POLYMER TYPE AFFECTS THE VERTICAL DISTRIBUTION OF MICROPLASTICS IN BOREAL LAKE SEDIMENTS

by

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Dalhousie University is located in Mi'kma'ki,
the ancestral and unceded territory of the Mi'kmaq.
Our study lakes are situated on Treaty 3,
the traditional lands of the Anishinaabe Nation and the Métis Nation.
We are all Treaty people.

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Abstract

Freshwater ecosystems serve as a receiver, transporter, and sink for microplastic pollution, as well as connect the terrestrial, atmospheric, and marine environment. Lakes are disproportionately affected by microplastic pollution, as microplastic concentrations tend to be higher in low energy environments, and particles are readily captured and deposited into sediments of enclosed waters. An estimated 99% of microplastic particles eventually settle in bottom sediments; yet, knowledge on the processes governing microplastic accumulation in lake sediments is limited, partially due to a lack of data from ecosystem-wide experiments. These processes likely vary with lake morphology, environmental conditions, and the particle size and polymer types (varying densities). This thesis provides a literature review and contributes to a Before-After-Control-Impact (BACI) whole ecosystem experiment called The pELAstic Project to elucidate the fate of microplastics in lake ecosystems. Three common microplastic polymers of different densities (polyethylene terephthalate [PET], polystyrene [PS], and low-density polyethylene [LDPE]) and sizes (15 – 1000 µm) were added to our experimental lake, L378 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA). Each application of microplastics to L378 occurred bi-weekly July – August, 2023, simulating stormwater. Here, I showcase data from the first year of additions, of a planned 3-year project, to assess the accumulation of microplastics of these three common polymer types in lake sediment. To analyze the migration potential of the polymers, I collected a combination of sediment cores and sediment grab samples across a water depth gradient. Sediment characteristics (water content, organic content, and mineral content) were determined, and showed significant differences in character between the littoral and profundal samples. From this I delineated the erosional, transitional, and depositional zones for L378. The most common polymer identified through microscopy was PS, followed by PET. LDPE was found in much lower concentrations; a negative association was found for water depth and LDPE. However, sediment depth was found to be a predictor of microplastic concentration across polymers. An interaction between sediment depth and size fraction was identified for PET and LDPE concentrations. Thus, polymer type as well as size fraction may impact microplastic accumulation within the sediment profile of boreal lakes. This work has contributed to a better understanding of the drivers of horizontal and vertical microplastic accumulation in sediment. Building on these findings, future experimental work to be completed in 2024 and 2025 will continue to explore these mechanisms, especially those driving vertical accumulation, to ultimately improve the prediction of microplastic hotspots in sediment and thereby direct the development and application of *in situ* remediation methods.

List of Abbreviations Used

AOPs …….. Advanced Oxidation Processes
BACI ……. Before-After-Control-Impact
$CaCl_2$ ……..…….. Calcium Chloride
CO_2 ……. Carbon Dioxide
DWTP ……...……... Drinking Water Treatment Plant
ELA ..……..……. Experimental Lakes Area
EPR ……..…….. Extended Producer Responsibility
ER …….. Ecological Risk
ERA …… Ecological Risk Assessment
FTIR ..…….…….……. Fourier Transform Infrared
H_2O …… Water
H_2O_2 ……...…….. Hydrogen Peroxide
IISD-ELA …….
……. International Institute for Sustainable Development-Experimental Lakes Area
KS test …….. Kolmogorov-Smirnov Test
L373 ……...…….. Lake 373
L378 ..……...…… Lake 378
LCA …… Life Cycle Assessment
LDPE ..……..…… Low-density Polyethylene
LOI ..……...…….. Loss-on-ignition
MP ……..……. Microplastic
MPs ……...……. Microplastics
NLA ……...….. National Lakes Assessment
NPs ..……...…… Nanoplastics
PET ..……... Polyethylene Terephthalate
PNEC …….. Predicted No-effect Concentrations
PPE ……...…. Personal Protective Equipment
PRF ……. Plastic Recycling Facility
PS ..…….…….. Polystyrene
QA/QC ……..…… Quality Assurance and Quality Control
ROV ……..….. Remote Operated Vehicle
SBS …….…… Social and Behavioural Sciences
SDGs …… Sustainable Development Goals
SDs …….. Standard Deviations
TOC ..……...……. Total Organic Carbon
WWTP …… Wastewater Treatment Plant

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CHAPTER I

Introduction

One of the most poorly understood types of aquatic pollution are plastics of various sizes, including microplastics (Jones et al., 2020; Koutnik et al., 2021; Schwarz et al., 2023; Tirkey et al., 2022). Global plastic production has grown 20-fold in the last 50 years and is predicted to continue its exponentially increasing trend for the foreseeable future (Borrelle et al., 2020). Circa 50-79% of plastics produced are unaccounted for, most of which have accumulated in natural ecosystems, which corresponds to an estimated 19-23 metric tons generated in just 2016 that are expected to have reached aquatic ecosystems (Borrelle et al., 2020; Zalasiewicz et al., 2016). This 'quest' for the missing plastic represents a major knowledge gap and illustrates the urgency of research in this area.

(Micro)plastic pollution research has its origins in oceanography (Rochman, 2020), with research in other ecosystem compartments, such as freshwater, growing rapidly in recent years (Allen et al., 2022; Munien et al., 2024). The first scientific findings of small plastic particles were reported in 1972 in the Saragasso Sea (Carpenter et al., 1972; Carpenter & Smith, 1972; Rochman, 2020); Carpenter and Smith (1972) predicted the stark increase of plastic particle concentrations in the marine environment as a result of expanding plastic production and insufficient waste disposal practices. However, plastic debris was only properly counted in 1986, ultimately setting in motion the discovery of the 'Great Pacific Garbage Patch' in 1996 (Moore et al., 2001), and inspiring the publication of the first long-term dataset on plastic debris in 2010 (Law et al., 2010; Rochman, 2020).

The modern term 'microplastics', defined as plastic particles smaller than 5 mm in size, was coined only 20 years ago by Richard Thompson who analyzed ocean sediments and surface waters and brought the urgency of increased research efforts to understand microplastic pollution to the attention of the scientific community (*International Marine Litter Research Unit*, 2024; Thompson et al., 2004). With microplastics accounting for up to 92.4% of floating plastics on the ocean surface, scientific focus has been shifting towards these tiny pollutants (Darabi et al., 2021), as

scientists are trying to make sense of the extent, effects, and fate of microplastic pollution (Allen et al., 2022; Besseling et al., 2017; Rochman, 2020).

Freshwater systems play a crucial role in the distribution and deposition of microplastics (Li et al., 2020). Lakes and rivers serve as the connector between terrestrial and marine ecosystems and provider of a significant portion of our drinking water, yet they are among the least studied systems in terms of microplastic contamination (Wagner et al., 2014). Within aquatic systems (including freshwater systems), sediments have been identified as the main sink for microplastics, with up to 99% of microplastic particles accumulating in bottom sediments of water bodies (Kane et al., 2020; Sandgaard et al., 2022). Whereas the impacts of microplastic pollution on individual aquatic systems may differ due to considerable variation of the effects and the long-term physical and chemical fate of microplastic particles (Karbalaei et al., 2018), many scientific studies proving the presence of microplastics in these ecosystems already warn about potential harmful environmental and human health impacts (Ajay et al., 2021; Hengstmann et al., 2021). Thus, numerous questions on the life cycle of microplastics, and more specifically their long-term fate in freshwater ecosystems, remain as we try to understand whether and how these systems interact with and recover from microplastic pollution (Rochman & Hoellein, 2020).

Here, I have examined microplastic concentrations and accumulation patterns in boreal lake sediments to better understand the drivers of horizontal and vertical microplastic accumulation that may direct microplastic fate in sediments. Characterizing these mechanisms, especially those driving vertical accumulation, is required to improve the prediction of microplastic hotspots in sediment and may be utilized to advance the development and application of *in situ* remediation methods of microplastic pollution. As such, I 1) demonstrate the importance of freshwater sediments to understand microplastic fate and remediate microplastic pollution in aquatic ecosystems (Chapter 2), 2) explore the sediment characteristics, microplastic contamination levels in sediment, and the relationship of both at the experimentally manipulated L378 of the IISD-ELA (Chapter 3), and 3) thematize issues of poor standardization and lacking data on mechanisms driving the transport of microplastics in the vertical sediment profile (Chapters 3 and 4). This will allow me to assess microplastic accumulation patterns and hotspots in

sediments and identify their potential predictors and remediation implications. This research is important because microplastic remediation is lacking reliable ecosystem-wide data to develop adequate technologies for the removal of microplastics from aquatic ecosystems and thus mitigate global microplastic pollution. In 2024-2025, the dosing of L378 will continue, and in the years 2026-2030 L378 will be monitored to quantify microplastic effects, determine microplastic fate in environmental matrices, and trial remediation technologies for microplastic removal, to ultimately help resolve knowledge gaps related to the impacts and accumulation patterns of microplastics in freshwater systems and direct the development of *in situ* remediation technologies.

This research is part of a larger project called The pELAstic Project. As a collaborative research project, The pELAstic Project brings together Canadian and US American scholars (based at eleven universities), government representatives, and experts from the International Institute for Sustainable Development Experimental Lakes Area (IISD-ELA) to better understand the effects and fate of microplastics in freshwater environments (*The pELAstic Project*, 2024). More specifically, the project is conducting a whole-lake experiment to describe effects, physical and chemical fate of microplastics across all levels of biological organization (molecular to whole ecosystem level) and monitoring the impacts on the lake ecosystem over a time span of ten years (Orihel et al., 2022; *The pELAstic Project*, 2024). This experiment may contribute to the development of national and international mitigation and remediation frameworks targeting microplastic pollution.

1.1 Thesis objectives and hypotheses

The goal of this thesis is to assess the accumulation of microplastics of three common polymer types in sediment from a whole-lake addition experiment. Determining the physical fate of microplastics in sediments across the lake has implications for the remediation of microplastic-contaminated lake sediments at this site and in a global context. To assess microplastic accumulation patterns in sediment and their potential relationship to sediment characteristics, I 1) characterize the composition of sediments in Lake 378 (water, organic, and mineral content) and assess how sediment composition varies across a water depth gradient spanning the littoral and profundal zones, 2)

examine the relationship between microplastic particle accumulation in sediment and water depth for three common polymer types of different densities: low-density polyethylene (LDPE), polystyrene (PS), and polyethylene terephthalate (PET), and 3) determine how deeply into the sediment microplastic particles of these three polymer types penetrate in the area where particles accumulate in the sediment. In conducting this research, I developed the following hypotheses and predictions:

1) Sediment composition (water, organic, and mineral content) most likely varies with water depth, differing between littoral and profundal sediments due to the processes of erosion and deposition in the lake. If this is the case, then I predict that sediment from deeper lake areas where depositional processes dominate will have higher organic content and lower mineral content than sediments collected from shallower areas of the littoral zone, where erosional processes prevail. This is important because depositional processes will influence the fate of microplastics in the lake.
2) The relationship between microplastic particle accumulation in sediment and water depth is likely driven by gravity. If microplastic particles are subject to gravity driven processes, then I predict that the concentration of microplastics will be greatest in the depositional zone of the lake, where organic content of the sediment is higher. I predict that microplastic concentration will generally increase with water depth. I further predict that, because the polymers differ in density, the slope of this relationship between water depth and microplastic concentration will be greatest for the densest polymer type (PET), compared with the mid-density (PS) and low-density (LDPE) polymers.
3) Finally, in the lake area where microplastic particles are accumulating, microplastics are expected to settle from the water column, resulting in their addition onto the sediment surface. Therefore, I predict that the concentration of particles will be highest in the surface sediment. However, due to a combination of gravity driven and biologically driven processes, particles may travel down deeper into the sediment profile. If bioturbation is the main driver, I expect to see nearly all (>95%) of the microplastic particles within the upper 3-5 cm of sediment, since this is the depth of active mixing by the benthos common to Lake

378 (chironomids and oligochaetes). I further predict that, if benthic macroinvertebrates are principally responsible for the mixing, all polymer types should penetrate equally deep into the sediment. If gravity drives the process, then I predict that particles may sink beneath this 3-5 cm active mixing horizon. I further predict that, if gravity is the dominant force driving this mixing, the densest polymer type (PET) will sink most deeply, followed by PS, with LDPE being limited to the upper layer of sediment. Finally, I predict that the smaller (53 μm) particles will be more mobile as they require less pore space and are impacted by friction to a lesser extent. Thus, smaller (53 μm) particles will penetrate the deeper sediment layers, followed by the medium-sized (106 μm) particles, with larger (212 μm) and thus less mobile particles being confined to the upper sediment layers.

CHAPTER II

Literature Review: The fate, transport, and remediation of microplastics in aquatic sediments

The long-term fate of microplastics (MPs) is an under-researched area of study that has been assigned top priority in recent studies of global fluxes, especially in freshwater ecosystems (Atugoda et al., 2022; Domercq et al., 2022; Malli et al., 2022; Peller et al., 2024; Rose et al., 2023; Sandgaard et al., 2023; Waldschläger et al., 2022; Wang et al., 2021a; Wang et al., 2021b; Wu et al., 2019). Microplastics (MPs) are particles of the size class <5 mm that can be distinguished by their origin as primary MPs, which are artificially produced to be small, or secondary MPs created through the breakdown of larger plastics (Kurniawan et al., 2023; Walker & Fequet, 2023). Recently, the term nanoplastics (NPs) has been added to the size classification of plastics, describing tiny particles smaller than 1 μm in size (Wang et al., 2021b); however, these ultra small particles constitute their own area of research. In addition to size, MPs can be distinguished by shape, colour, density, polymer, chemical additives, and to an extent source (see Figure 2.1). In global freshwaters and estuaries, the polymers with the highest environmental concentrations are low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS; Jones et al., 2020). While a hierarchical 'fit for purpose' categorization of MP morphologies has been proposed (Yu et al., 2023), no universally accepted shape characterization has emerged in MP research; some of the commonly described morphologies are fragments, fibres, and pellets (Rochman et al., 2019). Shape is closely linked to the source of the MP particle, as well as subject to changes based on weathering and other environmentally occurring biochemical and physical processes (Alimi et al., 2023; Dong et al., 2020; Karbalaei et al., 2018). Source categorization of MPs, and especially the identification of a singular point source is difficult; generally, distinctions are drawn between industrial effluents, wastewater, stormwater runoff, agricultural runoff, and illegal dumping / littering (Wang et al., 2022; Yu et al., 2024). Other MP particle descriptors such as polymer, additives, and colour may occur in any and all combinations, further complicating source

characterization and making MPs one of the most diverse classes of pollutants (Corami et al., 2021; Parida et al., 2023; Rochman et al., 2019).

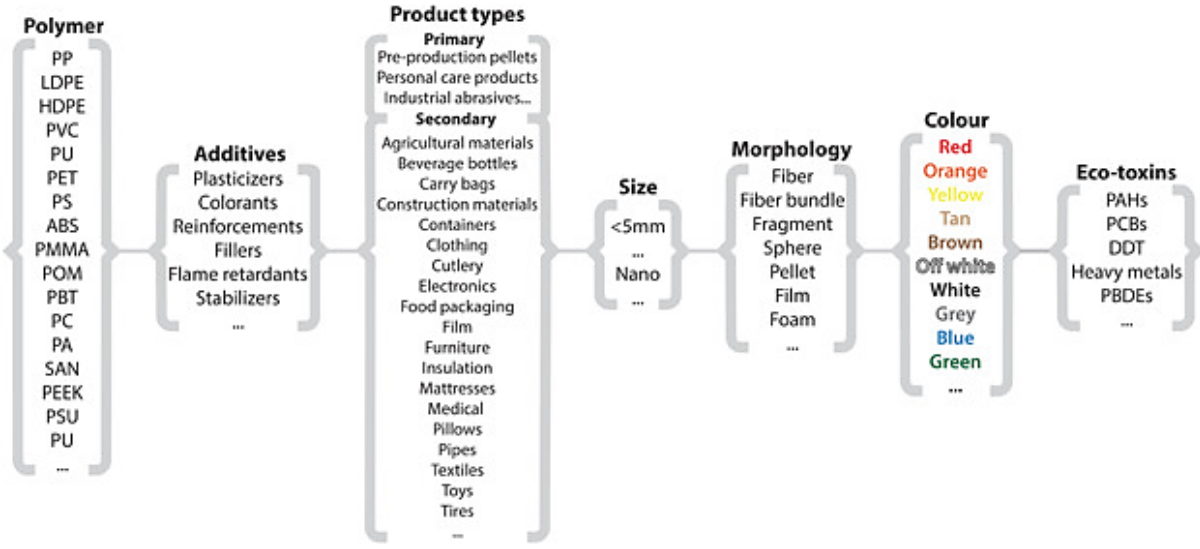

Figure 2.1 Distinguishing features of MPs; this list is not exhaustive (Rochman et al., 2019). Here, 'eco-toxins' refers to toxins absorbed from the surrounding environment and concentrated onto the MPs.

Due to their unique composition and enhanced durability, MPs, like all other plastics and independent of specific polymers, barely degrade (Briassoulis et al., 2024; Gaur et al., 2022). And even when degrading, MPs may exacerbate their potential for harm by generating the much smaller NPs that can penetrate organism's cell walls and exhibit higher ecotoxicity levels than MPs due to their larger surface areas (Kurniawan et al., 2023). In addition, new methods for biodegradation progress at a slower rate than MP pollution does (Briassoulis et al., 2024; Gaur et al., 2022). This is problematic as there is a growing body of evidence point towards the bioaccumulation and biomagnification of MPs and / or associated chemicals along the food chain (Krause et al., 2021; Provencher et al., 2022; Qaiser et al., 2023; Yang et al., 2022). The smaller and more dangerous MP particles are, the more likely and problematic becomes bioaccumulation (Provencher et al., 2022; Rochman et al., 2019). Therefore, MP degradation and active removal methods have been a priority in recent research efforts.

Adequately defining MPs matters for multiple reasons. Firstly, knowing the precise composition (and any other available data) about a specific particle may be helpful in estimating its effect on organisms. Some additives or morphologies may be more dangerous than others to specific biota; in addition, size may affect the potential of MPs to attract other pollutants as well as impacting their travel pathways (Ghaffar et al., 2022; Parida et al., 2023). Secondly, increased scientific knowledge on particle variations could aid in the creation of a MP particle database, and improve analytical methods for the isolation and detection of MPs. And finally, being able to estimate the most common and most dangerous types of MPs could be helpful in establishing priorities for policymaking involved with the reduction of MPs through upstream measures such as bans on individual items. Generally, the more is known about these novel pollutants, the better scientists and policymakers will be equipped to deal with them.

2.1 Microplastic in the environment: Effects, transport, and fate

The potential toxicity of MPs is a major factor in the environmental health considerations surrounding these contaminants. Toxicity effects have been recorded in marine, freshwater, and soil biota, among all levels of biological organization (Wang et al., 2021a); some specific examples include zebrafish (Hu et al., 2024; Rainieri et al., 2018), mice (Li et al., 2024), and in esophageal cells (Guanglin & Shuqin, 2024). MP toxicity has also been linked to mammalian fertility (He & Yin, 2023). With the recent COVID-19 pandemic, plastic litter related to face masks has received special attention in MP ecotoxicity studies (Hu et al., 2024; Oliveira et al., 2023). According to Castro-Castellon et al. (2022), freshwater studies are still lagging in this discipline; up to 2021 only 12% of MP studies had researched trophic transfer in freshwater, with invertebrates being the most studied group of organisms (61%) and *Daphnia*, a single genus, making up 21% of studies (Castro-Castellon et al., 2022).

MP toxicity is expressed across two main dimensions, namely the toxicity of the particles themselves (including chemical additives), and the toxicity of other pollutants that attach to the surface of MP particles (see ‘eco-toxins’ in Figure 2.1). The precise toxicity effects of plastics themselves on biota are still poorly understood (Walker et al., 2022; Wu et al., 2019); however, it is known that most plastic chemicals are harmful to

biota and can leach into the environment via MPs (Wagner et al., 2024). The better researched (and potentially more potent) factor, however, is the attaching of other contaminants to the surface of MPs due to their high surface-to-volume ratio (Parida et al., 2023), functional groups, surface topography, and point zero charge (Cássio et al., 2022). Especially well-documented cases include MPs as vectors of heavy metals (Hikon et al., 2021; Kaur et al., 2022; Yang et al., 2022), and cyanotoxins (Kaur et al., 2022; Moura et al., 2022). Non-traditional toxic contaminants, namely pathogens, parasites, and bacteria, can also be distributed through and released from the surface of MPs (Karbalaei et al., 2018; Nikolopoulou et al., 2023). The strongest evidence linking MP exposure to ecotoxicity effects in biota, however, is food dilution, which results in reduced growth and / or performance, and may even hinder survival (Provencher et al., 2022). Effects can also be caused by the translocation of smaller MP particles (roughly <70 μm) into tissues where they cause inflammation and oxidative stress (Provencher et al., 2022). Finally, the assessment of ecotoxicity can be achieved by using traditional toxicity data to derive predicted no-effect concentrations (PNEC), or non-traditional toxicity data to better include environmentally relevant concentrations and particle characteristics (Nam et al., 2023).

