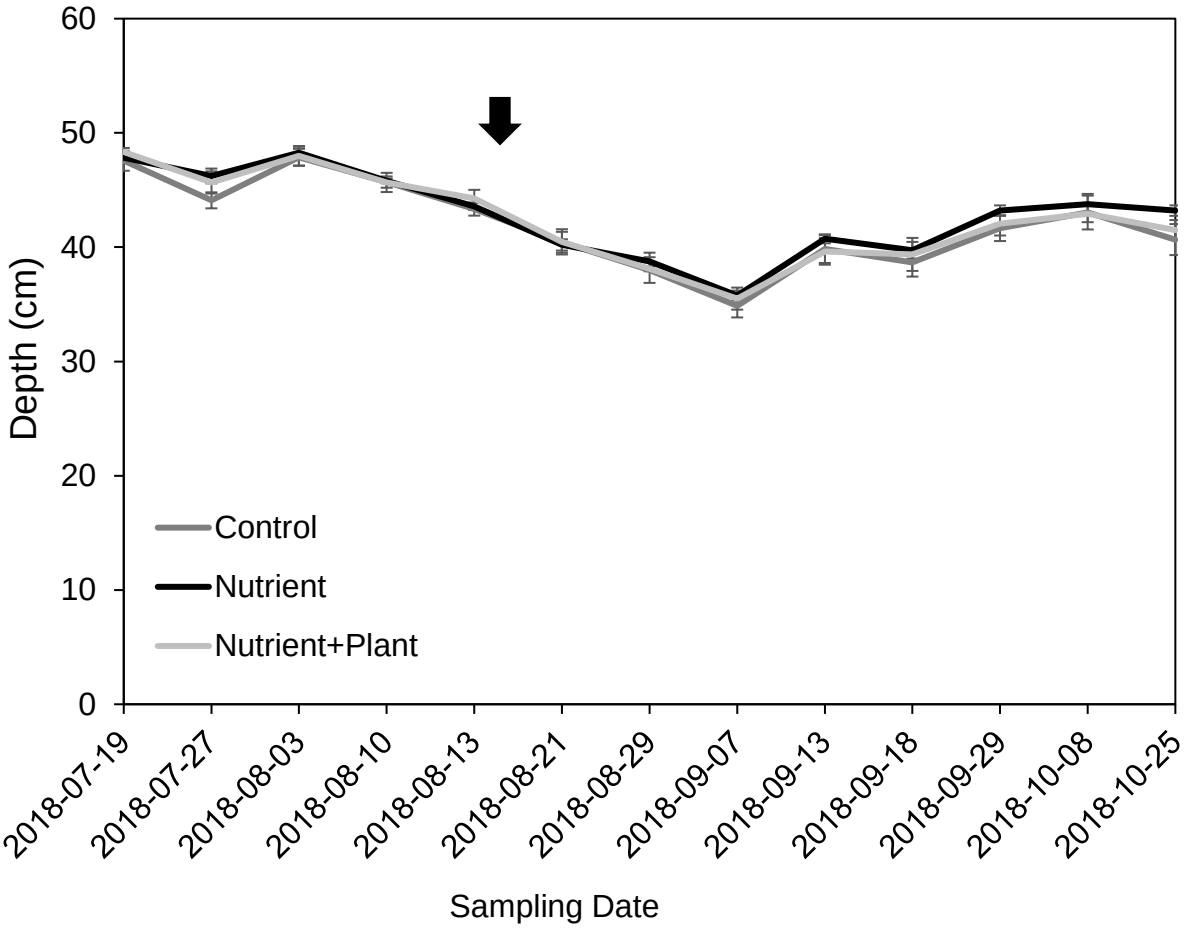


Figure C7. Mean±SE depths (cm) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Depth values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made with a meter stick weekly from July 19, 2018 to October 25, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