2.1.1 Effects on biota and interactions

Specific effects on biota are manifold and constitute a rapidly growing area of MP research. Most studies focus on ingestion of MP particles (Qaiser et al., 2023); however, bioadhesion has also been identified as a potentially problematic effect of MP pollution in biota (Kalčíková, 2023). Interestingly, body burdens based on MP ingestion do not necessarily reflect exposure levels in the environment, as some biota may be more skilled than others at egesting MPs (Vermeiren et al., 2021). Yet, negative impacts of MPs have been recorded for microalgae (Reichelt & Gorokhova, 2020), isopods (Izar et al., 2022), benthic biofilms (Lee et al., 2022; Wang et al., 2023), coral reefs (Huang et al., 2021), seabirds (Susanti et al., 2020), freshwater and marine fish (Forgione et al., 2023; Hossain & Olden, 2022; Lehtiniemi et al., 2018), freshwater and marine invertebrates (Pirillo & Baranzini, 2022), such as mussels and lugworms (Van Cauwenberghe et al., 2015), oysters (Dao et al., 2023), marine shrimp (Lehtiniemi et al.,

2018), sea cucumbers (Hartati et al., 2023), and sea snails (Li et al., 2020). Furthermore, MP effects on worms have been assessed along the marine-freshwater continuum (Vidal et al., 2023); note, that research on MP toxicity effects in biota has been expanding and this list is not exhaustive. An interesting addition here is that marine sponges can accumulate MPs over a long period of time and have been dubbed ‘libraries’ or ‘natural archives’ of MP pollution by researchers (Soares et al., 2022). Generally, studies tend to focus on marine organisms, which offer little transferability for freshwater ecosystems.

MP pollution impacts often stretch across matrices and can result in interaction effects of biotic, abiotic, and mixed nature (Cássio et al., 2022; Ma et al., 2023; Nguyen et al., 2023; Qaiser et al., 2023). Interaction effects with MPs particles have been proven for organic compounds (Mei et al., 2020), organic pollutants (Wang et al., 2020), pharmaceuticals (Arienzo & Donadio, 2023; Grgić et al., 2023), endocrine disruptors (Grgić et al., 2023), titanium dioxide nanoparticles (Kalčíková et al., 2023), trace elements (Binda et al., 2021), an antifungal agent in sediments (Lu et al., 2023), cyanobacteria and sedimentation processes (Leiser et al., 2021a), eutrophication in near shore lake waters (Yuan et al., 2021), and even climate change (Kakar et al., 2023). Arguably, weathering and other aging processes could be included in this list, as they change both the MP particle as well as the environmental connections it forms based on this change (Alimi et al., 2023; Binda et al., 2024; Dong et al., 2020; Hataley et al., 2022; Sun et al., 2020). Biochemical and physical interactions of MPs and sediments are especially poorly understood; this topic will be addressed in depth later in this chapter.

2.1.2 Transport mechanisms

Recent research has documented the presence of MPs in virtually all ecosystems of the planet, including remote areas such as the deep sea (Galgani et al., 2022), mountain glaciers (Schwarz et al., 2023), Mount Everest (Kurniawan et al., 2023), and the Arctic (Bergmann et al., 2022; Carlsson et al., 2021; Rose et al., 2023). MPs have been known to cycle through different abiotic media (Rose et al., 2023), including soil (Koutnik et al., 2021; Wu et al., 2023), sea- and freshwater (Carlsson et al., 2021; Jones et al., 2020; Koutnik et al., 2021; Wu et al., 2023), estuaries (Jones et al., 2020; Malli et al., 2022),

marine and freshwater sediments (Carlsson et al., 2021; Wu et al., 2023), and ice (Bergmann et al., 2022).

Common transport pathways of MPs include sea- and freshwater (Bergmann et al., 2022; Rose et al., 2023; Schwarz et al., 2023), biota (e.g., seabirds; Bourdages et al., 2021; Susanti et al., 2020), and the atmosphere (Bergmann et al., 2022; Rose et al., 2023; Ryan et al., 2023; Schwarz et al., 2023; Xiong et al., 2022). Newer research suggests MPs might be 'hitchhiking' the biological carbon pump of the Atlantic Ocean (Galgani et al., 2022). With water, air, and biological / weather-based pumps spanning the entire globe and connecting all ecosystems, it is no surprise that MPs have reached even the furthest corners of the natural environment. Concentrations and polymer abundances, however, vary greatly along the urban-remote scale and may depend on local industries (Koutnik et al., 2021; Jones et al., 2020). Heavily populated areas generally show higher MP concentrations throughout media (Koutnik et al., 2021); similarly, high-density polymers tend to accumulate relatively close to their source, whereas low-density polymers may be picked up by currents or air flows and can travel thousands of kilometres (Koutnik et al., 2021; Jones et al., 2020).

A lot less is known about the precise transport mechanisms governing MP distribution in the natural environment. So far, studies have relied on laboratory experiments and controlled micro- / mesocosms (Elagami et al., 2023; Sun et al., 2021) or modelling (Domercq et al., 2022; Elagami et al., 2023; Koutnik et al., 2021), with few examples attempting to track MP travel in the 'real world'. Peller et al. (2024) have provided a novel study monitoring the environmental loading and distribution of MPs released by a portable toilet manufacturer over the course of three years. Such studies are still the exception due to feasibility and resource constraints associated with MP tracking and processing.

Transport mechanisms are highly dependent on the specific ecosystem characteristics of any given system (e.g., biofouling rates, wind, turbulence, or levels of salinity), which have been proven to affect MP transport, vary greatly not only between freshwater and marine environments but even between oceans, estuaries, and individual lakes and rivers (Atugoda et al., 2022; Malli et al., 2022). Transfer between biotic and abiotic ecosystem components is similarly complex. The following list of processes is

non-exhaustive and especially pertains to freshwater systems such as lakes (see also Figure 2.2): Biotic-abiotic transfer (and the reverse) can occur via ingestion, egestion / excretion, predation, release after death of biota, bioadhesion, bioturbation, sedimentation, bottom turbulence, and resuspension (Ma et al., 2023; Sandgaard et al., 2023; *The pELAstic Project*, 2024; Waldschläger et al., 2022).

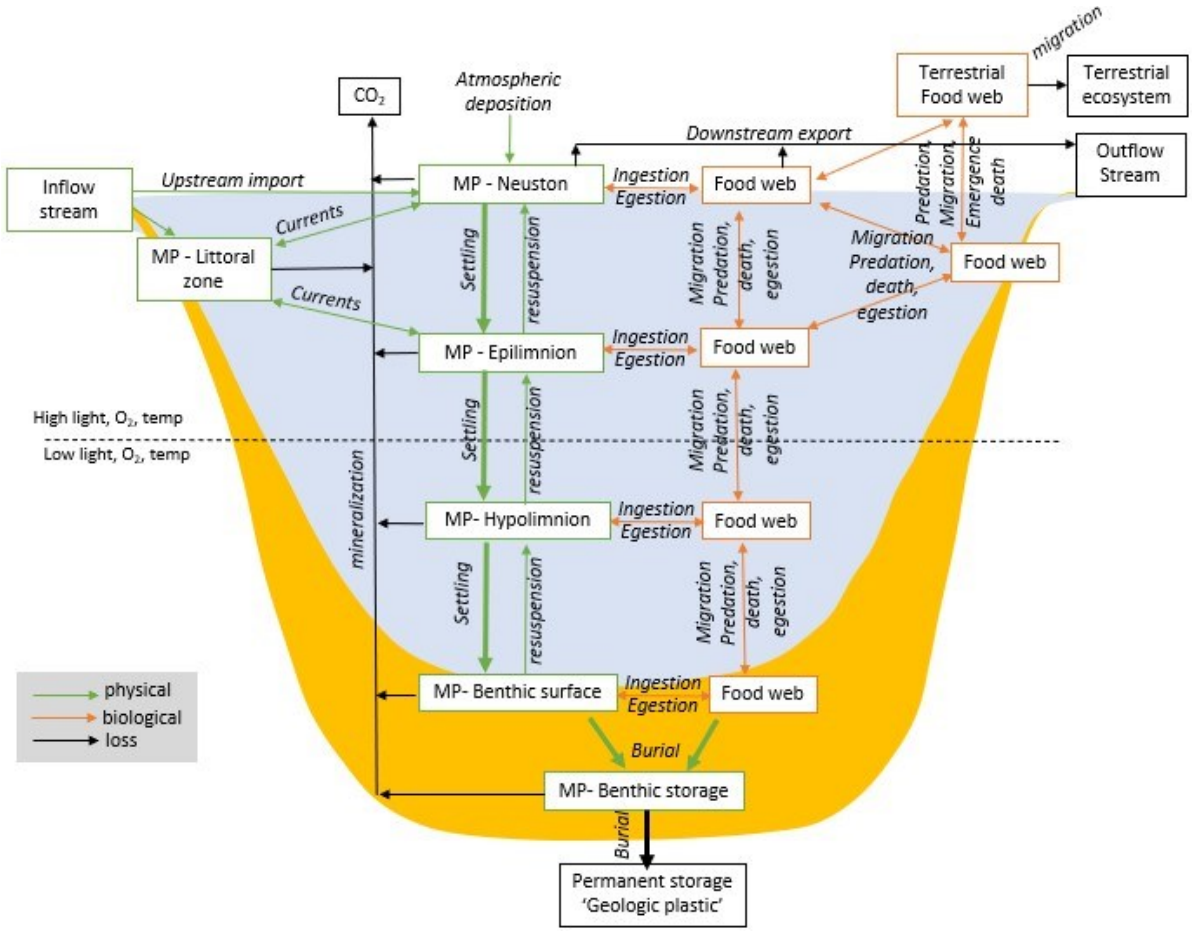

Figure 2.2 Biotic-abiotic transfer avenues of MPs in a lake environment (Hoellein & Rochman, 2021; *The pELAstic Project*, 2024).

MP particles are expected to settle in bottom sediments (Kane et al., 2020; Sandgaard et al., 2023), and may get buried permanently as geological plastic (*The pELAstic Project*, 2024) or transported back into other ecosystem components, e.g., the food web or the water column, via the variety of transport mechanisms and processes outlined above (Provencher et al., 2022; Sandgaard et al., 2023; Waldschläger et al.,

2022). Fate is a crucial piece of missing information needed to direct and inform mitigation and remediation.

2.1.3 The forgotten 'human' dimension of microplastic pollution

While MP pollution undoubtedly constitutes a scientific and environmental problem, there is also a human component to the issue of this widespread type of contamination. MPs research, like any other discipline, is subject to human error and biases, pertaining not only to sampling and processing but also the framing of the MP pollution problem, and therefore behavioural changes targeted at diminishing said problem. While negative impacts on biota, human health, and ecosystem health are widely accepted among scientific communities, policymakers, and the public, the causes and effects of MPs pollution in the economic and social domains have received less attention and tend to be debated more heavily (Diggle & Walker, 2022; Grünzner et al., 2023; Pahl & Wyles, 2016). Only few studies have used social and behavioural science (SBS) data and methods to make recommendations for MP research. Among these potentially crucial SBS methods and results are the insights generated by expert interviews related to the current state of evidence, risk, and solutions (Grünzner et al., 2023), the value of cross-sectional studies to expose the importance of social factors for key outcomes such as MP reduction strategies (Pahl & Wyles, 2016), the construction of communication and interventions based on scientific knowledge about human thought and behaviour (Pahl & Wyles, 2016), the formulation of economic thought as the center of the MP problem (Diggle & Walker, 2022), and finally, the systematic assessment of a path forward (Coffin, 2023).

While natural science has done a great job at collecting data on MP behaviour, effects, and fate in the natural world, it may be up to SBS to systematically assess the social, economic, and political blockages in place hindering MP mitigation and remediation and charting the way forward. Research, political discourse, and daily life decisions are affected by human error, biases, and personal motives to the same (or arguably higher) extent as are natural science methods such as sampling or processing. Therefore, research in this area must drastically increase and a collaborative space

between natural and social scientists must be established to jointly tackle the challenges of MP pollution.

2.2 Site characterization and sediment characteristics

Site characterization is the first necessary step for all well-designed and theoretically substantiated field work (De Vivo et al., 2017). Characterizing and sampling an environment can only be done when there is a reason for doing so; valid reasons include obtaining baseline data and / or assessing the level of contamination (De Vivo et al., 2017). In environmental science (and related fields), site characterization is used to identify and describe a site; often this is done to determine contamination levels, environmental impact, and implement or correct monitoring and mitigation strategies (Eslami et al., 2020; De Vivo et al., 2017). Generally, when dealing with contaminated sites, the following steps are completed in this order: Site characterization, risk assessment, and remediation (De Vivo et al., 2017; Pelletier et al., 2019). De Vivo et al. (2017) define eleven steps for adequately characterizing an environmental site, ranging from strategic mapping and desktop review to field sample collection, analysis, and reporting. Three major ways of sampling – namely exploratory sampling, monitoring, and presence / absence sampling – are used in the context of site characterization (De Vivo et al., 2017). They often inform one another, for example monitoring is only possible once one or several rounds of exploratory sampling have been concluded and interpreted.

More specifically, site characterization of sediments has been used in geotechnical studies to trace super-soft and sensitive sediments (Eslami et al., 2020) and in relation to water-quality management (Förstner, 2004). As the first step in remedial effort, contaminated sediments can be (and have been) characterized regarding metals (Baud et al., 2023; Ebinghaus et al., 2013; Pelletier et al, 2019; Zeman & Patterson, 2006) organic compounds (Pelletier et al, 2019; Zeman & Patterson, 2006), and radioactivity (Fesenko et al., 2009).

2.2.1 Physiochemical sediment characteristics

Sediments consist of particles of particulate matter that are carried through and ultimately settle at the bottom of aquatic systems (Sharma & Singh, 2014). The properties of sediments can differ widely based on location which tends to strongly affect the type of water body, geology, erosion rates, climate, abundance and community composition of biota, and many other factors (Grant et al., 2002; Sharma & Singh, 2014; Walker et al., 2008; Walker & Grant, 2009).

Common sediment characteristics used to describe sediment include water content, total organic carbon content, and grain size (Grant et al., 2002; Nedwell et al., 1993; Valentine, 2019). These measures are widely accepted as indicators of sediment properties and are often assessed routinely in environmental monitoring and contamination research (De Vivo et al., 2017; Förstner, 2004; Sharma & Singh, 2014). Flooded soils, such as the sediments at the bottom of a lake virtually consist only of two components: Solid particles and water filling up the pores between those particles (Avnimelech et al., 2001). Therefore, water content is an important measure to determine one of two major sediment components. The other one, solid particles, is generally divided into organic content, a measure capturing organic matter created by carbon-based life forms, and inorganic / mineral content (Avnimelech et al., 2001). Mineral content can then be further described by assessing the distribution of grain sizes; the most common classification system to divide grains according to their size classes is the Wentworth 1922 scale (Blott & Pye, 2012; Chotiros, 2017; Wentworth, 1922). According to this system, grains >1 mm are defined as gravel, grains of the size class 1 mm – 500 µm are classified as coarse sand, grains of the size class 500 – 250 µm are defined as medium sand, grains of the size class 250 – 63 µm encompass fine and very fine sand, and all those <63 µm are classified as silts and clays, with the upper cut-off for clays being debated but often drawn at 1 µm (Blott & Pye, 2012).

Sediment collection methods may at times affect the accurate reporting of these measures, as sediment core collection compresses the sediments and smearing occurs frequently (Dunnington & Spooner, 2017; Glew et al., 2001). Even minimal deformation of sediment layers caused by the corer itself can have effects on paleolimnological data (Dunnington & Spooner, 2017). However, depending on the sampling endpoints, these

effects are often negligible for contamination monitoring and can be partially mediated by subsampling from the least affected areas at the centre of the core.

2.2.2 The newest addition to sediment chronology: Microplastics

Traditionally, sediments and specifically sediment cores have been serving as chronicles of human history (Dimante-Deimantovica et al., 2024; Kilby & Batley, 1993; Martin et al., 2022). A variety of dating techniques allows researchers to track historical events in the environment, i.e., extreme droughts, or sudden influxes of nutrients, via sediment cores. Sediments can and have also been used to assess temporal changes in the input of contaminants into a given ecosystem (Kilby & Batley, 1993). As the ultimate aquatic ecosystem sink, sediments continue to serve as a natural archive of environmental changes, making them incredibly valuable for pollution research.

These methods of paleoenvironmental reconstruction have recently been harnessed in attempts to define the start of the Anthropocene in 1950 via the first appearances of plastics as a commonly manufactured and used material (Bancone et al., 2020; Dimante-Deimantovica et al., 2024; Martin et al., 2022). However, there are currently no standardized practices for dating sediment cores as part of MP studies and no official archives of MP-contaminated sediment cores exist, which constitutes a major data and knowledge gap (Bancone et al., 2020; Uddin et al., 2021). In addition, MPs seem to pose the first known case of particles that do not permanently stay in 'their' sediment layer. Dimante-Deimantovica et al. (2024) argue that MPs move downwards in sediment profiles of freshwater lakes, and that these effects constitute a true natural phenomenon as opposed to the often-argued procedural contamination such as core smearing and sample transfer. The processes driving this unique downward travel are still poorly understood, but floc formation (because of stratification) and gas exchanges have been theorized to increase the vertical distribution of MPs in lake sediments (Leiser et al., 2021b). Irrespective of the mechanisms, MP downward migration in sediments renders dating of cores with the purpose of establishing the beginning of the Anthropocene via MP occurrence impossible (Dimante-Deimantovica et al., 2024). However, sediment chronology might still be a useful contribution to MP research, if standardized practices for dating MP-contaminated sediment cores are being developed.

2.3 Microplastic-sediment interactions

The presence of MPs in aquatic ecosystems around the globe is underpinned by a massive body of literature (Ahmed et al., 2023; Ali et al., 2024; Ghaffar et al., 2022; Osman et al., 2023; Rochman et al., 2019; Rose et al., 2023; Schwarz et al., 2023; Tursi et al., 2022; Walker & Fequet, 2023). While concentrations may vary depending on location and ecosystem-specific variables such as currents and turbulence level, distance to urban centres, or connectivity to other systems, (Ahmed et al., 2023; Ali et al., 2024; Tursi et al., 2022), the fact that MPs are ubiquitous in all compartments of aquatic systems, including the benthic compartment, is undisputed (Kane et al., 2020; Sandgaard et al., 2023; Yao et al., 2019). MPs often outnumber plankton and fish larvae at ratios up to 30:1 in riverine and marine systems (D'Avignon et al., 2021), illustrating the size of the effect these particles may have on aquatic ecosystems.

Kurniawan et al. (2023) argue that freshwater systems could be considered MP conduits due to the ability of an individual ecosystem to simultaneously feature as a distributer, recipient, and potential sink for MP pollution, connecting all other environments (Kurniawan et al., 2023). As key factors in freshwater health, MP concentrations are ecologically relevant parameters that, according to a study by D'Avignon et al. (2021), should be included in standard limnological sampling protocols. MPs may affect nutrient concentrations and availability, as well as the performance of primary producers and herbivores, which has a cascading effect throughout the food web (D'Avignon et al., 2021). Within research focusing on MPs in freshwater systems, rivers receive the most attention, with more than three times the number of studies dedicated to them (N = 183) when compared to lakes (N = 64) in a 2021 review (Lu et al., 2021); however, these numbers may have changed now given the increase in MP freshwater research. Nevertheless, freshwater systems remain less studied than the ocean, despite their importance as ecosystem connectors (Kurniawan et al., 2023; Lu et al., 2021; Rose et al., 2023; Schwarz et al., 2023).

Freshwater sediments have recently been theorized to serve as the ultimate MP sink, with the majority of MP particles eventually settling in this section of the benthic compartment (Kane et al., 2020; Sandgaard et al., 2023). Yet, studies on freshwater sediments are still less common than those on marine sediments, fresh- and marine water,

and marine biota (Bellasi et al., 2020). Recent research in Malaysia (Zaki et al., 2023), Portugal (Rodrigues et al., 2018), and South Korea (Park et al., 2023b) revealed that MP abundance in freshwater sediments is primarily influenced by methodologies (lacking standardization of MP sample collection and processing), proximity to urban centres (which pose a higher annual loading), and seasonal and hydrodynamic conditions specific to the location and type of freshwater system (e.g., currents, boating, and the monsoon season). But regardless of variations, environmental loading of MPs remains exceptionally high in the benthic compartment of freshwater systems, compared to other environments (Park et al., 2023b; Rodrigues et al., 2018; Zaki et al., 2023).

Lakes are especially strongly affected by MP pollution. MP concentrations are generally higher in calmer (low energy) environments, and MP particles are easily captured and deposited into sediments of enclosed waters, as opposed to the oceans or even streams that offer more disturbances than lakes do (Uddin et al., 2021). Some studies also demonstrate that there are differences between MP concentrations in lakeshore and lakebed sediments, with lakebed sediments serving as a long-term sink of MPs whereas the MP abundance in lakebed sediments is subject to seasonal variations (Hengstmann et al., 2021). To further complicate things, a recent experiment with a novel augmentation method by Langknecht et al. (2023) suggests that MPs may be more abundant in sediments than current studies suggest, as extraction and identification protocols still lack standardization and therefore vary strongly.

The factors shaping MP concentrations in lake sediments intensify for semi-remote and remote lakes. These areas are sparsely populated or not populated by humans; nevertheless, semi-remote and remote mountain lakes and boreal lakes exhibit surprisingly high levels of MP contamination that largely relate to atmospheric deposition from distant urban centres, and the balance of depositional and erosional forces that result in a fine-grained sediment structure in the profundal zone where MPs tend to accumulate (Liang et al., 2022; McIlwraith et al., 2024; Neelavannan et al., 2022; Xiong et al., 2022; Zobkov et al., 2020). Evidence of MP depositional patterns of lakebed accumulation, in-shore seasonal fluxes, and the relevance of sediment characteristics in remote boreal lakes further exemplify the fact that their abiotic components are important puzzle pieces to understand MP effects and long-term fate (Liang et al., 2022;

McIlwraith et al., 2024; Neelavannan et al., 2022; Xiong et al., 2022; Zobkov et al., 2020). Being low energy systems with little environmental and human disturbances, remote lakes offer unique opportunities to isolate mechanisms driving MP fluxes and accumulation patterns. Yet, despite their setup to be the perfect study objects for better understanding MP travel and long-term fate, these lakes remain understudied.

2.3.1 Relevance: Why do microplastic interaction effects matter?

MPs are not a 'simple' class of contaminant that can be researched from an isolated vantage point. As this review has demonstrated so far, these pollutants are related to and influenced by many other factors in their environment and interact with ecosystems in complex and unexpected ways. Therefore, interaction effects with biotic and abiotic factors, and different processes within sampling media, should be prioritized to at least the same extent as MP quantification in different environments.

Surface sediments pose an important exposure pathway for biota, as well as a connector between the water column and deeper undisturbed sediment layers (Sandgaard et al., 2023), making them the ultimate connector in freshwater lakes. In addition, understanding sediment properties, behaviour, and interactions could increase scientific understanding and predictions of MP behaviour (Waldschläger et al., 2022), including accumulation which is arguably the most important factor for future remediation efforts. Consequently, potential interaction effects of MPs and sediments (especially lake sediments) should be explored in more depth, which the sections below will attempt to do.

2.3.2 Sediment properties: Grain size and nutrient cycling

Sediment grain size has long been theorized, and recently been proven to correlate with MP abundances and spatial distribution. MPs tend to be correlated with fine-grained sediments (Cera et al., 2022; Liang et al., 2022; Vermeiren et al., 2021; Zobkov et al., 2020), primarily of the silt and clay fraction which corresponds to grain sizes <63 μm (Blott & Pye, 2012; Wentworth, 1922). Related to this, MPs tend to be more abundant in sediments of deeper areas of lakes (Cera et al., 2022; Hengstmann et al., 2021; Shi et al.,

2022); those results are consistent with grain size, as deeper lake sections are classified as depositional and often exhibit high silt and clay levels (Cera et a., 2022). Whether these correlations are related to externalities such as wind and currents, or whether they unveil a crucial characteristic about MP behaviour is yet to be established. Nevertheless, knowing that MPs tend to cluster in substrates with small grain sizes could help predict MP depositional patterns.

Interaction effects have also been observed with microbial communities (Arias-Andres et al., 2018; Seeley et al., 2020; Yin et al., 2023; Zhang et al., 2022), biofilm (Arias-Andres et al., 2018; Salam et al., 2024), phosphorus cycling (Song et al., 2024; Yin et al., 2023), and nitrogen cycling (Salam et al., 2024; Seeley et al., 2020; Yin et al., 202; Zeng et al., 2023). Community structures as well as gas exchange rates were affected by MP abundance and polymer types in all six studies, strengthening previously introduced claims that biotic and abiotic environmental factors may interact or be affected to similar extents and must be examined with the same rigor. However, all these studies (except for Salam et al., 2024, a review / desktop study) base their claims on experiments conducted in controlled laboratory environments, which may have limited transferability to real aquatic systems. Another interesting dimension is introduced by Arias-Andres et al. (2018) who reframe the interaction between MPs and microbes / biofilm: MPs provide a new type of benthic substrate which may cause increased microbial attachment and biofilm formation, altering organic matter cycles within aquatic systems. In this scenario, MPs can also act as vectors for exogenous microbial groups, transporting them between ecosystems (Arias-Andres et al.; 2018). Serving as a new benthic substrate, MPs could potentially provide new niches and enhance heterotrophic activities within aquatic systems (Arias-Andres et al., 2018).

2.3.3 Horizontal transport mechanisms

Horizontal transport of MPs on sediments refers to the settling of MP particles in the top layer of the sediment across the water body, resulting in the creation of hotspots in some locations. Hotspots are of high interest to remediation: The better MP hotspots can be predicted, the easier it gets to make informed decisions on targeted remediation methods. However, MP hotspots are potentially more complicated to address than those of most

other contaminants due to their likelihood to simultaneously serve as biodiversity hotspots (Kane et al., 2020). This has only been proven for marine environments but is likely also applicable to freshwater systems. In either scenario, the two major processes governing horizontal MP transport are settling and resuspension.

The factors impacting MP settling in sediments are manifold and relatively well-document, although the implications are often less obvious and therefore poorly understood. MP settling factors can be roughly divided into environmental factors, such as currents, mixing, and windspeed (Berezina et al., 2021; Liu et al., 2022), or pertain to MP properties, such as shape, size, and density / polymer (Berezina et al., 2021; Lee et al., 2022; Liu et al., 2022; Lofty et al., 2023; Waldschläger & Schüttrumpf, 2019). Simply put, systems with high mixing activities and lots of wind are more likely to distribute MPs throughout the entire system, whereas calmer water bodies facilitate the sinking of MPs closer to their initial source. A high density, large size, and fragmental (as opposed to fibrous) shape further increase the likelihood of MPs sinking quickly and in relative proximity to point sources; lower density small fibers on the other hand are the MP class most likely to travel far and accumulate in any sediment substrate, depending more on environmental factors for accumulation (Berezina et al., 2021). However, not just the location but also speed of MP settling is directly affected by these factors. Settling and rise velocities tend to correlate with particle density, diameter, and shape (Waldschläger & Schüttrumpf, 2019), and trajectory characteristics of settling MPs are statistically different from sediments in only 1.4% of cases (Lofty et al., 2023). This suggests that MPs behaviour can, to an extent, be predicted by sediment behaviour, and that MP research can speed up its progress by learning from natural sediments (Paduani, 2020; Waldschläger et al., 2022).

Also related to settling is the tendency of MPs to heteroaggregate, that is, the formation of clusters with other particles such as sediment particles which increases sinking speed and thereby impacts sinking location. Natural kaolin particles and dissolved organic matter are especially likely to aggregate with MP particles (Reichelt & Gorokhova, 2023). This tendency to heteroaggregate has been recorded for both lake and sea waters and promotes faster sinking of low-density MP particles that would otherwise not settle or do so much later in time (e.g., lower density small fibers; Li et al., 2019;

Serra & Colomer, 2023). Multiple recent studies have observed this process and given it a range of interchangeably used names including 'heteroaggregation' (Christensen et al., 2020; Li et al., 2019; Serra & Colomer, 2023), the 'scavenging' of MPs by sediment particles (Mancini et al., 2023; Serra & Colomer, 2023), the 'co-settlement' of MPs with suspended sediments (Li et al., 2019), or the 'flocculation' of MPs with sediments (Laursen et al., 2023; Laursen et al., 2022; Leiser et al., 2021b). Heteroaggregation is also possible with other types of MPs (Christensen et al., 2020), and biofilm / organic matter, which increases density and decreases hydrophobicity of MP particles, causing them to sink faster (Lee et al., 2022; Leiser et al., 2021b; Provencher et al., 2022; Wang et al., 2023). In addition, biota, e.g., mussels or zooplankton, can impact the sinking location and speed of MPs via in- and egestion and surface changes (Berezina et al., 2021; Piarulli & Airoldi, 2020; Provencher et al., 2022). Similarly to the impacts of environmental factors, these biogeochemical processes affect low-density MPs to a higher degree than high-density MPs, which tend to accumulate in sediments quickly and close to their point source (Berezina et al., 2021).

Yet, MP settling is only one part of the equation. Resuspension of particles may also occur, with low particle density being the main determinator for resuspension and re-settling (Constant et al., 2023; Leiser et al., 2021b; Zhang et al., 2020). Grain size, nutrient cycles, and climate change may also interact with this process, as climate change may increase MP loading and resuspension events, which in return accelerates eutrophication (Zhang et al., 2020), and grain size affects what types of MP polymers (based on density) accumulate in sediment areas prior and post resuspension events (Liang et al., 2022; Park et al., 2023b). However, resuspension rates tend to be much lower than MP deposition rates, suggesting that most MPs get ultimately buried in sediments (Leiser et al., 2021b).

2.3.4 Vertical transport mechanisms

Vertical transport of MPs within sediments refers to the downward migration of MP particles to different sediment depths, resulting in their layering and temporary burial. The most widely known driver of vertical MP transport in sediments is bioturbation; during this process MPs get distributed in sediments through the feeding, burrowing, and

construction behaviour of benthic organisms (Frank et al., 2023; Wazne et al., 2023). The most common group among these organisms are benthic invertebrates such as worms and chironomid larvae (Frank et al., 2023; Song et al., 2024; Wazne et al., 2023). Benthic organisms tend to live and bioturbate in the upper sediment layers; this is represented in laboratory studies which were conducted with sediment depths of 0.5 cm (Frank et al., 2023), 6 cm (Song et al., 2024), and 8 cm (Wazne et al., 2023). Therefore, their impact on temporary MP burial is limited, focusing on the surface layers and sediment-water interface. Studies on bioturbation tend to focus on the toxicity effects of MPs on bioturbators, as opposed to the resuspension, deposition, and distribution of MPs in sediments because of this process (Waldschläger et al., 2022).

Other drivers of the downward migration of MPs in sediments, and specifically freshwater sediments, are still poorly understood. Anthropogenic activities may be a driver (Zheng et al., 2020), but that is to be expected, given that it hugely influences other transport mechanisms. In addition, MPs have been found in deep sediments (30-35 cm depth horizon) of semi-remote lakes (McIlwraith et al., 2024), rendering anthropogenic activities responsible for concentrations, but better classifying them as 'background noise' rather than an acute driver when it comes to burial within sediments. The molecular weight transformation of humic acid within sediments has been recently explored as a potential driver of vertical MP distribution (Tian et al., 2022), as have been gas exchanges (Dimante-Deimantovica et al., 2024). Similarly to horizontal transport, size, shape, and characteristics such as density or hydrophobicity may impact downward travel, i.e., small round particles with high contact angles (hydrophobic >90°, this includes MPs that have heteroaggregated and thereby changed their hydrophobicity) have the highest likelihood of migrating into the deepest sediment layers (Dimante-Deimantovica et al., 2024). Fibres, large particles, and less hydrophobic materials are more likely to remain in the upper sediment layers. Water content may be linked to this, with pore water potentially providing an easy travel route into deeper layers. However, there is very little research on the factors affecting the vertical transport mechanisms of MPs in sediments.

Note, that the distinction between horizontal and vertical transport of MPs in sediments is not entirely clear in the literature. The sediment surface layer (roughly 0-2

cm sediment depth horizon) is generally included in research focused on location-specific settling / hotspot formation across an aquatic system, yet studies investigating the vertical distribution of MPs in sediments start at the sediment-water interface (0 cm sediment depth horizon). Therefore, an overlap of theory and data exists between horizontal and vertical transport and accumulation of MPs in surface sediments. A clear separation of horizontal and vertical transport characteristics may not be feasible but should at least be attempted, as the mechanisms governing MP behaviour in this context could differ and would be highly informative for remediation efforts. Furthermore, studies almost exclusively provide laboratory experiments with sea water, which has questionable transferability for freshwater sediments in real life settings. Much research is still required to understand MP transport mechanisms.

2.3.5 A new dimension: Long-term fate = permanent burial?

The ability of sediments to serve as a potential MP sink has been addressed multiple times throughout this review. At this point, a clarification is necessary: while most MPs become permanently buried into sediments, a crucial characteristic of a permanent sink is its ability to retain a given contaminant without it being physically re-located or re-introduced into the food web (Sandgaard et al., 2023). This, however, is untrue for MPs (Sandgaard et al., 2023). The upper sediment layers are subject to the variety of transport and redistribution mechanisms outlined above, including the ingestion by organisms, thereby serving more as a reservoir than a permanent MP sink (Sandgaard et al., 2023).

Deeper sediment layers may be better candidates for permanent MP retention. Without bioturbators or any other disturbances, they effectively bind MPs, rendering these particles unavailable for organisms or continued transport as geologic plastic (*The pELAstic Project*, 2024). Related to this, MPs have been suggested as novel sedimentary particles (Paduani, 2020), which, if universally accepted, would define them as a regular occurrence in sediments. This, however, does not mean that accepting MPs as permanent aspects of sediment composition or using sediments as a permanent sink and creating geologic plastic is preferable to remediation. Given how little we know about these contaminants, and how quickly and unhinderedly they migrate within sediments, even reaching sediment layers deposited before the first plastics were produced (Dimante-

Deimantovica et al., 2024; Zalasiewicz, 2023), it would be unlikely that the increasing presence of MPs has no long-term negative effects on aquatic benthic ecosystems. MPs have now also been recorded in archaeological sediment samples, opening more and more questions and conversations about the reach of MP pollution (Rotchell et al., 2024). While MPs may get permanently buried in deeper sediment layers, this is not the preferable options. The fate of MPs in the benthic compartment is now largely dependent on the scientific community's progress in the field of MP removal and remediation.

2.4 Ecosystem-wide experiments and their urgency in microplastic research

To date, MP research has focused on two main components: collection of data on the extent and types of MP pollution in a variety of ecosystems (Castro-Castellon et al., 2022; Hengstmann et al., 2021; Koutnik et al., 2021; Osman et al., 2023; Rose et al., 2023; Uddin et al., 2021; Walker & Fequet, 2023), and experimental studies conducted in laboratory settings to pinpoint precise effects and travel mechanisms of MPs (Constant et al., 2023; Elagami et al., 2023; Leiser et al., 2021b; Lofty et al., 2023; Lu et al., 2023; Mancini et al., 2023; Waldschläger & Schüttrumpf, 2019). There are multiple problems with this. Firstly, there is a mismatch between those two components relating to exposure levels and types of plastics used, what is present in the environment is often quite different to the MPs used in laboratory studies (Burns & Boxall, 2018; Phuong et al., 2016; Weis & Palmquist, 2021). Some researchers have started using MPs collected in the field (as opposed to pristine MPs manufactured for scientific research) for experiments to partially mitigate this issue (Islam & Nishi, 2023). Yet, among the papers included in this analysis, only two studies have attempted to bridge the gap between laboratory results and their direct application in real-life settings (Dao et al., 2023; Park et al., 2023a). Secondly, the results obtained by modeling and laboratory experiments may not apply to real-life settings, as they should adequately represent field complexity, which is rarely the case for MP studies (Mouneyrac et al., 2017). In addition, some ecosystem components and processes, especially freshwater sediments, abiotic processes, and organisms at higher levels of biological organization, are still systematically understudied (Burns & Boxall, 2018; Mouneyrac et al., 2017). Similarly, presence-absence studies and review articles are very common in MP research, promoting a

circular development focused on re-identifying the same research priorities and recommendations, with little change or concrete steps being taken in these clearly identified directions. Finally, common flaws in both components of MP research must be addressed; such flaws include the lack of particle controls used in experimental studies (the environment is not 'particle-free', and using only MPs and filtered water skews the results; Ogonowski et al., 2018), huge variations in limits of detection (Lu et al., 2021; Prata et al., 2020; Ye et al., 2022), and the much-called-for standardization of field collection methods, analytical tools, and reporting of results (Coffin, 2023; Kurniawan et al., 2023; Lu et al., 2021; Phuong et al., 2016; Weis & Palmquist, 2021; Wong et al., 2020).

All these issues can, to an extent, be addressed by two changes: the increased standardization of MP research, towards which a trend has emerged in recent years, and the conducting of real-life experiments targeted at especially poorly understood ecosystem components and processes. Standardization should focus on sample analysis and reporting of results, harmonizing limits of detection, chemicals used, quality controls, and data units used for reporting results. These tweaks would improve comparable scientific knowledge on MPs the quickest and are most urgently needed to inform remediation protocols and policies. Real-life experiments refer to MP effect and transport studies conducted in the environments they wish to draw inferences on. These studies could provide insights into the complexity of MP-environment interactions of specific ecosystems and their associated processes and inhabitants. A major consideration or limitation of this approach would be research ethics. Actively contaminating habitats to better study impacts of MP pollution comes with the ethical trade-off of research not being supposed to cause unnecessary harm but this harm potentially being required to advance MP research sufficiently to drastically upscale remediation efforts. In addition, study places where such real-life experiments could be conducted are extremely limited globally. Yet, this type of research would provide most of the insights research is currently still lacking when it comes the effects, distribution and travel mechanisms, and long-term fate of MPs in the natural environment.

2.5 Risk assessment strategies and remediation of microplastic pollution sites

Pollution mitigation and the remediation of already polluted sites are essential components of environmental monitoring and ecotoxicological assessments. A common tool in this context is ecological risk assessment (ERA) which aims to assess ecological risk (ER) related to a potential stressor or pollutant by defining contaminants of concern, characterizing and and analyzing risk, and recommending options to mitigate this risk (Hooge et al., 2024; Redondo-Hasselerharm et al., 2023). In the context of MP pollution, 'risk' is understood as "the likelihood that observed effects are caused by past or ongoing exposure to MP" (Redondo-Hasselerharm et al., 2023, p. 2). Assessing ER for MP pollution is difficult due to complicated and therefore poorly understood exposure routes in freshwater systems as well as a lack of comparable data when it comes to measured environmental concentrations of MPs (Adam et al., 2019; Burns & Boxall, 2018; Qiao et al., 2022; Redondo-Hasselerharm et al., 2023). In addition, knowledge gaps related to MP properties must be addressed to better assess ER (Hooge et al., 2024). Redondo-Hasselerharm et al. (2023) argue that these issues are even more pronounced for MP-contaminated freshwater sediments as they may pose higher risk to benthic organisms than the water column.

Standardized remediation (i.e., the isolation or removal of a contaminant from a site), does currently not exist for MP contaminated sites (Cheng et al., 2022). However, the groundwork has been laid via the exploration of upstream and downstream solutions. Upstream solutions (sometimes called direct solutions) refer to the prevention of MPs entering the natural environment, whereas downstream solutions (used interchangeably used with indirect solutions) revolve around the active recovery and removal of MPs from ecosystems (Wong et al., 2020; Zhao et al., 2023). Upstream solutions typically involve policies and management solutions (i.e., bans on single-use plastics) (Xanthos & Walker, 2017), or the exploration of bioplastics as a sustainable alternative (Wong et al., 2020). Downstream solutions range from biological and chemical to physical MP removal technologies and may include filters, biodegradation accelerators (e.g., bacteria capable of breaking down plastics), membrane technology, and many more (Rose et al., 2023; Wong et al., 2020). Whereas recommendations usually point towards the necessity of upstream solutions to prevent pollution loads rising even more dramatically (Wong et

al., 2020), a combination of both would arguably yield the best long-term results by preventing ‘new’ pollution while simultaneously reducing existing environmental stocks. Currently, roadblocks are in place for both upstream and downstream solutions, as governments and manufacturers are slow to take decisive action against plastic production, and removal technologies still lack scalability. The sections below will introduce existing and potential policies and management solutions (upstream), filter technologies (downstream), biomimicry and biotreatments (downstream), and other recent innovations geared towards MP removal from the environment (downstream).

2.5.1 Policies and management solutions

On the global level, the most prominent framework related to environmental sustainability are the UN sustainable development goals (SDGs). These 17 SDGs were launched in 2015 to “address the most important global threats of our time and provide a vision for achieving a sustainable future” (Walker, 2021, p. 1). Plastic pollution, and MPs more specifically, are central to the sustainable use of oceans and freshwater to preserve ecological integrity for future generations. Despite the widespread effects of MP pollution on the environment, society, and the economy, and their direct and indirect impacts on at least 12 of the SDGs, only one of these, Goal 14 (‘Life Below Water'), specifically relates to reducing these impacts. However, MPs are never addressed directly, and none of the other goals mention reduction targets or indicators with which to assess reduction progress of (micro)plastic pollution (Walker, 2021). So far, international policies have focused on single-use (micro)plastics, banning plastic bags, microbeads, and other nonessential plastic items since 1991 (Diggle & Walker, 2022; Xanthos & Walker, 2017). At the time of writing this thesis, Canada’s Zero Plastic Waste 2023 initiative, which was overturned in November 2023, is being re-examined and could, if accepted, ban a wide range of single-use plastics including plastic cutlery.

Bans, levies, and taxes are upstream interventions targeting specific stages of the plastic life cycle, namely market entry, retail distribution, post-use / disposal, and trade regulations (Diggle & Walker, 2022; Jiao et al., 2024). These interventions are specific to the national and local levels they are implemented at. Life cycle assessment (LCA), a standardized tool in environmental decision-making, is used to evaluate the

environmental impacts caused by a product throughout its life cycle and has recently been examined as a managerial option to mitigate the effects of MP pollution (Jiao et al., 2024). However, the environmental, ecological, or human health effects caused by MPs and plastic degradation by-products are still poorly understood and have consequently not yet been addressed in LCA studies (Jiao et al., 2024).

The economic and socio-economic impacts of MPs are as wide-ranging as the environmental ones, spanning reduced revenues from tourism caused by beach litter, impacts on fishing, direct costs incurred by industry and governments, loss of goods and services related to aquatic debris, and dependence on raw material extraction (Chaudhry & Sachdeva, 2021; Diggle & Walker, 2022; Mahmud et al., 2022). Consequently, economic, and socio-economic solutions are as urgently needed as technological ones. A circular (micro)plastics economy, while possible, has not been prioritized and instead of improving recycling- and recovery-rates for the re-use of MPs focus has shifted towards the development of bioplastics (Elsamahy et al., 2023; Osman et al., 2023; Parida et al., 2023; Prata et al., 2019). However, the sustainability of these materials has not been tested sufficiently yet, making a confident judgement on their value as a long-term alternative difficult (Elsamahy et al., 2023). Other socio-economic measures suggested for the mitigation of MP pollution can be divided into short-term, mid-term, and long-term measures and may include improved waste management systems (Mahmud et al., 2022; Osman et al., 2023; Prata et al., 2019; Rose et al., 2023), education (Mahmud et al., 2022; Osman et al., 2023; Prata et al., 2019), and behavioural changes such as decreased consumption and opting for clothes made from natural fibres (Mahmud et al., 2022; Osman et al., 2023; Prata et al., 2019). Ultimately, the responsibility for reducing (micro)plastic cannot and should not lie entirely with the consumer. Concepts such as the extended producer responsibility (EPR) principle, like the polluter pays principle in climate justice, would place the responsibility for financing the end-of-life management of their products and materials on businesses instead of consumers (Cowger et al., 2024; Diggle & Walker, 2022; Prata et al., 2019; Thacharodi et al., 2024). This concept has proven successful in other product industries, e.g., the automotive industry, and electronic waste (Diggle & Walker, 2022; Prata et al., 2019; Thacharodi et al., 2024), and could, if applied globally, fully shift the costs of recycling from the taxpayers to the

industries responsible for producing unsustainable materials such as (micro)plastics. Similarly, responsibility to drive behavioural changes has relied on activism and citizen engagement for too long. While recognizing the complexity of MP pollution and the consequently required multidimensional approach involving the public, industries, governments, and international organizations, governments must step up to lead this change by implementing rules targeting the reduction of MPs both up- and downstream (Thacharodi et al., 2024). Only then can industries be regulated, and international agreements ratified.