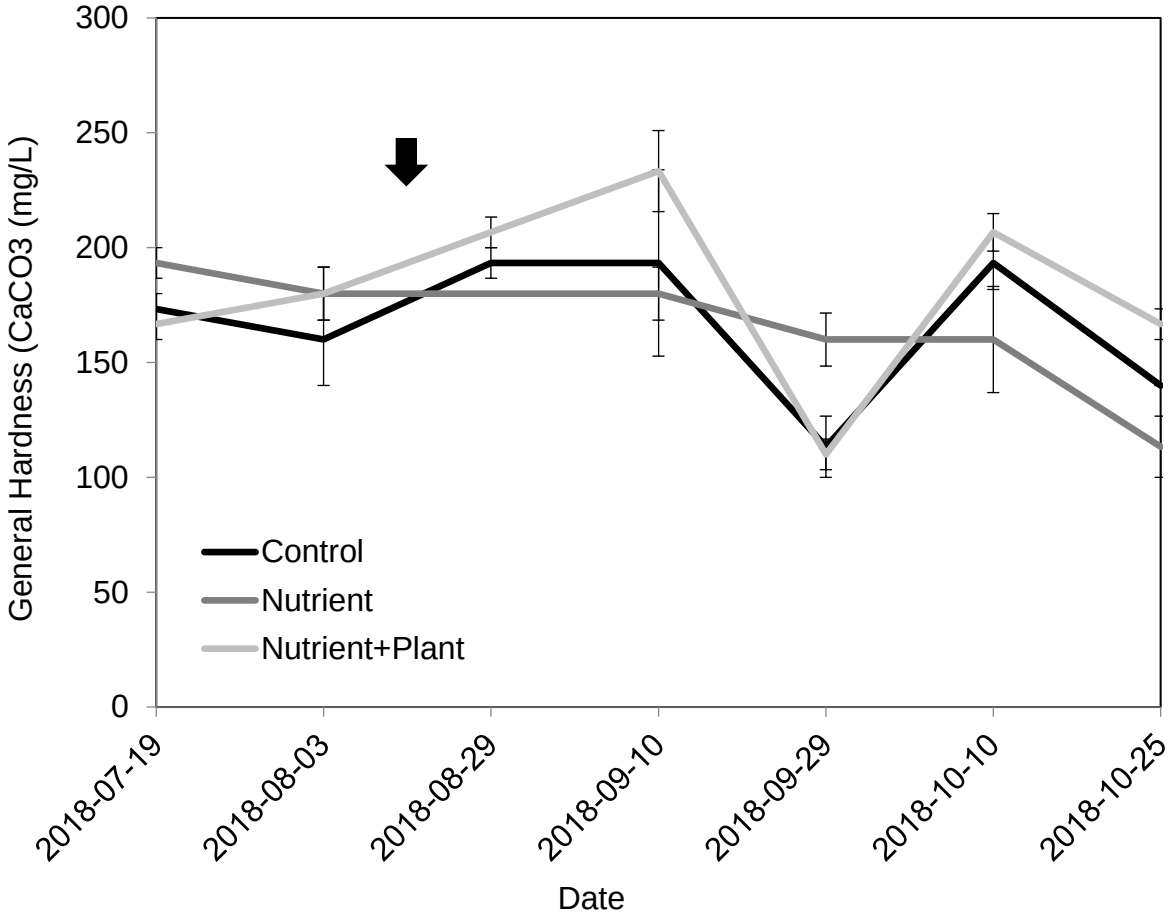


Figure C8. Mean±SE general hardness ($CaCO_3$) concentration (mg/L) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. General hardness values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). General hardness was measured using Nutrafin aquarium test kits, weekly from July 19, 2018 to October 25, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