Some studies have argued that policy must be informed by and cannot proceed without reliable findings of specific MP-associated risks identified by RA frameworks (Burns & Boxall, 2018; Redondo-Hasselerharm et al., 2023). In response, national and local RA frameworks geared towards MP pollution have been developed in China (national scale; Qiu et al., 2023), Indonesia (case study of river sediments; Ilmi et al., 2023), Canada (specific to tire and road wear particles in surface waters; McCarty et al., 2023), the US (for aquatic systems in California; Mehinto et al., 2022), for the Laurentian Great Lakes in Canada and the US (bi-national scale; Koelmans et al., 2023), and in a holistic way based on data of global MP distributions in freshwater (Adam et al., 2019) and more specifically freshwater sediments (Redondo-Hasselerham et al., 2023). While these RA frameworks are thorough and provide great recommendations, they fall into the same trap as scientific studies attempting to characterize MP abundances and effects: Standardization and therefore comparison is lacking. Each framework uses slightly different parameters to define ‘hazard’, ‘exposure’ or ‘risk’, accounts for other confounds (e.g., modelling uncertainty in MP risk; Koelmans et al., 2023), and focuses on a different scope (global to case study level), making comparable inferences and recommendations difficult to derive.

Given their unique role as ‘conduits for MPs’ (Kurniawan et al., 2023), it is not surprising that research on the remediation of MPs in freshwater systems has spiked in recent years. Between 2016 and 2020 scientific publications on MP mitigation and remediation in freshwater systems increased by 1700%; this stark surge may be related to the higher total number of studies published on MPs, which increased by 2323% in 2019 relative to 2009 (Munien et al., 2024), and partially reflects the perceived urgency of

addressing the MP pollution problem. However, no standardized RA frameworks nor widely accepted protection policies of freshwater system have yet emerged in relation to MP pollution.

2.5.2 Filters

Filters are among the best-known and most conventionally used MP removal strategies of freshwater systems. They are primarily associated with the collection of plastic fibres released into household wastewater, e.g. by washing machines or the disposal of personal hygiene products. Recently, MP filters have been developed to fit into washing machines and prevent the release of fibrous MPs at one of their main sources (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024); the collection of MP fibres is complicated and often escapes other remediation methods which makes source prevention crucial for this sub-category of MPs. Similarly to these filters, small catchment balls, which can be added into a load of laundry and serve as an *in situ* filter, have been developed (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). In addition, MP filters have been tested in a wastewater treatment plant (WWTP) in Spain and showed a MP removal efficiency of up to 98.3%, with fibres being the most abundant MP shape (Martin-Garcia et al., 2022). WWTPs are a hotspot of MP filter technology, often combining and perfecting methods already used for the removal of other contaminants (Rose et al., 2023). MP filters have also been deployed at a plastic recycling facility (PRF) in the UK, finding that filtration, although removing bigger MPs, was not efficient at retaining the environmentally highly relevant particles <10 µm (Brown et al., 2023). Finally, filter systems have been tested in experimental set-ups: A combination of meshed steel frames, plankton nets, carbon-block filters, and a reverse osmosis filter ranging from 5 mm to 0.001 µm was installed in a sequence of tanks mimicking the flow rate of a river in Japan (Islam & Nishi, 2023). Testing two measurement methods across multiple experimental conditions with this filter system, the experiment determined average capturing efficiencies of 81% for the particle counting method, and 83% for the weight measurement method across cases, providing one of the first successful capturing methods for MP fragments (Islam & Nishi, 2023).

Considering these success stories as well as the relative cost-efficiency and simplicity of production of filters, they could provide a great solution combining downstream and upstream solutions. With the right policies, filters could prevent most MPs released in wastewater from households, WWTPs, and, if further improved, PRFs. They could even be employed in rivers and channels to capture MPs that have already entered the environment, although their efficiency as a downstream MP removal technology could still be improved. Filter-related policies could include a regulation mandating washing machine manufacturers to include built-in MP filters in their products and provide services to retrofit already existing models. Similarly, WWTPs and PRFs could be mandated to add a MP-specific treatment to their process, with regular check-ups on efficiency.

2.5.3 Biomimicry and biotreatments

Bioremediation is the most common technique for the remediation of aquatic systems, as it is cost-effective and environmentally friendly (Milološa et al., 2022). Biotreatments of MP pollution often rely on biomimicry or the harnessing of organisms for MP removal. Within this category of biotreatments, common avenues are plants, algae, worms, microbes, bacteria, and fungi; this brief review will also introduce a miscellaneous collection of less common bio-based treatments.

Aquatic plants, and especially floating plants, have excellent phytoremediation potential, trapping even ultra-small NPs (Yuan et al., 2023). The efficiency of entrapment by aquatic plants is related to their root systems and leaf morphology, rendering some species better suited for the tracking and mitigation of MPs in aquatic environments (Tan et al., 2023; Yuan et al., 2023). With some aquatic plants floating and others rooting in sediments, they have the potential to bioremediate both surface waters and surface sediments (Mahmud et al., 2022; Yuan et al., 2023).

Although not yet employed as a remediation method, lichens and mosses have been found to be effective biological monitors of atmospheric MP pollution (Jafarova et al., 2023). This ability could, similarly to the retention of MPs in aquatic plant roots and leaves, serves not only as a monitoring tool but also as a potential entrapment method.

Algae and microalgae have been used in two major ways related to MP remediation: as biodegrading agents, and as bioflocculants. As biodegrading agents, green algae have been found to degrade low-density plastics with an average weight loss rate of 8.18% per month at 27°C (Nguyen et al., 2023). Microalgae are typically added as bioflocculants during the wastewater treatment process and could capture low-density MPs through the process of flocculation due to the tendency of MPs to heteroaggregate (Mahmud et al., 2022; Miloložа et al., 2022; Thanigaivel et al., 2024).

Waxworms have been found to decompose low-density polyethylene at impressive speeds, reducing 92 mg of weight from a PE bag within 12h (Nguyen et al., 2023). Other worm species and invertebrates (e.g., beetle larvae) also possess the ability to biodegrade different plastic polymers such as low-density polyethylene, and polystyrene (Rose et al., 2023). Similar abilities are known for many different types of bacteria, microbes, and fungi, which have also been assessed in terms of their ability to degrade MPs (Hu et al., 2021; Leiser & Wendt-Potthoff, 2022; Mahmud et al., 2022; Miloložа et al., 2022; Nguyen et al., 2023; Rose et al., 2023). Over 20 genera of bacteria display MP-degradation abilities, as do two species of cyanobacteria, although degradation speed and type of polymer suitable for microbial or bacterial degradation vary (Nguyen et al., 2023). Fungi have been reported to be more efficient at MP breakdown than bacteria, reducing low-density polyethylene weight by up to 58% over 90 days (Nguyen et al., 2023).

While degradation of MPs by small organisms seems promising, one must keep in mind that by-products are not always reported. Sometimes, the degradation of plastics refers to the diminishing of a larger plastic piece into smaller ones, which in the case of MPs and NPs only increases the environmental risk. In addition, introducing microbes / organisms into ecosystems they usually would not naturally occur in may disrupt these systems and could have unforeseen implications. Therefore, biological degradation methods should be assessed carefully before employing them on a larger scale.

Other promising biotreatments for MP removal include biochar (Dong et al., 2023; Mahmud et al., 2022; Nepal et al., 2023), starch (Gao et al., 2024), and jellyfish mucus (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). Biochar, due to its unique honeycomb structure, is capable of capturing and

immobilizing MPs, as well as removing metals attached to them using its catalytic and magnetic removal properties (Nepal et al., 2023). At higher production temperatures, biochar can also accelerate the degradation of MPs, sporting a degradation rate of 22-31% (Dong et al., 2023). WWTPs and drinking water treatment plants (DWTPs) could easily make use of this efficient and safe method. Starch, and specifically cationic-modified starch, was used as a coagulant for MPs, once again utilizing the tendency of MPs to heteroaggregate for their immobilization and removal (Gao et al., 2024). While being abundant, cheap, biodegradable, and easily chemically modified to have enhanced coagulation properties, starch outperformed commercial coagulants (e.g., polyaluminum chloride) with a mean MP removal rate of 49.8-62.4% (Gao et al., 2024). Removal rates also depended on MP particle size and polymer, and were reduced when kaolin clay and / or humic acid were added (Gao et al., 2024); this is in line with the sediment-MP interaction effects, as MPs are likely to heteroaggregate with kaolin particles (Reichelt & Gorokhova, 2023) and display an increased ability to 'hitchhike' when humic acid is present (Tian et al., 2022), thus no longer needing to coagulate with the starch. Finally, the GoJelly Project has been employing jellyfish mucus to trap and remove MPs from wastewater (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). Although jellyfish mucus with its ability to quickly bind MPs has been turned into a filter, it is a strong example of the harnessing of organisms and their naturally occurring products and therefore included in this section.

Finally, MPs can be bioremediated using bionanomaterials, which are defined as "nanomaterials of biological origin or inorganic nanomaterials coupled with organic components" (Chellasamy et al., 2022, p. 5). The application of bionanomaterials to MP remediation is still in its early stages but has shown great potential under laboratory conditions. Controlling the size of the nanoparticles is a major issue, but, if achieved at a consistent level, these materials could provide a remediation option with low energy costs, enhanced performance, easy processing, and a longer service life (Chellasamy et al., 2022).

2.5.4 Chemical processes and lesser-known technologies

This section will introduce technologies and methods that are not yet employed on as large of a scale as filters and biotreatments; most of these are of a chemical and / or mechanical nature. The first subgroup of these methods, chemical and biochemical processes, are aimed at the destruction of MPs. Advanced oxidation processes (AOPs), including photodegradation (Ahmed et al., 2023; Assis et al., 2023; Hu et al., 2021; Mahmud et al., 2022; Rose et al., 2023; Wang et al., 2024), Fenton and Fenton-like systems (Hu et al., 2021; Mahmud et al., 2022; Wang et al., 2024), ozone oxidation, electrochemical oxidation, persulfate advanced oxidation, and discharge plasma, can produce strong reactive oxygen species with a high redox potential, allowing them to decompose MPs into carbon dioxide (CO_2) and water (H_2O) via a chain of reactions (Wang et al., 2024). Other chemical processes, such as electrocoagulation (Ahmed et al., 2023; Krishnan et al., 2023; Rose et al., 2023; Shen et al., 2020) or the sol-gel process (Ahmed et al., 2023; Rose et al., 2023), bind MPs by capturing them with electrodes and in silica gels respectively, which can be separated from the MPs with simple filter techniques later. Constituting biochemical processes, membrane reactors (Ahmed et al., 2023; Krishnan et al., 2023; Miloloža et al., 2022; Rose et al., 2023) and absorption (Ahmed et al., 2023; Assis et al., 2023) have been used to filter and immobilize MPs primarily from wastewater. Most of these techniques have been adapted from and / or tested in WWTPs and DWTPs and are therefore tailored towards the removal of MPs in water in a specialized and controlled environment.

Magnetic extraction of MPs is a chemo-mechanical process that uses magnetic seeds and acid with an external magnetic field to faster separate MPs from water (Ahmed et al., 2023; Hu et al., 2021; Rose et al., 2023; Shen et al., 2020). Some adaptions of this process do not require acid anymore, using oil and magnetite powder, or magnetic coils coated with nitrogen and manganese to achieve the same reaction (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). Other chemo-mechanical processes have managed to convert MPs into nanostructures and value-added products that can be re-used, e.g., as fuels, construction materials, for energy recovery, in films and coatings, as carbon nanostructures, and many more (Hu et al., 2021; Thacharodi et al., 2024). Many of these products would contribute to a circular economy; however, if

MPs are being re-applied as e.g., coatings they will eventually break down and become secondary MPs again, re-entering the natural environment and thereby creating an endless pollution cycle. Therefore, repurposing MPs should be considered carefully by evaluating their secondary breakdown potential.

Targeting MP emissions at one of its lesser-known sources, a mechanical device called The Tyre Collective prevents the leakage of MP particles from spinning wheels using electrostatics and aerodynamics (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). The device attaches to a wheel and collects tyre particles as they are emitted.

Finally, a variety of technologies have been invented to remove MPs from the natural environment. Water remains the main target medium, although some inventions also offer MP removal from sand via vibrating screens or vacuuming (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). Freshwater-based technologies range from large screen drum units installed in a river (Fuchs et al., 2024) to PolyGone's Plastic Hunter, a floating wetland unit mimicking plant roots as biofilters, all the way to simple small-scale solutions such as towable MP collection nets or bubble curtains (Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024).

2.5.5 Problems with microplastic remediation

To summarize, much attention has been directed to remediating MP-polluted waters via an array of political, biological, chemical, mechanical, and combination methods, of which many show great potential. However, complexity and scale of MP pollution in aquatic systems have prevented the development of a universally effective treatment solution (Tursi et al., 2022). Finding inventions and treatments that balance resource availability and environmental sensitivity is of the utmost importance, but has yet to be achieved (Munien et al., 2024). Problems related to a marine- and prevention-based focus, treatment end points, scalability, and cultural changes persist.

The number of policies as well as technologies applicable to marine ecosystems towers the number of methods available for MP mitigation in freshwater and terrestrial systems. This is a huge oversight, as land-based sources are what contaminates

freshwater and terrestrial systems AND supplies MPs to the oceans; targeting freshwater, the ‘conduit of MPs’, for remedial efforts would address these issues simultaneously.

Furthermore, much of the political and scientific attention has been placed on upstream solutions to prevent further environmental loading of MPs. Yet, even if plastic production came to a complete halt today, most of plastics that have been produced since the 1950s (of which 50-79% are unaccounted for; Borrelle et al., 2020) would continue to build up in ecosystems and serve as MP sources until remediated. In addition, while it is undeniable that plastic production must be reduced and / or transformed, it is unrealistic to *expect* politics to progress quickly and fully eliminate plastics in the near future; pushing for political change must continue, but we should do so under the assumption that nothing (or very little) will happen and therefore explore as many other options as possible to tackle MP pollution. This would mean a focus shift, or more so a focus division, towards the advancement of downstream solutions. Related to this is the current state of these options: Many of the above-described downstream solutions require highly controlled laboratory settings and cannot be employed in ecosystems without severely endangering these systems. More focus and funding should be dedicated to those technologies that can be used to clean natural environments.

Similarly, current environmental solutions focus on MP removal from the water column, which only retains low-density MPs for the time it takes to employ these solutions. High-density particles as well as weathered and ‘scavenged’ MPs will sink into sediments and accumulate there. No methods for the removal of MPs from natural sediments exist to my knowledge, which is highly problematic considering that they serve as long-term MP sinks. Focus must be shifted to address MP remediation in the most relevant medium / treatment end point, which, based on the current state of knowledge, is sediment.

In addition, the scale of political and technological remediation options is insufficient. Scalability, while becoming an increasingly important research priority, has not been achieved for most up- and downstream solutions. If a remediation method is to be successful it must be widely applicable and easily adjustable to different scales and contexts; most options currently operate well at a small scale but have not been expanded to the scale required for universal and / or environmental applications. While not all

plastics are the same and a one-size-fits-all solution for the mitigation and remediation of MPs is unlikely, the often suggested 'hybrid treatments', combining multiple solutions / approaches (Ahmed et al., 2023; Rose et al., 2023), are subject to this criticism as well. The development of treatments offers a great opportunity to dedicate more attention to scalability from the get-go.

And finally, the importance of behavioural and cultural changes cannot be overstated. A consumerist society based on an industrial system where manufacturing from scratch is cheaper than repairing, recycling, and reusing is unsustainable and will eventually face pollution and / or material bottlenecks (Walker & Fequet, 2023). MP pollution is a stark reminder of this fact and should be taken as a warning sign and reminder that there are other ways. Natural science must collaborate with SBS to determine and address psychological and social blockages in the way of a more sustainable society in general, and upstream solutions for MP prevention, mitigation, and remediation more specifically.

2.5.6 Assigning ecological risk – practical and moral considerations

Many studies and policy frameworks tasked with recommending MP remediation options heavily base themselves on defining ecological risk thresholds (Adam et al., 2019; Ilmi et al., 2023; Koelmans et al., 2023; McCarty et al., 2023; Mehinto et al., 2022; Qiu et al., 2023; Redondo-Hasselerharm et al., 2023), however, this is rarely questioned from a practical and moral standpoint. Maybe the question is not 'how can we best characterize risk related to MP pollution?' but rather 'SHOULD we characterize risk related to MP pollution?', i.e., whether the use of 'risk' as the primary concept to ground MP mitigation and remediation is useful and justifiable. So far, RA frameworks have resulted in the same conundrum as scientific studies on MPs, lacking standardization and therefore comparability (Adam et al., 2019; Ilmi et al., 2023; Koelmans et al., 2023; McCarty et al., 2023; Mehinto et al., 2022; Qiu et al., 2023; Redondo-Hasselerharm et al., 2023). This could certainly be changed, but should risk be at the center of these changes, or would another concept be better suited for the job? Historically, the concept of risk and RA frameworks have helped control contaminants quite effectively (Baud et al., 2023; De Vivo et al., 2017; Ebinghaus et al., 2013; Fesenko et al., 2009; Pelletier et al, 2019;

Zeman & Patterson, 2006). However, given that MPs are a unique suite of contaminants unlike any we have ever seen, critical evaluations of how to best remediate MP pollution could, similarly to critical evaluations of field sampling / sample analysis tools, benefit from including practical and moral re-examinations of the conceptual tools we plan on using.

Considering practicality, risk has three advantages. Firstly, it is commonly used in ecotoxicology and environmental management (De Vivo et al., 2017; Pelletier et al, 2019; Redondo-Hasselerharm et al., 2023; Zeman & Patterson, 2006), meaning it does not have to be defined as thoroughly and debated by many different actors over the time span of years before it becomes a universally accepted term. Related to this, saving time at this first stage of RA framework development, where priorities are set and terms get clarified (Pelletier et al, 2019; Redondo-Hasselerharm et al., 2023), could speed up the entire process of making MP-specific policies and deriving set goals and environmental law from them. Finally, risk exhibits the characteristics of a buzz word: It combines 'a strong belief in what the notion is supposed to bring about' (Rist, 2013, as cited in Cairns & Krzywoszynska, 2016) with a vagueness and multiplicity of definition that enables the mobilization of broad support (Cairns & Krzywoszynska, 2016). It thereby has the potential to generate media attention and can communicate a level of urgency even in short briefing documents common in politics (Cairns & Krzywoszynska, 2016).

There are also disadvantages of continuing the use of 'risk' in MP remediation. From a practical standpoint, these disadvantages mostly relate to the implicit assumptions that come with the word itself. Risk thresholds are defined differently in most studies (Adam et al., 2019; Ilmi et al., 2023; Koelmans et al., 2023; McCarty et al., 2023; Mehinto et al., 2022; Qiu et al., 2023; Redondo-Hasselerharm et al., 2023), and negotiating an 'objective' middle ground specific to the risk associated with MPs could be time- and resource-consuming, both of which could be spent on the development of action plans and / or technologies. Furthermore, risk is based on probabilities, models, and eventualities (Adam et al., 2019; Koelmans et al., 2023; McCarty et al., 2023), making it an inherently vague concept; working with probabilities and eventualities also implicitly communicates that MP pollution effects and measures to combat them are mere options as opposed to necessities for well-being and survival (see effects on mice

[Li et al., 2024], in esophageal cells [Guanglin & Shuqin, 2024], and on mammalian fertility [He & Yin, 2023]). Finally, and most importantly, an 'absolute zero' of risk cannot be established for MP exposure / pollution levels. The precise effects and travel pathways of MPs are still unclear, and accumulation of the physical particles as well as other effects (e.g., toxicity) have been going on for many years (Domercq et al., 2022; Elagami et al., 2023), often not adhering to standard dating techniques in media such as sediments (Dimante-Deimantovica et al., 2024). Therefore, a neat new definition of risk can only capture a late-stage exposure, and thereby suboptimal environmental state, of MPs in the natural environment.

Moral arguments surrounding risk are much harder to make. Environmental justice encompasses its own field of study with a huge body of literature; I will not be making this section another literature review but rather an attempt at constructing my own simplified but sound argument. Generally, environmental ethics emerged as a critique of human-centred ethics and concerns itself with defining subjects of moral concern (Brennan & Lo, 2021), which then affects our decisions and behaviour in relation to those subjects. The term 'moral considerability' describes the ethical obligations we have towards other entities (Dussault, 2018); as a result, entities that are a subject of moral concern become ethically relevant. Environmental justice scholarship has long struggled with clearly defining ecosystems and therefore making sound claims that these systems (and abiotic ecosystem components in general) are subjects of moral concern (Vonberg, 2022). In the absence of prescribed moral obligations, decisions relating to environmental problems on the ecosystem level, such as MP pollution, have been grounded exclusively in economic and managerial thought and become exposed to poorly characterized concepts such as risk.