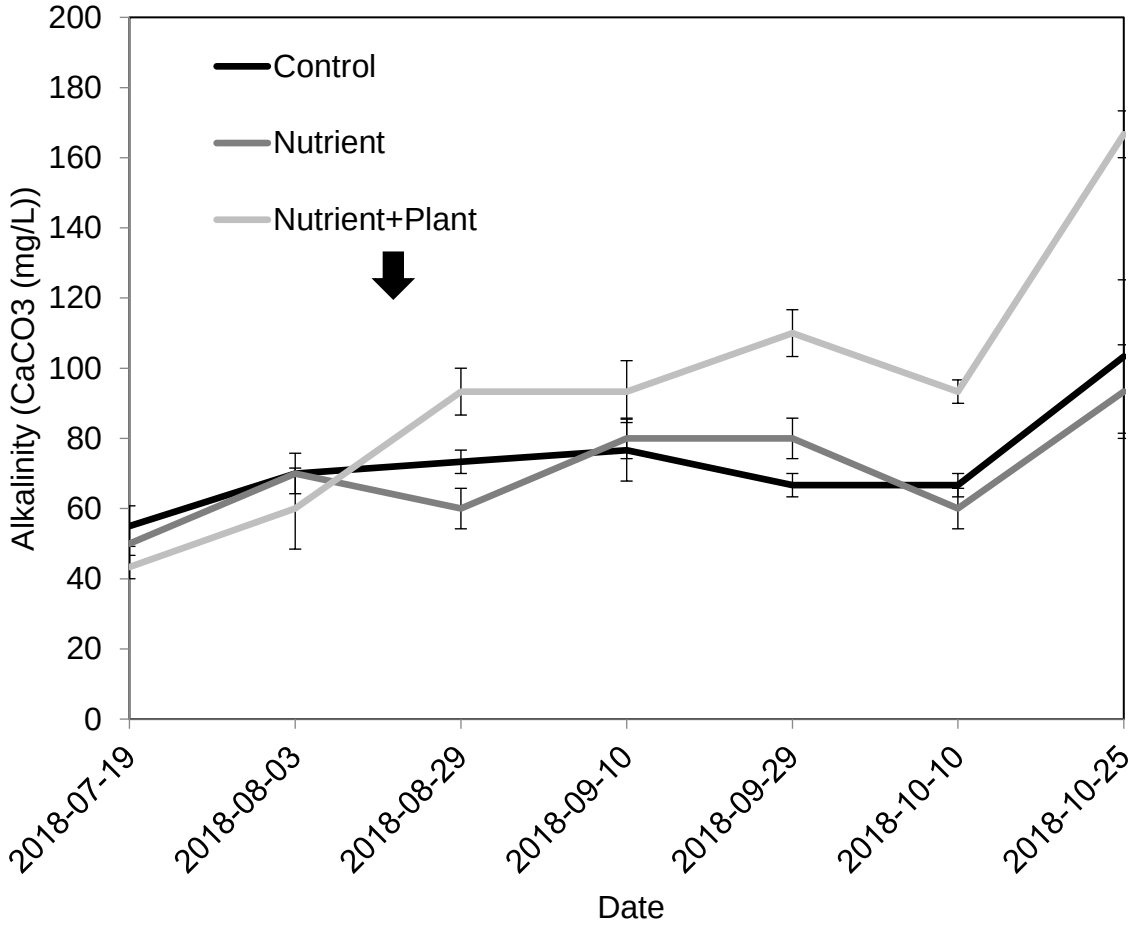


Figure C9. Mean±SE alkalinity ($CaCO_3$) concentration (mg/L) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Alkalinity values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Alkalinity was measured using Nutrafin aquarium test kits, weekly from July 19, 2018 to October 25, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).

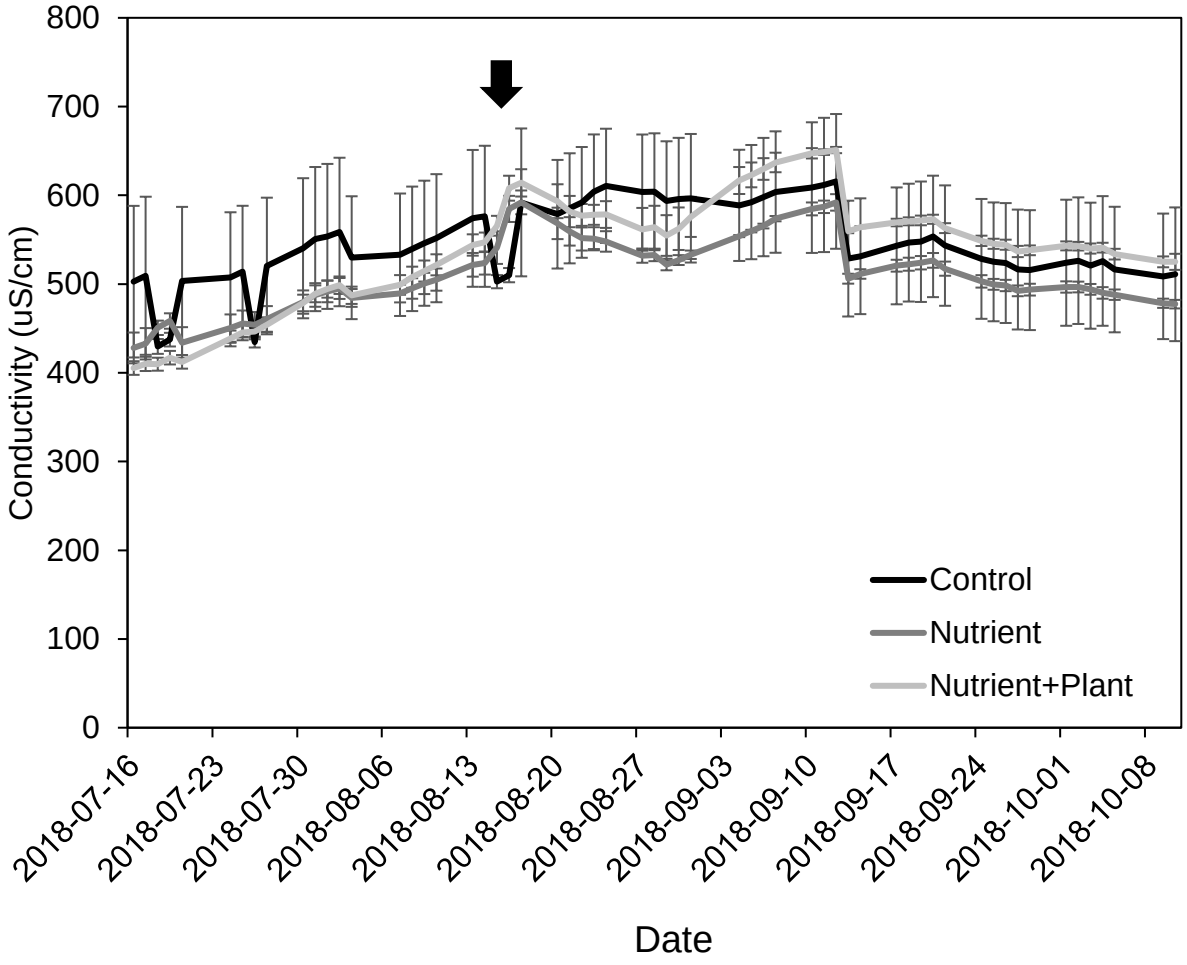