From an ethical perspective, 'risk' is flawed in two major ways related to anthropocentrism. Firstly, environmental management and the definition of risk are entirely based on Western human perception, assuming it to be a universally true and relatively objective baseline capable of recognizing all potentially relevant effects of MP pollution. That is likely untrue. Human perception (and Western thought) is inherently flawed, and accounting for potentially negative effects of MP pollution by utilizing a concept that encompasses practical likelihoods (e.g., measurements may have been taken

inaccurately) but not ethical likelihoods (e.g., issues are either overlooked or directly caused by working from an anthropocentric viewpoint) is ethically weak. Of course, accounting for factors hidden to human perception when the people working on MP pollution are in fact human is impossible. What would be required, however, is the use of concepts that allow for perceptual expansion and explicit (rather than implicit) ethical assumptions about why those concepts have been chosen to best represent the moral dilemma of MP-polluted ecosystems. Secondly, 'risk' is typically used as a vector for instrumental values; a major driver of wishing to preserve and remediate ecosystems are the ecosystem services they provide for current and future generations, and MP pollution risk is primarily expressed along this axis of impacts on current and future generations. Intrinsic values of wishing to preserve and remediate nature for its own sake and based on the ethical obligation we have towards ecosystems because moral considerability has not been established in MP pollution research. This missing level of ethical equality and the resulting binary human-nature division is a general problem in environmental science and a strongly debated topic in environmental ethics and becomes further enshrined by utilizing highly anthropocentric concepts such as risk. Based on these two major flaws, the use of risk as a central concept in environmental management of MP pollution should not be continued in its current state. 'Risk', or a potential successor concept, must become less anthropocentric by re-examining the role of human perception and its related implicit assumptions, and becoming more equal by expressing intrinsic values more clearly. Only then can risk be used as an ethically sound expression of the moral considerability of MP-polluted ecosystems.

To summarize, assigning ecological risk has not been sufficiently questioned from a practical or moral perspective. Both reveal problem areas within this concept that should be further clarified and addressed before centering policy and management frameworks for the mitigation and remediation of MP pollution in the natural environment around 'risk'.

2.6 Summary of knowledge gaps

MPs come in many different sizes, shapes, and polymers (varying chemical composition and density), all of which can potentially alter the type and gravity of effects on the environment (Rochman et al., 2019; Rose et al., 2023; Tursi et al., 2022). Standardized data collection on MPs pollution and remediation thereof (including risk assessments) has been complicated by the diversity that constitutes this class of contaminants (Adam et al., 2019; Lu et al., 2021). Recently, trends towards standardization have begun to emerge but the pace of this development must increase, as comparable data and being able to clearly define the problem are crucial to move forward with remediation efforts. A first step could be to develop standardized frameworks for sub-groups of MPs, e.g., based on polymer or shape. These frameworks are already in the making but require more data input (Burns & Boxall, 2018). Similarly, protocols for the dating of MP-contaminated sediment cores must be standardized to adequately understand and compare historical and current MP distribution trends in sediments across the globe.

Until very recently, little attention has been given to MPs in sediments, and especially freshwater sediments. Freshwater systems (and their sediments) still tend to get lumped together as one big category, despite the large differences that can exist between estuaries, rivers, and oligotrophic and eutrophic lakes. More differentiation is needed in this area, as we are trying to understand the precise ecosystem connections relevant to MP pollution. Similarly, interaction effects and inner-sediment travel pathways (especially vertical transport) are still poorly understood (Dimante-Deimantovica et al., 2024), which is especially problematic in the light of optimistic claims about lake sediments functioning as a natural MP sink removing MP pollution from aquatic systems. While MP predictable MP hotspots in sediments would be incredibly useful for targeted remediation, caution and increased research efforts are irreplaceable in this context (Sandgaard et al., 2023). As we learn more every day about this relatively new class of contaminants, potential solutions must provide a high degree of confidence that no additional harm is caused. As of now, this can only be estimated. Convincing MP remediation frameworks must be provided with the best available information and respect the rule of unnecessary harm avoidance. Therefore, more studies focusing on MP-sediment interactions and vertical transport mechanisms, drawing

implications for the wider ecosystem through interconnectivity, are needed to adequately inform remediation. Similarly, more focus should lie on thinking outside the box and learning from environmental matrices to inform our understanding of MP behaviour; sediments are not only a crucial end point / sink for MP pollution but also provide highly transferable knowledge that can guide researchers to draw parallels between natural sediments and MPs in terms of shape, transport, and deposition behaviour (Sandgaard et al., 2023; Waldschläger et al., 2022). Using natural sediments, we can learn for our MP sampling methods, particle descriptions, and environmental effects (Waldschläger et al., 2022).

Presence-absence studies and review articles are very common in MP research. However, this has not exactly fuelled progress; a move towards more real-life experiments could provide the data needed to bridge the gap between laboratory experiments and field studies. Furthermore, real-life experiments can trial mitigation and remediation options in medium-scale applications and thereby expand our 'catalogue' of available MP treatments; these learnings can then be applied to other contexts and may contribute to the standardization of MP remediation.

Despite a surge in MP publications, the contributions of SBS (i.e., the 'human dimension' of MP pollution) and applications of environmental ethics ('concept-checks') to the field of MP pollution research are still minimal. Natural sciences, including environmental science, must become more interdisciplinary if they want to adequately address the complex environmental problems of an increasingly globalized and connected society.

Finally, mitigation and remediation via up- and downstream solutions face a multitude of problems related to focus areas (marine vs freshwater; prevention vs removal), treatment end points (lack of strategies for media with most MP retention, e.g., sediments), scalability, cultural changes, and the lack of ethical foundations for their suggested concepts (e.g., risk). These issues, in combination with unclear MP effects and unstandardized reporting of MP data, have prevented serious progress towards standardized remediation frameworks and wide-ranging policy recommendations (Adam et al., 2019; Burns & Boxall, 2018; Qiao et al., 2022; Walker, 2021).

CHAPTER III

Experimental evidence that polymer type affects vertical distribution of microplastics in boreal lake sediments

3.1. Abstract

Lake sediments are especially affected by microplastics pollution compared to other aquatic media; yet knowledge on the lifecycle of microplastics in lake systems and the processes driving accumulation patterns in sediment is limited. These processes likely vary with environmental parameters (e.g., sediment and / or transport characteristics) and the particle size and specific densities of different polymer types of the microplastics. Here, I aim to help fill some of these knowledge gaps by contributing to a Before-After-Control-Impact (BACI) whole ecosystem experiment called *The pELAstic Project*. In our experimental lake, L378 at the International Institute for Sustainable Development – Experimental Lakes Area (IISD-ELA), microplastics were added throughout the ice-free season (which lasts June through October) of 2023 to simulate stormwater. Every two weeks low-density polyethylene (LDPE), polyethylene terephthalate (PET), and polystyrene (PS) microparticles, ranging in size from ~15 – 1000 µm, were added. Sediment cores and sediment grab samples were collected across a water depth gradient in July and August 2023. Sediment characteristics and variations were analyzed and microplastics identified via microscopy. Results revealed that water content, organic content, and mineral content differed between the littoral and profundal sediments. Water depth was negatively associated with only one of the three polymers (LDPE), but sediment depth was a helpful predictor of microplastic concentration across polymers. An interaction between sediment depth and size fraction was detected for PET and LDPE concentrations. Polymer type as well as size fraction may therefore influence the vertical transport of microplastic fragments within the sediment profile of boreal lakes. This information is crucial to identify microplastic hotspots within the sediment profile and advance the development and application of appropriate remediation methods.

3.2. Introduction

Microplastic fate and depositional patterns are a highly variable and poorly understood global problem. Microplastics are a suite of contaminants comprising plastic particles <5 mm in size and can vary greatly in size fractions, shapes, polymers, and additives (Almas et al., 2022; Rochman et al., 2019). Microplastics are classified as primary, intentionally manufactured microplastics such as microbeads, or secondary, which are generated through the breakdown of meso- and macroplastics (Walker & Fequet, 2023). While an estimated of 92% of the globally available 269 million tonnes of plastic particles constitute microplastics (Rose et al., 2023), point sources as well as travel pathways of microplastics are often difficult to trace (Wang et al., 2022; Yu et al., 2024). Microplastics can be distributed via an array of transport pathways, e.g., traveling through aquatic systems (Rose et al., 2023; Schwarz et al., 2023), adhering to or being in- / egested by biota (e.g., seabirds; Bourdages et al., 2021; Susanti et al., 2020), and even hitch-hiking winds which results in atmospheric deposition (Rose et al., 2023; Ryan et al., 2023; Schwarz et al., 2023; Xiong et al., 2022). With microplastics being recorded in ecosystems across the globe, including extremely remote areas (e.g., the deep sea [Galgani et al., 2022], and Mount Everest [Kurniawan et al., 2023]), and increasingly urgent warnings about their effects on human and environmental health (Ajay et al., 2021; Hengstmann et al., 2021), the relevance of understanding and combatting microplastic pollution for research and to develop effective reduction policies cannot be overstated.

Freshwater systems have been nicknamed ‘conduits of microplastic’ due to the ability of a singular ecosystem to serve as a transporter, receiver, and potential sink for microplastic particles, connecting the terrestrial, atmospheric, and marine environment (Kurniawan et al., 2023). Lakes are especially heavily affected by microplastic pollution, due to the tendency of microplastics to accumulate in low energy environments, and particularly enclosed waters, where particles can be easily captured and deposited into bottom sediments without much disturbance (Uddin et al., 2021). Consequently, lake sediments are theoretically among the most affected media globally by microplastic pollution. Recent data from the Great Lakes confirms this theory: 27,830 particles / kg of dry sediment, the highest microplastic concentration in nearshore sediments worldwide,

have been detected in Lake Ontario (Yao et al., 2019). The most abundant polymers in global freshwaters and estuaries are low-density polyethylene (LDPE), polypropylene (PP), polyethylene terephthalate (PET), and polystyrene (PS; Jones et al., 2020).

An estimated 99% of the globally available microplastic particles are expected to settle in sediments (Kane et al., 2020; Sandgaard et al., 2023) and may get buried permanently as geological plastic (*The pELAstic Project*, 2024), or transported back into other ecosystem components (e.g., the food web or the water column), via a variety of transport mechanisms and processes (see Figure 3.1; Provencher et al., 2022; Sandgaard et al., 2023; *The pELAstic Project*, 2024; Waldschläger et al., 2022). Recent research suggests that there may be interaction effects occurring between microplastics and sediments, impacting the horizontal (spatial distribution of MP concentration gradients in sediments) and vertical (downward migration within the sediment profile) distribution of microplastics in sediments (Paduani, 2020; Sandgaard et al., 2023; Waldschläger et al., 2022). Sediment properties and behaviours could also be highly informative for microplastic research, as there is much transferable sedimentology knowledge that could be applied to microplastic behaviour (Waldschläger et al., 2022). In addition, sediment properties such as grain size (Cera et al., 2022; Liang et al., 2022; Vermeiren et al., 2021; Zobkov et al., 2020) and nutrient cycling (Salam et al., 2024; Seeley et al., 2020; Song et al., 2024; Yin et al., 2023) are often correlated with microplastic abundance and specific polymer types, allowing inferences about microplastic deposition based on known sediment parameters.

Here, I examine microplastic accumulation patterns of three common polymer types (low-density polyethylene [LDPE], polystyrene [PS], and polyethylene terephthalate [PET]) in sediment from a whole-lake addition experiment of lake 378 (L378) at the International Institute for Sustainable Development-Experimental Lakes Area (IISD-ELA). Microplastic fate was expected to be affected by depositional processes. As such, sediment composition was characterized and common sediment zones (based on depositional process) across the water depth gradient were identified. Microplastic accumulation across the lake was expected to be driven by gravity, and thus microplastic concentrations of the three polymers across the water depth gradient were assessed by comparing microplastic concentrations between littoral and profundal

sediments. Microplastic accumulation within the vertical sediment profile was also expected to be driven by gravity or bioturbation, which we examined through 1) microplastic concentrations in surface sediments compared to deeper sediment layers, 2) the effect of polymer type and associated density on sinking depth, and 3) the effect of particle size (≥212, 212-106, and 106-53 µm; referred to by the lower size fraction cut-off from here onwards) on sinking depth. Thus, we address the drivers of microplastic accumulation in sediment, ultimately improving the prediction of microplastic hotspots in sediment and thereby directing the development and application of in situ remediation methods. This is required to mitigate microplastic pollution in aquatic systems across the globe.

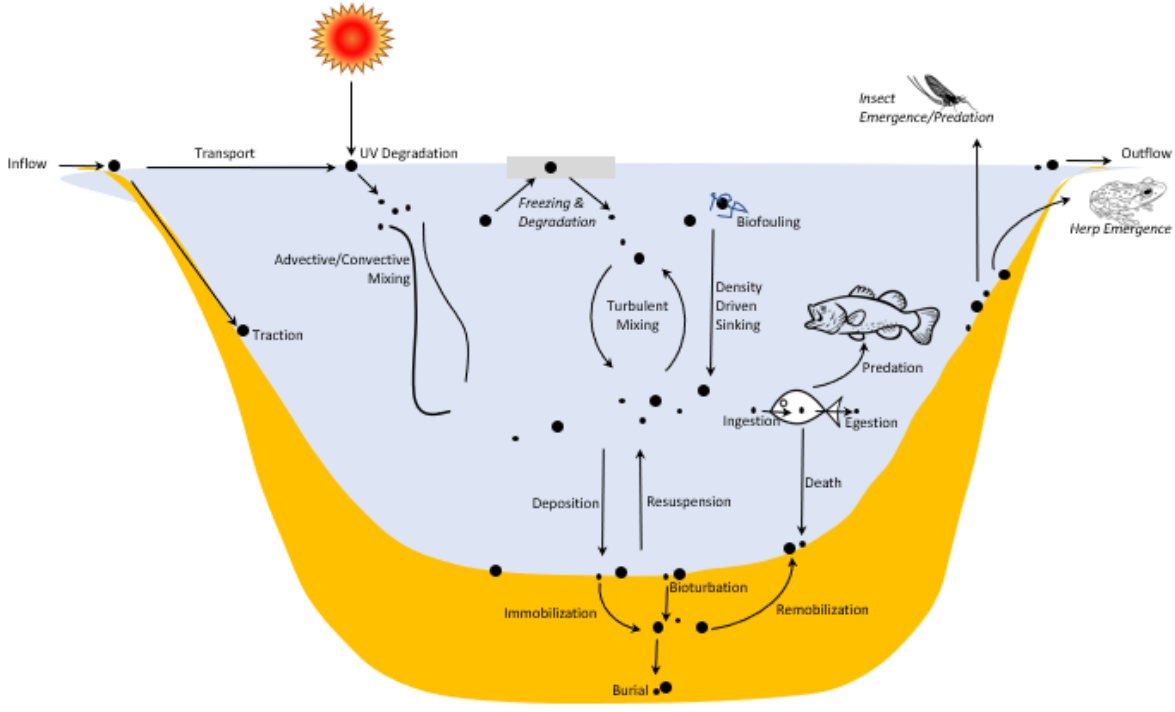

Figure 3.1 Aquatic-benthic processes as identified by the pELAstic team (Timothy Hoellein, Matthew Hoffman, and Melissa Duhaime, personal communication, March 3, 2023).

3.3. Methods

3.3.1. Study area

The pELAstic Project and this research have taken place at the IISD-ELA in North-Western Ontario, Canada (49.6603°N, 93.7283°W). Nicknamed 'The world's freshwater laboratory', this unique and remote research facility, consisting of 58 freshwater lakes, allows researchers to examine environmental stressors as isolated variables in real-life settings, without confounding anthropogenic factors (*IISD Experimental Lakes Area*, 2024). Within the IISD-ELA, The pELAstic Project has been working on the lakes 373 (L373) and 378 (see Figure 3.2), with L373 being designated as a control and L378 being subject to the experimental manipulation with microplastics (*The pELAstic Project*, 2024). L373 was chosen based on its relative similarity to L378 regarding many ecosystem characteristics (e.g., community structure); however, the research of this thesis was based exclusively in L378 and will therefore not go into any more depth regarding L373. L378 was selected as the appropriate site for this experiment on the basis of three main characteristics: 1) It is easily accessible from the research station (located just off pine road, no portaging with equipment required), 2) it has a healthy and representatively diverse fish community, and 3) there have been no previous experiments resulting in a long-term contamination that might skew the results of The pELAstic Project.

L378, the research lake sampled for this study, has an area of 260,000 m^2 and a volume of 2,000,000,000 litres (L) of water; it has a maximum water depth of 18 m and possesses one outflow. L378 is characterized by low productivity rates, as would be expected due to its northern and undisturbed location, and is best described as a boreal, remote, and oligotrophic headwater lake that has experienced little to no human disturbance for decades. The littoral regions are comprised of bedrock and sand whereas the profundal sediments have exceptionally high water content, high organic matter content and, correspondingly, low mineral content (Michael Paterson, personal communication, December 6, 2022). The sediment characteristics of L378 have to my knowledge never been analyzed systematically; the only sediment sampling in L378 was done in 2006 for a dinoflagellate cysts study (Danesh et al., 2024) and a diatom study

(Enache et al., 2011), and 2019 to collect data on baseline microplastic contamination (Dias, 2020; McIlwraith et al., 2024), and none of these specifically characterized sediments.

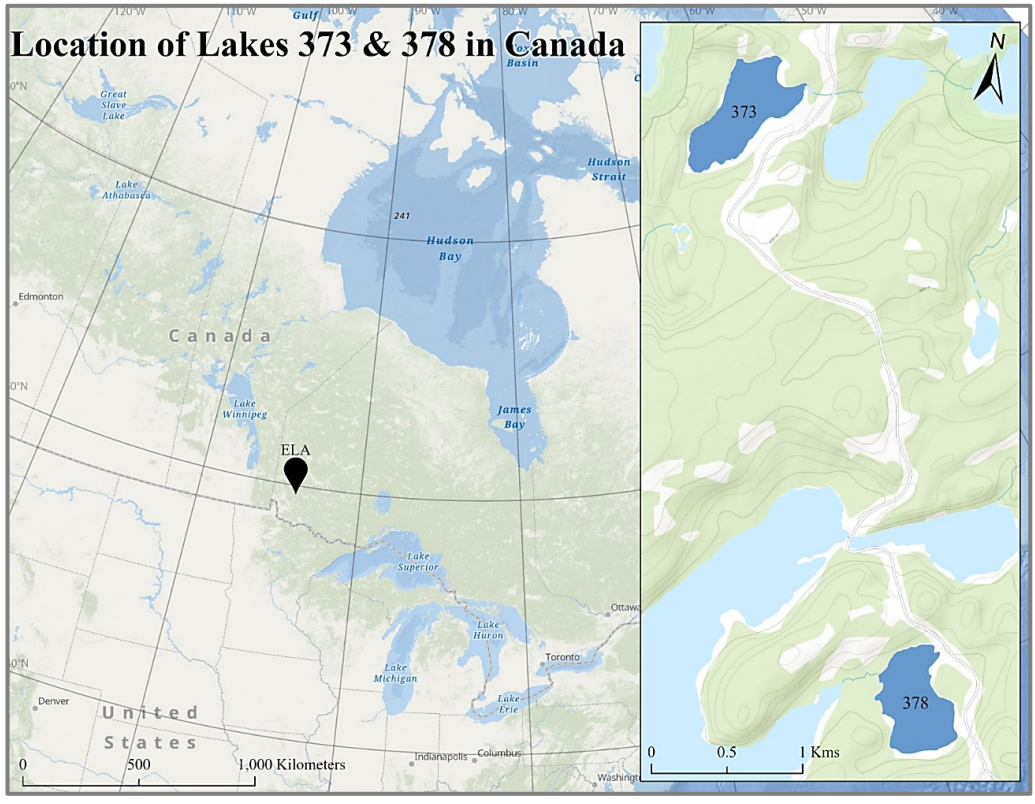

Figure 3.2 Location of L373, L378, and the IISD-ELA in Canada.

3.3.1.1. Experimental manipulation

The experimental manipulation of this lake commenced in early summer of 2023 and is set to continue for three years in total. Particle diameter ranges from 15-1000 µm and all fragments are dyed in vibrant colours according to polymer (LDPE in yellow, PS in pink, PET in blue). The polymers have different densities resulting in LDPE being positively buoyant (density = ~0.93 g/cm³), PS neutrally buoyant (density = ~1 g/cm³), and PET negatively buoyant (density = ~1.4 g/cm³). The yearly loading is comprised of 330 billion plastic particles, adding up to a total amount of 916 kg of plastics being added to the lake per year (344 kg PET, 292 kg PE, and 280 kg PS). This is comparable to adding

one gallon of plastic to an Olympic size swimming pool every year. These numbers were determined by aligning data from the Laurentian Great Lakes with the ecosystem requirements of L378. Dosing takes place bi-weekly throughout the ice-free season and started on 7 June 2023. In combination, the microplastic concentration per addition and the bi-weekly dosing schedule replicate the effect of stormwater inflows into a lake. At the time of sediment sample collection, four full additions had taken place, with the fifth occurring after core collections were completed and before grabs had been taken (see Figure 3.3).

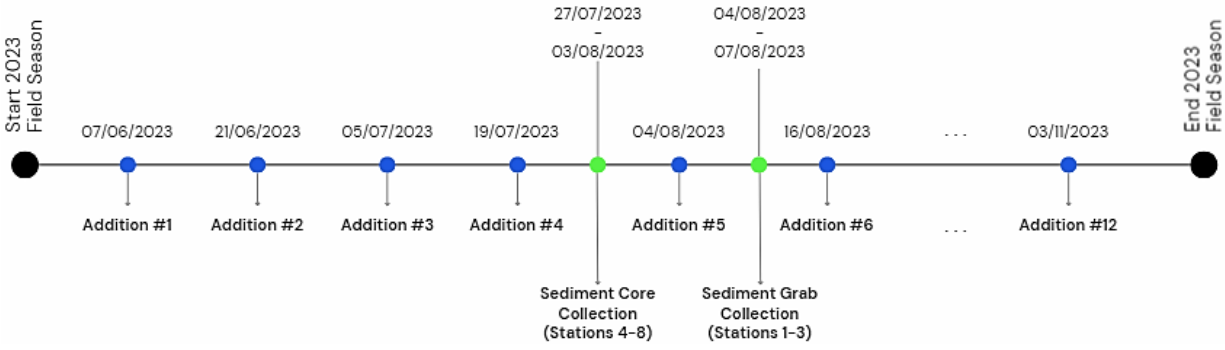

Figure 3.3 Microplastic additions and sediment sampling events during the 2023 field season.