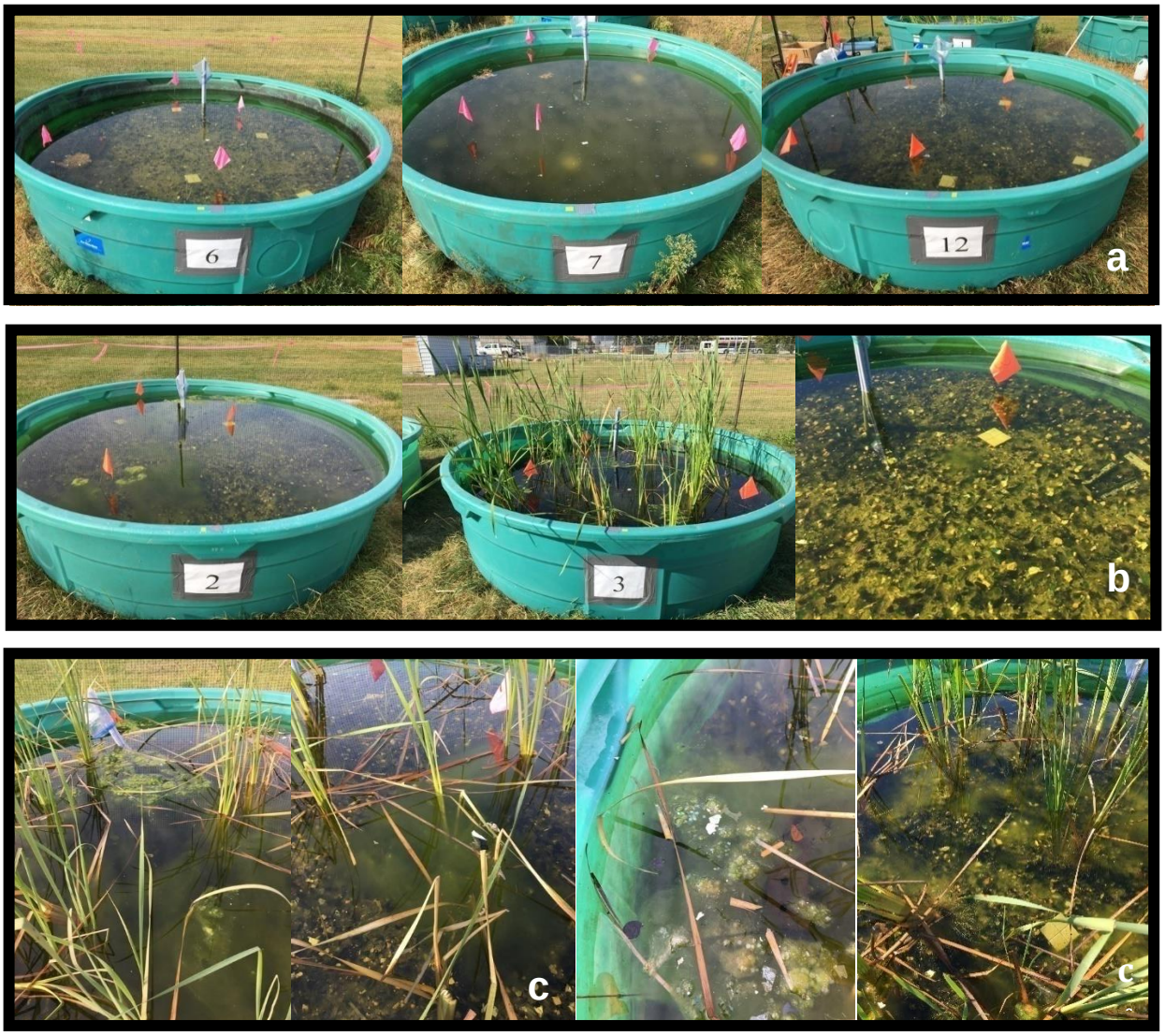