3.3.1.2. Field collection

Samples were collected mid-season, in the period of 27 July 2023-7 August 2023, to maximize the time span since the first microplastic addition while minimizing resource constraints (space at camp and MES timeline). Stations for sediment coring were established across transects running through the water depth gradient, resulting in six depth zones (0-2 m, 2-5 m, 5-8 m, 8-11 m, 11-14 m, 14-18 m) from nearshore (<1 m) to maximum lake depth (18 m) (see Table 3.1). One station was sampled per zone, and an additional station was added for the deepest and shallowest depth zones respectively to adequately reflect the focus of the hypotheses, producing a total of 8 stations. Due to the highly limited availability of baseline data (maps or databases) of L378, exact locations required some trial and error and could only be accurately determined in the field due to the types of benthic substrate encountered and high local variability. Based on these large

variation in substrate encountered, three different sampling methods were employed: a T-bar vacuum corer at <1 m water depth for Stations 1-2, a Petite Ponar® grab sampler at 1-7 m water depth for Station 3, and a National Lakes Assessment (NLA) gravity corer in areas of >7 m water depth for Stations 4-8 (Glew et al., 2001; Baud et al., 2023) (see Figures 3.4-3.5). These collection methods produced samples of the type 'composite in-shore core', 'grab sample', and 'sectioned sediment core' (6.8 cm diameter) (see Figure 3.6).

Table 3.1 Stations according to depth zone and precise water depth.

Station #	Depth zone #	Depth zone (m)	Water depth (m)
S1	1	0-2	0.6
S2	1	0-2	0.8
S3	2	2-5	3.6
S4	3	5-8	7.1
S5	4	8-11	10.6
S6	5	11-14	12.4
S7	6	14-18	14.8
S8	6	14-18	16.8

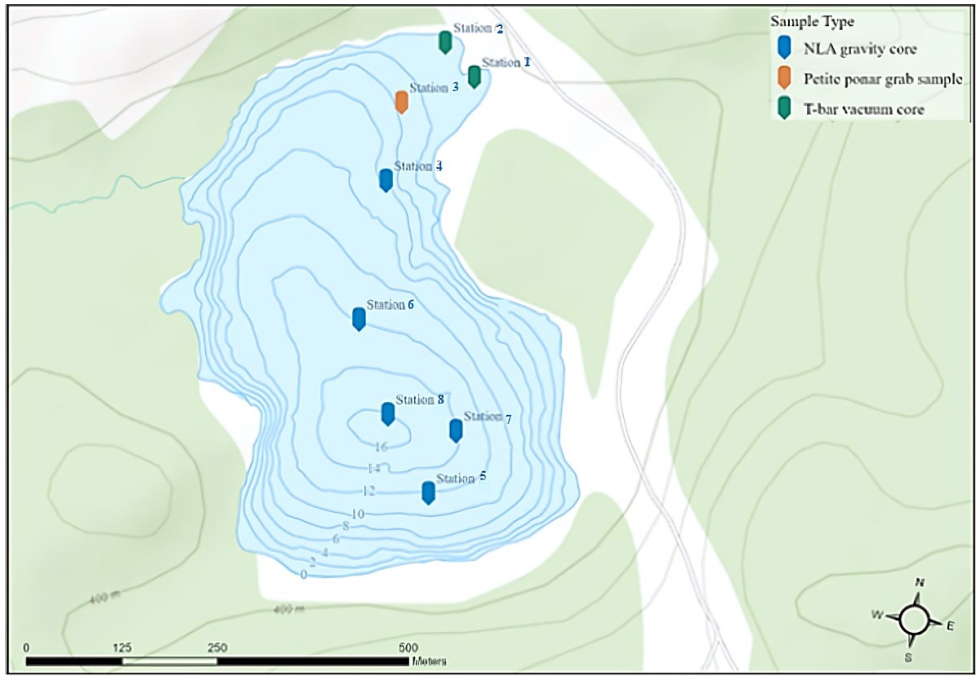

Figure 3.4 Sediment sampling stations established in L378 for this study.

Figure 3.5 Sample collection methods: T-bar vacuum corer (left), Petite Ponar® grab sampler (middle), and NLA gravity corer (right).

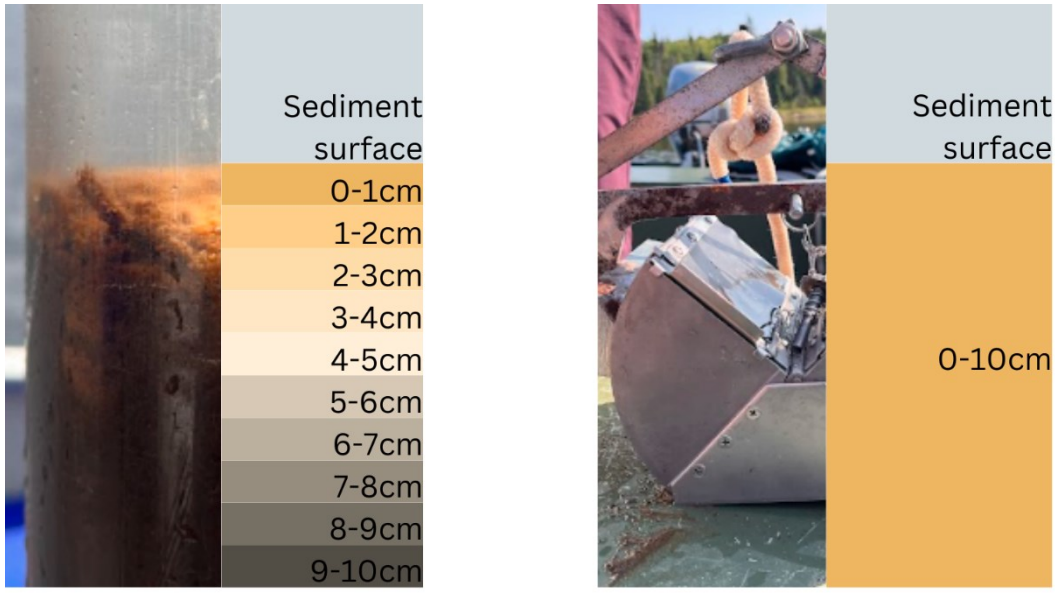

Figure 3.6 Visualization of sample types 'sectioned sediment core' (left) and 'composite in-shore cores / grabs' (right).

Plastic was, whenever possible, avoided as a material in field (and later the laboratory) equipment; if plastic was an irreplaceable component of equipment (e.g., the plexiglass tube of the NLA corer for sample collection) or the only feasible option (e.g., Whirl-Pak® bags for sample storage and reliable cross-country transport), colours resembling those of the polymers used during the experimental manipulation were

circumvented. Prior to sample collection, procedural field blanks were collected at each station: all equipment used for sampling was cleaned and then triple rinsed with MilliQ,collecting the particle-water mixture in a Whirl-Pak® bag, to assess contamination between samples during collection; one field blank was collected per station (N = 8).

Sediment cores collected with the NLA gravity corer were extruded in the field down to 10 cm sediment depth at 1 cm intervals and stored in Whirl-Pak® bags. Each sediment core slice had an initial volume of 36.32 cm^3, with varying amounts of rinse water added. Grab samples collected using the Petite Ponar® grab sampler were rinsed into a plastic bucket in the field, and transferred into large labelled double-sealed ziplock bags. In-shore cores were collected on foot in hip waders and extruded in the field as composite samples (0-10 cm sediment depth) into labelled double-sealed ziplock bags. Triplicate samples for both sediment and microplastic endpoints were collected at each station, resulting in six cores / grabs per station and a total of 48 cores and grabs (total sample N = 318). All sediment samples and blanks were stored in a cooler with ice in the field and frozen at the field station to prevent any chemical reactions occurring (which could impact sediment analysis results) until the laboratory analysis; similarly, samples were transported by airplane from the field station to Dalhousie University (estimated travel time: 12 hours) in an iced cooler and immediately re-frozen upon arrival.

3.3.2. Sediment analysis

Water content, organic content, and mineral content were analyzed for all sediment samples (N = 159). Quality assurance and quality control (QA/QC) measures for the collection and processing of samples for the sediment analysis endpoint were focused on wearing appropriate personal protective equipment (PPE) such as nitrile gloves and a cotton laboratory coat and cleaning the laboratory equipment between samples to avoid cross contamination.

Sediment samples were left to thaw overnight at room temperature (circa 12 h at 18°C). Sediment dry weight was determined gravimetrically using a fixed volume subsample of wet sediment (6.4 mL) that was dried to constant weight at 65°C for 24 h (Fisherbrand Gravity Oven 51030520); constant weight was previously determined by

drying trial samples for 12, 24, 36, and 48 h, with no change occurring after 24 h. Mass was measured with a Sartorius Competence Analytical Balance CPA225D with a precision level of 0.0001 g (calibrated with 2 g standardized weight). Water content was calculated by subtracting sample dry weight from wet weight and expressed as a percentage.

Mineral content and organic content were determined via the loss-on-ignition (LOI) method (Frank et al., 2023) at 445°C overnight (circa 12-18 h; Thermolyne Sybron Corporation Furnace F-A1730, Series 85/153855). These furnace times exceeded the minimum furnace time of six hours for complete LOI as determined by the literature (Andersen & Krysell, 2005; Schumacher, 2002). The weight following LOI (Sartorius Competence Analytical Balance CPA225D, precision level of 0.0001 g, calibrated with 2 g standardized weight) was recorded and the percentage of mineral content was calculated by dividing the weight following LOI by the wet weight of the sediment. The percentage of organic content was determined by subtracting the weight following LOI from the sediment dry weight and dividing this result by the wet weight.

Finally, a core from centre buoy (Station 8) was sent to the University of Ottawa for 210Pb radiometric dating analysis (TBD). Determining the age of the individual sediment layers will have implications for recommendation concerning the long-term contamination of sediments with microplastics.

3.3.3. Microplastic extraction and identification

Due to resource and time constraints, only 33% of collected microplastic samples (one core / grab per station; N = 8) were analyzed. QA/QC measures for the microplastic endpoint samples consisted of identification training, PPE, matrix spikes, blanks, and duplication. Before processing real samples, extraction and quantification training were completed with Rochman Lab at the University of Toronto to minimize processor error (confirmed with Fourier transform infrared [FTIR] spectroscopy and Raman spectroscopy); once training was completed, I switched to identifying polymers by colour using microscopy. Three picked sediment samples have been sent to Rochman lab for FTIR confirmation to provide a post-training microplastic ID rate (TBD). Whenever working in the laboratory, nitrile gloves, a cotton lab coat, and non-plastic clothing (e.g.,

cotton, linen, wool) were worn. All equipment and surfaces were washed with laboratory-specific dish soap and a coconut fibre sponge, as well as triple rinsed with MilliQ before use. Three matrix spikes were sent alongside the samples and results reported back to Rochman Lab to determine recovery rates from sediment samples. Recovery rates (available in Appendix A) should range between 70-130% and were deemed excellent, allowing for additional confidence in reporting results. To assess the total external contamination of samples during sample collection and processing, combined field and lab blanks were sent alongside samples (one blank per field sample); to achieve combined field and lab blanks, the collected field blanks were processed for MPs in the exact same way as the samples. One blank sent per field sample reflected a higher frequency of blanks than required by the pELAstic QA/QC document (minimum of one blank per every ten field samples). Blank correction was achieved by subtracting the mean quantity of each polymer type per size fraction within the blanks from the corresponding samples (available in Appendix A). A duplicate core was processed for Station 8 (centre buoy) to account for variability of microplastic distribution and therefore results; only one of those cores, the initial sample (C13), was included in the analysis on the basis of exhibiting lower microplastic counts and therefore producing a more conservative estimate (averaging was avoided to maintain the same level of representativeness with the other samples); information about the second core, the duplicate (C15), and variability data between the two cores is available in Appendix A. All methods and documentation of results were presented to the pELAstic team and determined to be in accordance with the larger project's objectives as well as compatible with the general QA/QC methods put down by Rochman Lab.

Microplastics were extracted from the sediments and quantified following the methods detailed in *Rochman Lab – Resources for microplastics research* (2021) and Thornton Hampton et al. (2023). Organic matter was digested by adding triple the sample volume in 30% hydrogen peroxide (H_2O_2) to samples and holding them at 47°C (Fisherbrand Gravity Oven 51030520) for 24 h. Temperature was closely monitored, as an environment exceeding 55°C may damage or destroy some plastic polymers (*Rochman Lab – Resources for microplastics research*, 2021). Once digestion was complete, the sample was poured over a 53 μm sieve and rinsed thoroughly with MilliQ

to not carry over any residual hydrogen peroxide. To separate remaining small grains and debris from microplastics, a three-step density separation protocol (2 x 2 h, 1 x 20 h) with 300 mL $CaCl_2$ solution (ρ = 1.4 g/cm³) per step and sample was employed. Strong focus was placed on rigorous stirring to re-distribute even the finest particles, and thorough rinsing of the beaker walls as to not lose any trapped particles. Samples were size fractioned onto polycarbonate membrane filters (Sterlitech 1270175, pore size 20 µm, inner diameter 47 mm) using a sieve stack of 212, 106, and 53 µm sieves and vacuum filtration pump (Sigma-Aldrich Vacuum Filtration Assembly Z290408). Filters were then sealed in a petri dish (one per size fraction) until analysis.

Microplastic particles were dry-picked off the filters and their polymer type identified with microscopy, based on the colour coding of the three polymers added to the lake: yellow for LDPE, pink for PS, and blue for PET. The magnification level was 20-40-80x and the microscope used was a Omax Trinocular Stereo Microscope G24T-X2-L56S-C720WF. A subsampling method was employed for some samples (see Table A16 in Appendix A for details): A total of ten particles per colour and size fraction were picked; all additional particles of the same colour and size fraction were tallied but not picked. This method was only used a few times, but tremendously helped cut down the time spent on microplastic quantification.

Finally, microplastic concentrations (reported in particles / g of dry sediment) were derived by dividing microplastic counts by dry sediment mass. Dry sediment mass was determined by calculating the mean sediment dry weight from the triplicate cores processed during the sediment characterization analysis.

3.3.4. Statistical analyses

To analyze the variation of sediment composition across a water depth gradient, an independent samples t-test was conducted, which compared group means between the littoral (Stations 1-3) and profundal stations (Stations 4-8) for the variables water content, organic content, and mineral content (all measured in percent) using SPSS (IBM SPSS Statistics 29.0, 2022).

To assess whether the concentration of microplastic particles depended on water depth in the lake and/or vertical depth within the sediment profile, three generalized

linear models were constructed for each polymer type with the use of R v4.2.2 (R Core Team, 2022). This recognized that the three polymer types, having different densities, may accumulate differently within the lake. The linear models for each polymer were structured in the same way, including microplastic concentration as the response variable, water depth of the sampling station and sediment depth from within the profundal cores as continuous predictors, size fraction as a categorical predictor, and an interaction between the sediment profile depth and particle size fraction. The generalized linear models were run using the glmmTMB package (Brooks et al., 2017), with model fit being assessed using simulated residual diagnostics with DHARMa (Hartig, 2022). Microplastic concentrations for PET and PS were log transformed to fit the assumptions of normality (Kolmogorov-Smirnov [KS] test for PET: $p = 0.787$; KS test for PS: $p = 0.836$) and homogeneity of variance (see PET and PS DHARMa residual plots in Appendix B). For the microplastic concentrations of LDPE, log transformations were not enough to achieve a sufficient model fit; therefore, the Poisson distribution was used (KS test for LDPE: $p = 0.438$; see LDPE DHARMa residual plots in Appendix B). Model predictions were calculated as estimated marginal means using the ggeffects package (Lüdecke, 2018). All significant differences were determined and plotted using the emmeans function. To estimate to what depth within the average sediment profile 95% or more of the microplastic particles were contained, 95% of the total emmeans for each size fraction and the cumulative sum of emmeans across sediment depths were calculated (holding water depth constant); the sediment profile depth cut-off where 95% of the counted microplastics could be found for each size fraction was defined as the number where 95% of the total emmeans is exceeded by the cumulative sum across sediment depths (slice depth). An alpha value of 0.05 was used for all statistical tests.

3.4. Results

The examination of the sediment composition and microplastic concentrations in sediment samples from L378 led me to the identification of sediment zones and detection of horizontal and vertical microplastic accumulation patterns. As such, I expected to find different trends between the littoral and profundal samples for sediment composition variables (water content, organic content, and mineral content) and microplastic

concentrations (total, PET, PS, and LDPE). I base this on the depositional processes in the lake, and gravity and / or bioturbation being the main drivers of horizontal and vertical microplastic accumulation in sediments.

3.4.1. Sediment composition

The water content in the littoral sediments (water depth above 7 m, Stations 1-3; N = 9) was low, ranging from 15.7% to 29.5% and averaging 23.1% ± 4.6% (mean ± standard deviation [SD]), whereas water content levels in the profundal sediments (water depths below 7 m, Stations 4-8; N = 150) were exceptionally high, ranging from 81.5% to 94.0% and averaging 87.6% ± 2.4%. This difference in group means between littoral and profundal sediments for water content was statistically significant ($t(8.268) = -41.518$, $p < 0.001$). The effect size, as measured by Cohen's d, is $d = -24.895$, indicating a very large effect.

Organic content levels, similarly to water content levels, were low in the littoral sediments, ranging from 0.4% to 1.4% sediment wet weight and averaging 0.9% ± 0.4% sediment wet weight. In contrast, the profundal sediments exhibited markedly greater organic content levels that ranged from 1.4% to 5.0% sediment wet weight with a mean of 3.7% ± 0.6% wet weight. The difference in organic content levels between littoral and profundal sediments was statistically significant ($t(10.347) = -22.132$, $p < 0.001$) and the effect size was very large ($d = -5.268$).

In contrast, mineral content was the largest component in the littoral sediments, ranging from 69.2% to 84.0% wet weight with a mean of 76.1% ± 4.9% sediment wet weight, but made up a much smaller portion in the profundal sediments, ranging from 3.5% to 14.9% sediment wet weight and averaging 8.7% ± 2.1% sediment wet weight. The difference in group means between littoral and profundal sediments for mineral content was statistically significant ($t(8.179) = 40.757$, $p < 0.001$) and the effect size was very large ($d = 28.689$). More data (minimum, maximum, and range) on sediment composition across water depth are available in Table 3.2; additional visualization is provided in Figure D1 (Appendix D).

Table 3.2 Range, minimum and maximum values, and mean of water content, organic content, and mineral content between station clusters (N = 159).

	Measure	N	Range (%)	Minimum (%)	Maximum (%)	Mean (%)	SD (%)
Stations 1-3	Water content	9	13.8	15.7	29.5	23.1	4.6
	Organic content	9	1.0	0.4	1.4	0.9	0.4
	Mineral content	9	14.8	69.2	84.0	76.1	4.9
Stations 4-8	Water content	150	12.4	81.5	94.0	87.6	2.4
	Organic content	150	3.6	1.4	5.0	3.7	0.6
	Mineral content	150	11.5	3.5	15.0	8.7	2.1

Based on these sediment characteristics and sampling experiences, sediment zones common in the literature – erosional, transitional, and depositional (Bloesch, 1995; Cera et al., 2022; Rowan et al., 1995) – were determined for L378. The erosional zone ranges from 0-2 m water depth, the transitional zone from 2-8 m water depth, and the depositional zone spans the areas deeper than 8 m water depth (see Figure 3.7), with the primary benthic substrates consisting of sand, bedrock-sand mixture, bedrock-organic material mixture, and organic material.

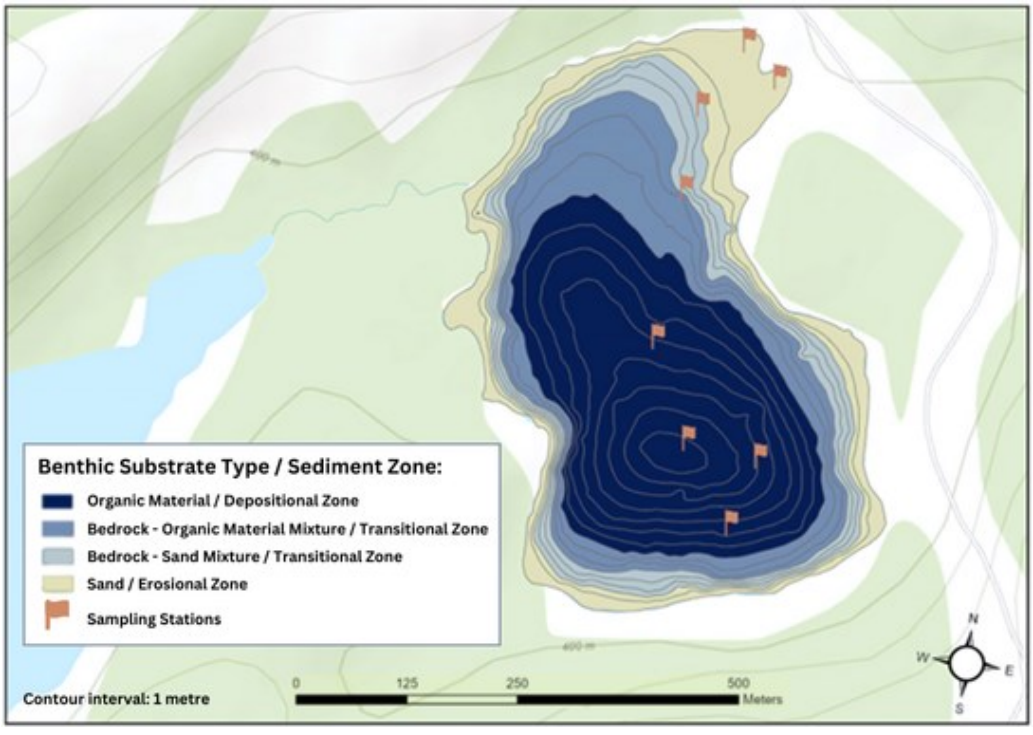

Figure 3.7 Benthic substrate types and sediment zones across water depth gradients in L378; orange flags symbolize sampling stations.

3.4.2. Microplastic accumulation along the water depth gradient

A total of 1059 microplastic particles – corresponding to an average microplastic concentration (in particles / g of dry sediment) of 14 ± 18 (mean ± SD) – was found during this analysis, with particles detected at every water depth. The highest microplastic concentration across polymers was detected at a water depth of 10.6 m; at this station a mean of 21 ± 25 microplastic particles were captured. This water depth also exhibited the highest PET and PS concentrations with averages of 8 ± 9 particles per g sediment dry weight and 12 ± 15 particles per g sediment dry weight, respectively. However, the highest LDPE concentration, with a mean of 1 ± 2 particles per g sediment dry weight, was detected at 7.1 m water depth.

The lowest microplastic concentration across polymers, with a mean of 2 particles per g sediment dry weight, was detected 0.6 m water depth (no SD's are available for the composite in-shore and bulk samples at 0.6 m, 0.8 m, and 3.6 m water depth). The station established at 0.6 m water depth also exhibited the lowest PET (mean: 0 particles per g sediment dry weight) and LDPE (mean: 0 particles per g sediment dry weight) concentrations. In contrast, the lowest PS concentration with 1 particles per g sediment dry weight, was detected at 3.6 m water depth.

Overall, PS concentration was the highest among the three polymers at all water depths except for 3.6 m and 14.8 m where PET concentration dominated slightly. LDPE concentration was the lowest among the three polymers at all water depths. Additional details on microplastic concentrations per polymer and across polymers for each water depth are available in Figure 3.8 (additionally, see Table C1 in Appendix C).

Testing these effects statistically revealed that water depth was not significantly correlated with PET concentration ($z = -1.126$, $p = 0.260$) or PS concentration ($z = -1.377$, $p = 0.169$), meaning that, contrary to our expectations, water depth was not a helpful predictor for the accumulation of these polymers. LDPE concentration, however, was significantly and negatively correlated with water depth ($z = -3.073$, $p = 0.002$), suggesting decreasing LDPE concentration with increasing water depth (see Figure 3.9).

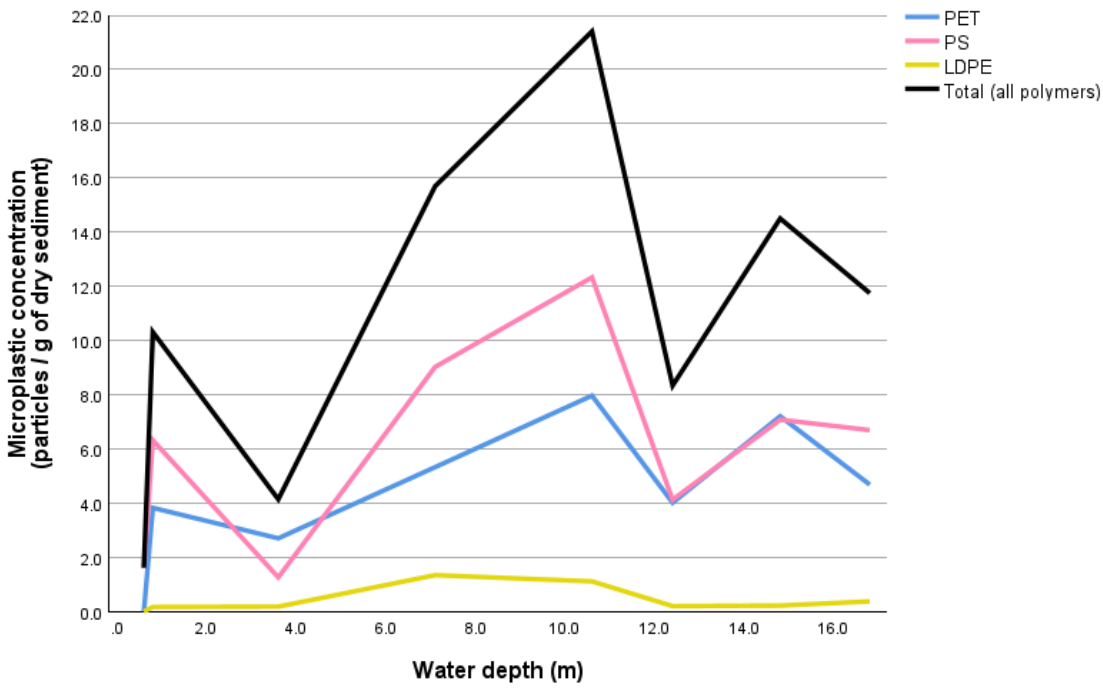

Figure 3.8 Microplastic concentration by water depth (across polymers and size fractions).

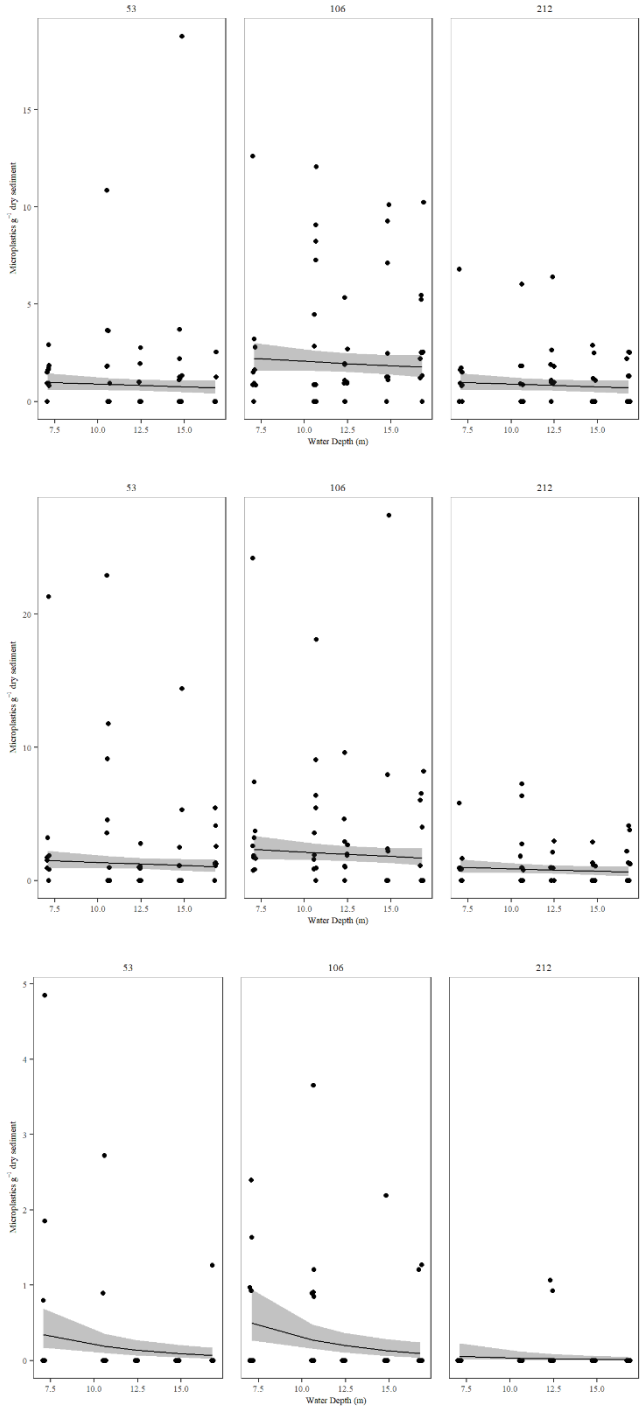

Figure 3.9 Visualization of the relationship between microplastic concentration and water depth by size fraction for PET (top plot), PS (middle plot), and LDPE (bottom plot). There is no significant relationship between water depth and PET or PS concentrations in the sediment (averaged across sediment depth), and a slight negative relationship between LDPE concentration (averaged across sediment depth) and water depth. The black line shows the predicted relationship between water depth and LDPE concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

3.4.3. Microplastic accumulation within the sediment profile

Microplastic particles were also detected at every sediment depth (down to 10 cm below the sediment-water interface) from the NLA corer collected samples (i.e., from water depths >7 m). The highest microplastic concentration (in particles / g of dry sediment) across polymers, with a mean of 57 ± 29 (mean ± SD) units was detected at the sediment surface. The sediment surface also exhibited the highest PET and PS concentrations with averages of 21 ± 10 and 35 ± 19 units, respectively. The highest LDPE concentration, with a mean of 1 ± 2 units, was detected in the 1-2 cm sediment depth subsample.

The lowest microplastic concentration across polymers in the sediment profile was detected in the deepest layer (9-10 cm sediment depth subsample), averaging 3 ± 1 units. The deepest sediment layer also exhibited the lowest PET and PS concentrations with means of 2 ± 1 units and 2 ± 1 units, respectively. The lowest LDPE concentration, 0 ± 0 units, was detected at the deepest layer as well as at 8 cm sediment depth. Overall, PS concentration was the highest among the three polymers at all sediment depths except the 3-4 cm, 7-8 cm, and 9-10 cm sediment depth subsamples where PET concentration dominated slightly. LDPE concentration was the lowest among the three polymers at all sediment depths. Microplastic concentrations per polymer and across polymers for all sediment layers are visualized in Figure 3.10 (additionally, see Table C2 in Appendix C).

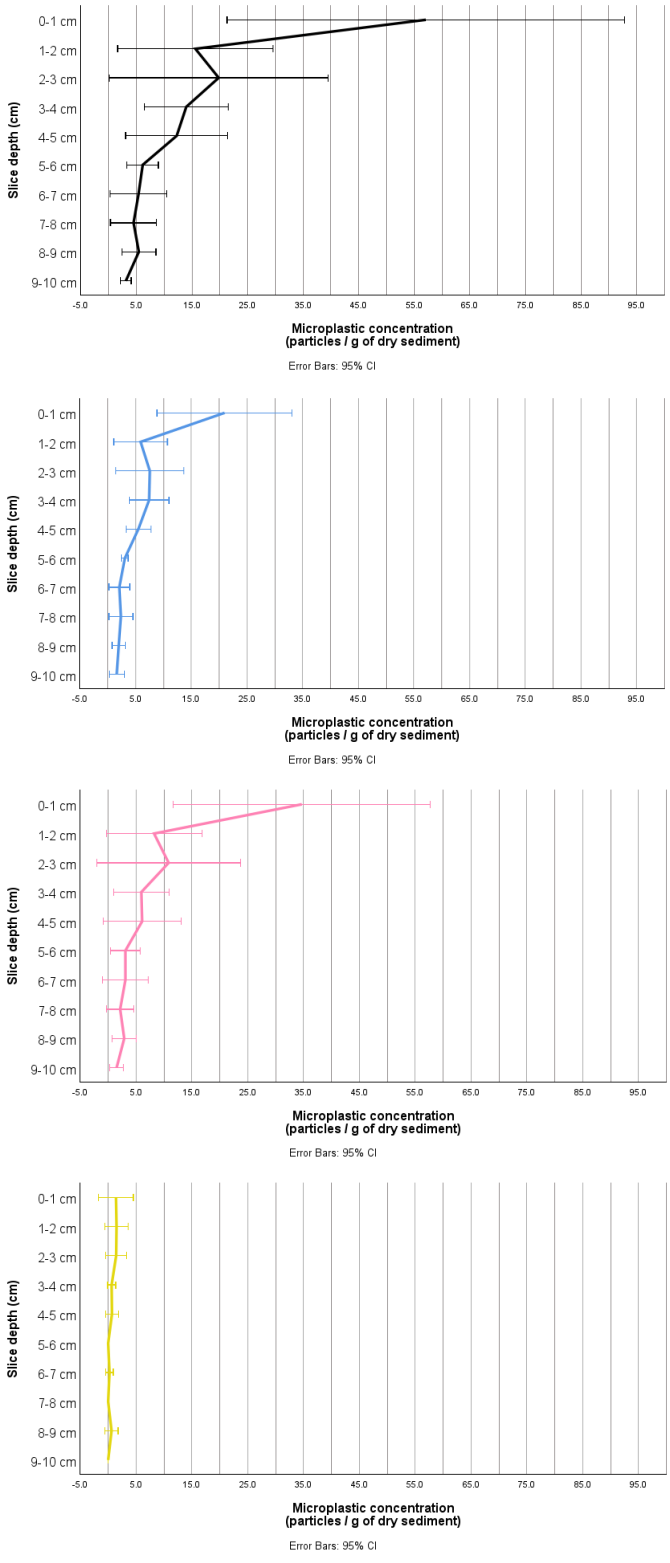

Figure 3.10 Vertical distribution profile of microplastic concentration in depositional sediments (water depth > 7 m) in total (top plot) and for each polymer across size fractions (PET, PS, and LDPE from second to bottom plot).

These effects were statistically tested for each polymer, revealing distinct patterns for the vertical penetration of microplastic particles in the sediment profile related to polymer type and size fraction. PET concentrations could better be explained by the linear model including the interaction between sediment depth (i.e., slice depth) and size fraction than the model without the interaction; the interaction was marginally significant ($X^2 = 5.810$, $p = 0.055$). Since sediment depth and size fraction were included in an interaction, these coefficients and p-values could not be interpreted separately. Instead, water depth was held constant at its mean and a pairwise comparison (contrast function from emmeans) for size fraction was fitted to assess the variation of PET concentration across size fractions at different sediment depths. Whereas the 212 µm and 53 µm size fractions did not differ significantly from each other among different sediment depths (t-ratio = -0.004, p = 1.000), the 106 µm size fraction differed highly significantly from both the 212 µm and the 53 µm size fraction (t-ratio = 4.320, p <0.001; t-ratio = -4.325, p <0.001 respectively). PET concentration decreased with sediment depth across all size fractions, although more rapidly for the 106 µm fraction. These relationships are plotted below in Figure 3.11. Finally, 95% of the PET particles counted occurred in the upper 8 cm of sediment for all three size fractions (53, 106, and 212 µm).

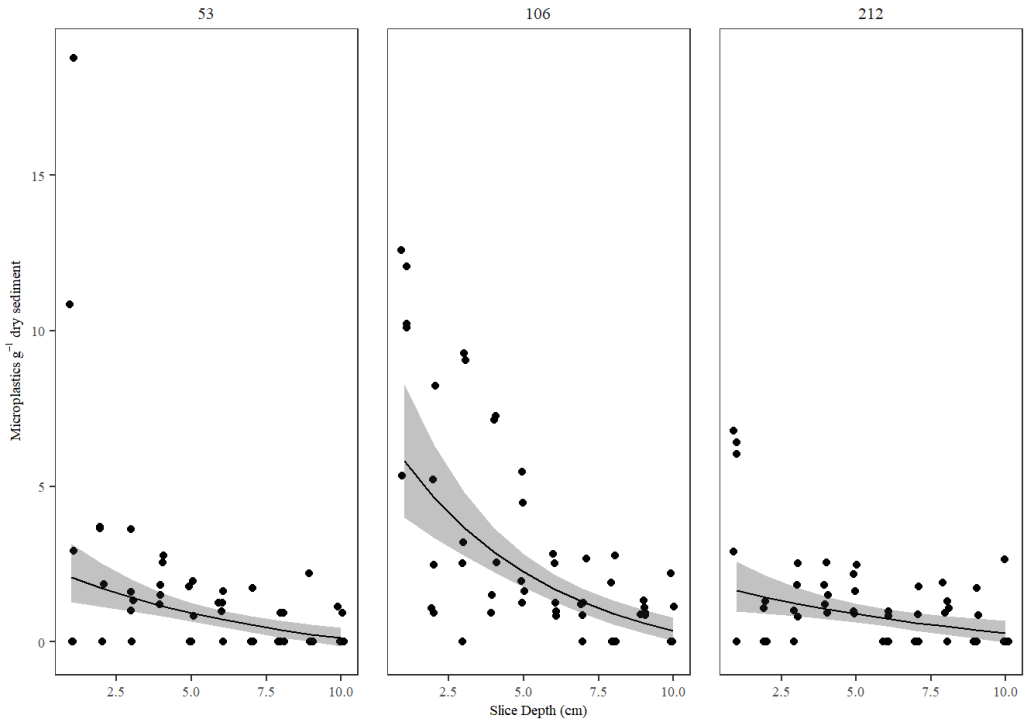

Figure 3.11 Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for PET concentration. The 106 µm size fraction exhibited a much steeper decline in concentration with depth within the sediment core than either the 53 µm or the 212 µm size fractions, which were generally less concentrated and more consistent in concentration across the upper 10 cm of the sediment core. The black line shows the predicted relationship between sediment depth and PET concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

While PS concentrations could also better be accounted for with the linear model including the interaction between sediment depth (i.e., slice depth) and size fraction than the model without the interaction; the interaction was not significant ($X^2 = 1.462$, $p = 0.481$). Water depth was again held constant and a pairwise comparison for size fraction was fitted to assess the variation of PS concentration across size fractions at different sediment depths. The 53 µm size fraction differed marginally from the 106 µm size fraction (z-ratio = -2.141, $p = 0.085$), and not from the 212 µm size fraction (z-ratio = 1.732, $p = 0.200$). The 106 µm and 212 µm size fractions were highly significantly different from each other between different sediment depths (z-ratio = 3.864, $p < 0.001$). PS concentration decreased consistently with slice depth across all size fractions. These

relationships are plotted below in Figure 3.12. Finally, 95% of the PS particles counted occurred in the upper 7 cm of sediment for the 53 µm and 212 µm size fractions, and in the upper 8 cm of sediment for the 106 µm size fraction.

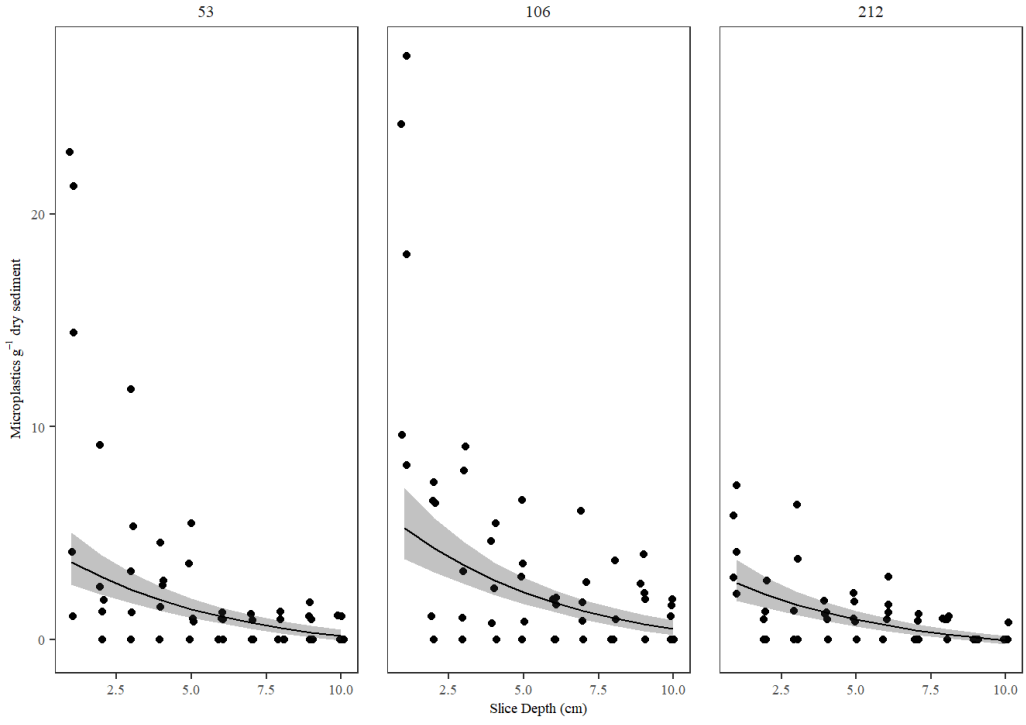

Figure 3.12 Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for PS concentration. The 106 µm size fraction exhibited a marginally steeper decline in concentration with depth within the sediment core than the 53 µm and 212 µm size fractions, which were less concentrated and slightly more consistent in concentration across the upper 10 cm of the sediment core. These effects are most prominent in the shallower sediment depths. The black line shows the predicted relationship between sediment depth and PS concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

LDPE concentrations, similarly to PET and PS concentrations, could also better be accounted for with the linear model including the interaction between sediment depth (i.e., slice depth) and size fraction than the model without the interaction; the interaction was marginally significant ($X^2 = 5.830$, $p = 0.054$). Another pairwise comparison for size fraction was fitted, holding water depth constant, to assess the variation of LDPE concentration across size fractions at different sediment depths. The 53 µm size fraction did not differ from the 106 µm size fraction (z-ratio = -1.054, $p = 0.543$), but the 53 µm

and 212 µm size fractions differed marginally from each other (z-ratio = 2.464, p = 0.037). The 106 µm and 212 µm size fractions were highly significantly different between sediment depths (z-ratio = 3.028, p = 0.007). LDPE concentration decreased consistently with slice depth across the 53 µm and 106 µm size fractions; however, this trend was not visible in the 212 µm due to the very low LDPE concentration of this size class. These relationships are plotted below in Figure 3.13. Finally, 95% of the LDPE particles counted occurred in the upper 4 cm of sediment for the 53 µm size fraction, in the upper 8 cm of sediment for the 106 µm size fraction, and in the upper 3 cm of sediment for the 212 µm size fraction.