Figure C10. Mean±SE conductivity concentration (uS/cm) of nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Conductivity values were averaged across replicates (n=3) for each of the three treatments (Control, Nutrient and Nutrient+Plant). Measurements were made using a YSI 6600 V2 Sonde every weekday morning from July 16, 2018 to October 10, 2018. The arrow indicates when the synthetic wastewater was added to the exposed treatments (excluding controls).



C11. Photos of filamentous algae in the Control, Nutrient and Nutrient+Plant treatments in nine mesocosms at the Prairie Wetland Research Facility (PWRF) in the short-term study. Examples of low to high (1 to 3), where (a) low (1)= no algae present, (b) medium (2)= distinct algal masses visible, (c) high (3)= algal colonization.

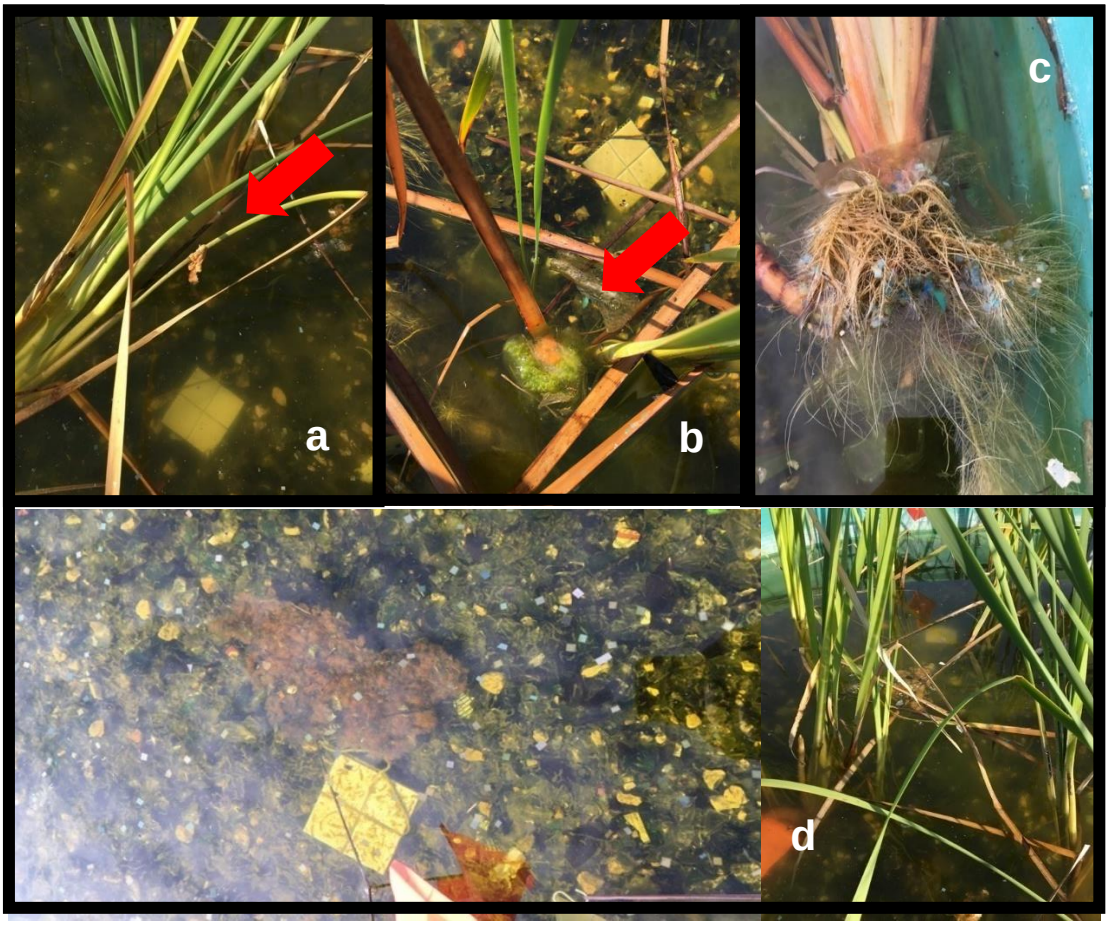


Figure C12. Fibre and film behviour in the mesocosms (a) fibres stuck to cattials, both fibres and films in hetero-aggregates stuck in filamentous algae, (b) and filamentous algae trapping films and fibres, (c) cattail roots trapping films, (d) fiber and film hetero-aggregates floating on surface and settling to the crushed glass.

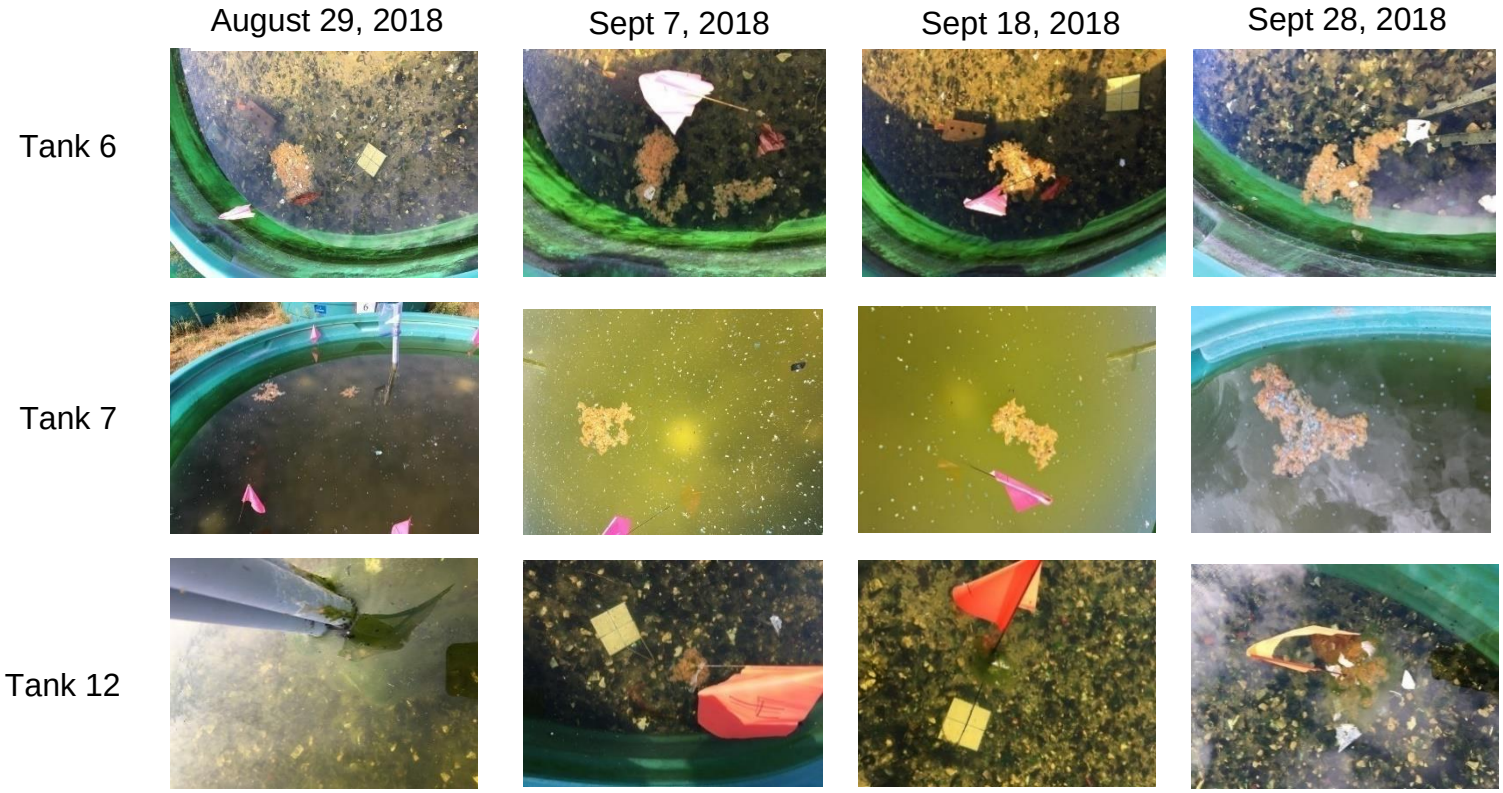


Figure C13. Visual observation photos of the Control treatment tanks taken August 29, 2018 to April 18, 2019.

Figure C13.Continued.

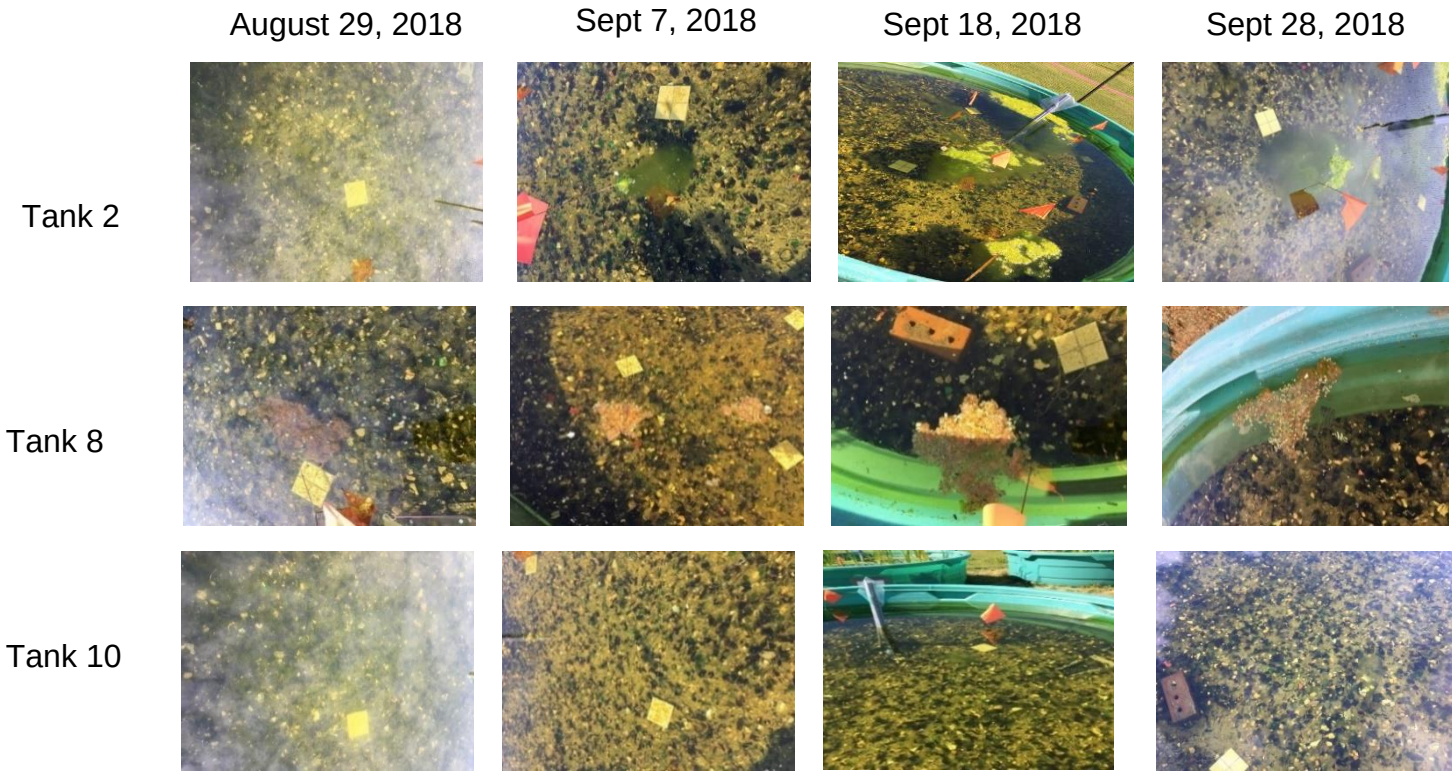


Figure C14. Visual observation photos of the Nutrient treatment tanks taken August 29, 2018 to April 18, 2019.

Figure C14. Continued.

Figure C15. Visual observation photos of the Nutrient+Plant treatment tanks taken August 29, 2018 to April 18, 2019.

Figure C15. Continued.

Figure C16. Microplastics frozen in surface layer of ice.