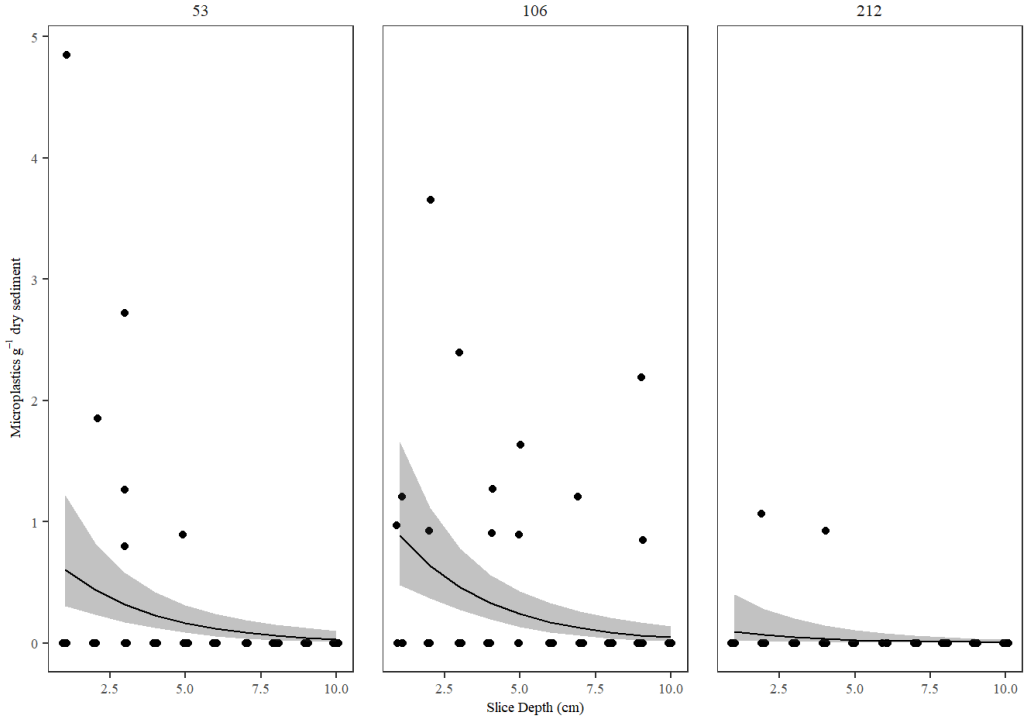

Figure 3.13 Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for LDPE concentration. The 106 µm size fraction exhibited a marginally steeper decline in concentration with depth within the sediment core than the 53 µm size fraction, and a much steeper decline than the 212 µm size fraction. The 53 µm size fraction was less concentrated and marginally more consistent in concentration across the upper 10 cm of the sediment core than the 106 µm size fraction; the 212 µm size fraction exhibited a very low concentration (close to zero) and was notably more consistent across the sediment core than the other two size fractions. The black line shows the predicted relationship between sediment depth and LDPE concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

3.5. Discussion

The findings of this study regarding sediment composition of benthic substrates of L378 were consistent with predictions. The expected trends of microplastic concentrations, however, were only partially observed: A significant relationship with water depth could only be demonstrated for LDPE; vertical microplastic accumulation within the sediment profile appears to be partially driven by polymer density, but some questions regarding PET accumulation and the particle size distribution remain.

3.5.1. Sediment characteristics and variations of benthic substrate

My first objective was to characterize the sediment composition and the variation of benthic substrates across a water depth gradient (spanning the littoral and profundal zones) in L378. I found highly significant differences between the littoral and profundal stations for all three variables, with high mineral content (and correspondingly low water and organic content) in the littoral sediments, and a high water and organic content (with correspondingly low mineral content), in the profundal sediments. Consequently, sediment zones were characterized as erosional at 0-2 m water depth, transitional at 2-8 m water depth, and as depositional in all areas deeper than 8 m.

These results are consistent with other studies that have characterized sediments of similar lakes at the IISD-ELA which have described coarse-grained mineral-content-dominated littoral sediments and fine-grained profundal sediments exhibiting high water and organic content levels (Cooley, 1997; Cooley & Franzin, 2008; Rowan et al., 1992; Stephenson et al., 1995). Where precisely the erosional, transitional, and depositional zones lie differs for each lake based on maximum water depth, basin shape and slope, and sedimentation and mixing rates (Cooley & Franzin, 2008; Rowan et al., 1992; Stephenson et al., 1995).

These depositional processes and patterns of lake sediments at the IISD-ELA, and specifically L378, likely affect microplastic deposition. Having been classified as novel sedimentary particles (Paduani, 2020) and demonstrated to behave similarly to natural sediments (Lofty et al., 2023; Paduani, 2020; Waldschläger et al., 2022), microplastics could be subject to the same depositional factors that are used to define the

erosional, transitional, and depositional sediment zones. Soft, porous sediments with a high water content could provide avenues for the vertical movement of microplastics, facilitating the downward migration through pore water to deeper sediment layers (Dimante-Deimantovica et al., 2024). Sediment composition could also impact microplastic transport across the sediment-water interface, as different substrates affect the availability of microplastics in the top sediment layer, i.e., sandy and rocky substrates enable re-distribution across the lake floor whereas softer substrates 'capture' microplastics and enable sinking within the sediment (Laursen et al., 2023; Leiser et al., 2021b; Li et al., 2019; Mancini et al., 2023; Serra & Colomer, 2023). Similarly, sediment grain size has been proven to be correlated with microplastic concentration; specifically, the finer grain classes such as silt and clay tend to co-occur with microplastics in deeper lake zones (Cera et al., 2022; Liang et al., 2022; Vermeiren et al., 2021; Zobkov et al., 2020). While benthic substrates high in organic content likely also contain small grain sizes, associating them with microplastics accumulation (Zobkov et al., 2020), this needs to be further investigated and the hypothesis of grain size being an important microplastic deposition predictor should be tested for L378. As substrates differ highly between and within aquatic systems, analyses of sediment composition should be included as standardized measures in all studies focusing on microplastics in sediments.

3.5.2. Horizontal microplastic accumulation patterns

My second objective was to examine the relationship between microplastic particle accumulation in L378's sediments and water depth for PET, PS, and LDPE. As expected, microplastic concentrations were highest in the depositional zone, peaking at 10.6 m water depth. Interestingly, PS was the most prominent polymer in the sediment (8 ± 11 units [mean ± SD]), followed closely by PET (6 ± 6 units), but contrary to my hypotheses, I found no relationship between PS and PET concentrations, respectively, and water depth. LDPE was least prominent, and I found a negative relationship between this polymer and water depth, meaning that fewer LDPE particles were detected in the depositional zone when compared to shallower lake areas.

While the depositional zone served as a microplastic accumulation area, the reason for the peak in microplastic concentration at specifically 10.6 m water depth

could be that the bottom slope of L378 between 2 m and 10 m water depth is steep (see bathymetry in Figure 3.4). This could potentially result in a down-sliding of particles accumulating in this water depth range; similar effects have been recorded by Cera et al. (2022). Although polymer seems to have been a relevant factor for microplastic accumulation, not all hypotheses could be confirmed. Essentially, the lower the polymer density of microplastic particles, the more likely they are to be redistributed within the water column, being subjected to water movements and related meteorological factors (e.g., wind and the following wave action; Berezina et al., 2021; Liu et al., 2022; Waldschläger & Schüttrumpf, 2019). Therefore, the densest polymer (PET) was expected to be homogenously distributed with water depth, as it should have been sinking close to the point source, as argued by Berezina et al. (2021) and Waldschläger and Schüttrumpf (2019). PS, being neutrally buoyant, was expected to accumulate in the deeper lake zones as it was moved there by water currents and slowly settled under gravity. A noticeable resuspension and redistribution at the deeper water layers or on the sediment surface was unlikely due to the relatively high densities of PET and PS particles (Constant et al., 2023; Leiser et al., 2021b; Zhang et al., 2020) and the low energy environment that L378 poses (Uddin et al., 2021). However, no relationship between PS and PET concentrations, respectively, and water depth was detected in this study, suggesting there may be different distribution factors at play. The joint effect of density and meteorological factors could also be a possible explanation of the negative relationship between LDPE concentration and water. LDPE's low density allows it to float for extended periods of time, only sinking once biofouling has occurred (Lee et al., 2022; Leiser et al., 2021b; Provencher et al., 2022; Wang et al., 2023) which makes the accumulation pattern of this polymer highly dependent on meteorological redistribution events (Berezina et al., 2021; Liu et al., 2022; Waldschläger & Schüttrumpf, 2019) and enables sinking in the littoral areas where biofilm is abundant. This has been observed recently by other pELAstic team members: LDPE accumulation has been recorded in the littoral zone (especially the shoreline), forming rings around the rocks lining the shore and even blowing into the surrounding forest (*The pELAstic Project*, 2024). In this study, the four shallowest stations were all located in the same area of the lake (see Figure 3.4) due to sampling difficulties, potentially enabling meteorological factors as a confound of

LDPE accumulation patterns; if this is the case, the sampling location within the lake and along a constant water depth gradient in the littoral zone would be more relevant for accumulation than the water depth itself.

The large effect of buoyancy on the fate of microplastics (especially that of positively buoyant particles) is highly relevant for environmental monitoring and remediation. On the downside, tracing pollution sources via concentration gradients may be severely hindered with positively buoyant and therefore highly mobile polymers, complicating the monitoring of low-density microplastics in the environment. However, *in situ* remediation is likely easier for positively buoyant polymers, as most existing microplastic remediation methods (e.g., filters, or PolyGone's Plastic Hunter) target the upper water column (Islam & Nishi, 2023; Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). The reverse applies to negatively buoyant polymers, where point sources are usually easier to trace but removal from sediments is extremely difficult. Neutrally buoyant microplastic pose an interesting case as they are both mobile (i.e., it is difficult to trace their source) and bound for deposition in sediments (i.e., it is difficult to remove them from the aquatic environment).

The mean microplastic concentration in sediments captured in this study is exceptionally high, compared to sediments of other remote cold-climate lakes (see Table 3.3). This could be because the other lakes in this comparison (except for Lake Ontario) are relatively pristine ecosystems largely unaffected by anthropogenic activity which is reflected in their microplastic loading that is primarily due to airborne microplastic deposition. While L378 is also a remote lake, the experimental manipulation is aimed at producing microplastic concentrations similar to those of the Laurentian Great Lakes, which exhibit much higher pollution levels due to a variety of anthropogenic factors (e.g., agriculture, population centres, tourism; McIlwraith et al., 2024). These differences in microplastic loading and subsequent detection could have been further entrenched by the varying lower size detection limits which complicate comparisons across studies. Interestingly, the lakes in the Tibetan Plateau (Liang et al., 2022) and those sampled at the IISD-ELA (McIlwraith et al., 2024) display similar microplastic concentrations; this could be due to comparable remoteness from anthropogenic activity and / or the similar detection limits used in those studies. Finally, sediment sampling depth and intervals are

not standardized in microplastics research. Only one other study in this comparison (Ballent et al., 2016), in addition to mine, used 1 cm intervals during sediment core extrusion; all other studies combined core layers into bulk samples or collected grab samples using other equipment (which is typically less precise than a corer; Glew et al., 2001). This could potentially lead to imprecision in assigning the exact depth were microplastics were found at and further result in a failure to capture microplastic particles in deeper sediment layers (see the recovery of microplastics particles from 30-35 cm sediment depth in Table 3.3 by McIlwraith et al., 2024), which is highly relevant if the determination of microplastic fate and / or environmental loading is the research goal. However, if organisms are the research target, sampling too deeply within the sediment and combining samples into bulks could skew results by diluting exposure concentrations. Thus, precise sediment sampling depth and a justification of the lower depth horizon tailored to the research objectives should be reported in future scientific studies and monitoring reports. In the context of this research, comparability with the studies in Table 3.3 is complicated not only by the high microplastic concentrations of the experimental manipulation but also by these different sampling techniques, varying lower depth horizons, and the previously addressed detection limits. The case of Zobkov et al. (2020) illustrates this nicely: Their sampling was focused on the sediment surface and recovered relatively high microplastic concentrations, therefore they may have found similar microplastic concentration to this study if they had adopted a lower microplastic size limit (e.g., 50 µm); the different sampling techniques, however, may limit transferability.

Table 3.3 Comparison of the mean microplastic concentration in L378 with other remote lake sediments and Lake Ontario. Microplastic concentration in L378 has been converted to particles / kg of dry sediment to match the other studies in their reporting.

Lake (Country)	Mean microplastic concentration (particles / kg of dry sediment)	Lower size limit	Depth range of sediment samples (sectioning type)	Reference
L378, This study (Canada)	13830 ± 18182	53 µm	0-10 cm (1 cm core slices, grab sample)	This study
Three lakes of the IISD-ELA (Canada)	551 ± 354.42 177 ± 103	53 µm	0-5 cm (bulk core) 30-35 cm (bulk core)	McIlwraith et al., 2024
Lake Ontario (Canada)	760 (range: 20-27830)	25 µm	Multiple (1 cm core slices, bulk core, grab sample)	Ballent et al., 2016
Lake Onego (Russia)	2188.7 ± 1164.4	174 µm	0-5 cm (grab sample)	Zobkov et al., 2020
Multiple (12) lakes on the Tibetan Plateau (China)	544.62 ± 297.99	50 µm	0-5 cm (bulk core)	Liang et al., 2022

In conclusion, while the highest microplastic concentrations in this study were recorded in the depositional sediments of L378, no significant relationship could be found between the denser polymers (PET and PS) and water depth. Thus, potential effects of down-sliding of particles should be monitored further. The negative relationship between the low-density polymer, LDPE, and water depth may have been confounded by meteorological factors; exploring the settling patterns of LDPE in relation to biofilm and the rock rings could be another avenue for future research.

3.5.3. Vertical microplastic accumulation patterns

My final objective was to determine how deeply into the sediment microplastic particles of these three polymer types penetrate in the area where particles accumulate in the sediment. As expected, microplastic concentrations were highest in the surface sediment, averaging 57 ± 29 particles per g sediment dry weight (mean ± SD). Furthermore, there

was a negative relationship between sediment depth and microplastic concentration for all polymers. PET and PS particles sank down to 10 cm sediment depth, with similar levels being observed below 5 cm (PET: 2 ± 1 units; PS: 2 ± 1 units); in the upper layers PS was slightly more prominent. There was little LDPE present, which was detected primarily in the top 5 cm. Furthermore, 95% of microplastic particles occurred in the upper 8 cm of sediment, with some deviations to the upper 3 cm (LDPE particles of the 212 µm size fraction), the upper 4 cm (LDPE particles of the 53 µm size fraction), and the upper 7 cm (PS particles of the 53 and 212 µm size fractions). These findings, namely the sinking down of particles to 10 cm sediment depth, the prominence of PET at that depth, and the 95% threshold generally extending beyond 5 cm sediment depth, support the hypothesis of gravity being the main driver for mixing within the sediment profile. The interaction between sediment depth and size fraction was marginally significant for PET ($p = 0.055$) and LDPE ($p = 0.054$), but not for PS ($p = 0.481$). Contrary to my hypothesis of the smaller particles penetrating deepest within the sediment, the medium (106 µm) size fraction was prominent across polymers, being the only significantly different size fraction in the comparison; this is mainly visible at the shallower slice depths.

The negative relationship between sediment depth and microplastic concentration across polymers has also been recorded in the literature (Ballent et al., 2016; Dimante-Deimantovica et al., 2024; Leiser et al., 2021b; Uddin et al., 2021), although in one case the opposite effect, i.e., increased microplastic concentrations with increasing sediment depth, has been recorded (Tian et al., 2022). More importantly, Dimante-Deimantovica et al. (2024) have argued that results of microplastic penetration into the deeper sediment layers (in the case of their study back to the 17th century time horizon which is far beyond the 1950s when plastics were first produced) are representative of a true natural phenomenon as opposed to contamination during sampling such as core smearing.

However, the speed at which the microplastics in our experiment have entered the deeper sediment layers is concerning. Considering that the first addition took place on June 7, 2023, and particles were detected as deeply as 10 cm down within the sediment, the microplastics migrated to a sediment depth that is not even consistently sampled in studies (the majority of which stop at the 5 cm depth horizon, see Table 3.3) in less than

two months. While the microplastics in L378 were representative of a two-month-long contamination at the time of sampling, the sediment layers they were found in are much older. A sediment core from L378 that was collected in 2006 and dated by Jeziorski et al. (2014) suggests that the layer at a sediment depth of 10 cm consists of material deposited in the late 1930s (see Figure 3.14). The sedimentation rate of L378 (expressed as mass accumulation rate in g/cm²/year, see Baud et al., 2023) ranges from 0.035 g/cm²/year (when including all stations) to 0.027 g/cm²/year (when only including the depositional zone); this is marginally higher than the very low sedimentation rate of similar oligotrophic lakes at the IISD-ELA (e.g., 0.013 g/cm²/year for the pELAstics reference lake L373) and could be related to the addition of microplastics. While some microplastic particles could have been damaged or destroyed during the drying process, a portion of the dry weight used to calculate the mass accumulation rate could have been plastic, potentially explaining the higher sedimentation rate. Considering Jeziorski et al.'s (2014) dated sediment cores from L378, the curve of sediment age with sediment depth flattens in recent years due to the compression of sediment over time, so that one centimetre of recently deposited sediment corresponds to roughly 7.5 years (with 'recently' referring to the last 50 years, see Figure 3.14). In this case, the sediment depth of 10 cm collected in this study was likely deposited in the 1950s (to be confirmed with 210Pb radiometric dating analysis of one of my cores). The microplastic particles detected in these decade-old sediments, however, were not a result of atmospheric deposition since the 1950s but rather added via the experimental manipulation throughout the two months prior to sample collection.

The implications of this sinking speed of microplastic particles within the vertical sediment profile are drastic for microplastic sediment sampling, environmental monitoring, and remediation. Firstly, sediment sampling for microplastic studies and monitoring may be missing long-term contamination if it continues to focus on the surface layers. As microplastics behave differently than other sedimentary particles and are not contained in the sediment layer they were initially deposited into, the age of the microplastics in sediments is unlikely equivalent to the age of sediments (Dimante-Deimantovica et al., 2024); thus, sampling deeper is required to understand the extent of microplastic contamination in sediments. This is also relevant for remediation. If a long-

term contamination with microplastics is occurring in sediments, penetrating much deeper layers much quicker than previously thought, the likelihood of 'geologic plastic' (*The pELAstic Project*, 2024) existing and layering up in the Earth's crust is high. Remediating microplastics in sediments is already tricky, which most techniques focussing on more accessible media (e.g., the water column; Islam & Nishi, 2023; Duke – Nicholas Institute for Energy, Environment & Sustainability, 2024). Extracting microplastics from sediments, especially the deeper layers <5 cm sediment depth, without damaging the surrounding ecosystem significantly is challenging; this would be even more complicated for the depth that would need to be reached to access geologic plastic. To my knowledge, no *in situ* remediation techniques exist for the removal of microplastics from sediments of any depth.

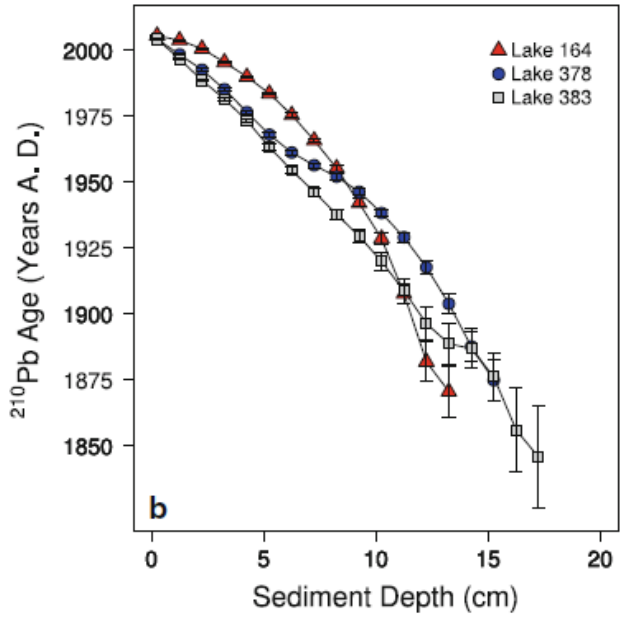

Figure 3.14 Sediment age (determined by 210Pb radiometric dating analysis) and corresponding sediment depth for three lakes of the IISD-ELA (L164, L378, L383); error bars indicate one standard deviation in sediment age (Jeziorski et al., 2014).

Some questions regarding the accumulation of PET particles remain. PS was prominent in the upper sediment layers but PET and PS levels are becoming more similar in the deeper layers; in addition, the lower threshold of penetration by these polymers was not captured by this study (both polymers are still present in the 10 cm sediment layer), leaving room for speculation how the distribution of PET and PS particles looks

like further down in the sediment (<10 cm). Since PET is negatively buoyant, it should be accumulating almost exclusively in the sediments (Berezina et al., 2021; Constant et al., 2023; Dimante-Deimantovica et al., 2024) and therefore exhibit higher levels than the neutrally buoyant PS which requires more time before sinking to the lake bottom; yet this effect was not observed in our experiment. Moreover, particles are added in fixed numbers that are the same for each polymer, rather than the mass of particles, due to the different densities and weights; thus, the dosing protocol is not responsible for the polymer distribution trend observed in this study. An explanation could be that PET particles sink directly where they were added into the water ('streaks' of PET were observed by pELAstics team members on the lake bottom following an addition), creating hotspots that can be 'hit or miss' during sampling, whereas PS gets distributed more evenly in the water column before eventually settling in bottom sediments (Koutnik et al., 2021; Jones et al., 2020). In this case the PET would actually be in the sediment but was not fully captured by sampling; this would likely be a random effect that could be controlled for by using a remote operated vehicle (ROV) before and / or during sediment sampling to verify whether 'streaks' are still present on the lake bottom. Another explanation could be that PET sinks faster and deeper *within* sediments (see the levelling of PS and PET counts around 5 cm sediment depth), which would suggest that the 'missing' PET may be even deeper in the sediments than the portion that was sampled (Dimante-Deimantovica et al., 2024). Similarly to the previously mentioned concerns regarding overall penetration depth of microplastics in sediments and the resulting potential long-term contamination, sampling deeper would allow research and monitoring to confirm whether high density particles like the 'missing' PET sink into the deeper (<10 cm) sediment layers that are currently not sampled as reliably as the surface layers. Determining whether these lower depth horizons do in fact vary for different polymers is crucial in this research stage, as closing these knowledge gaps would allow directing the development of appropriate and differentiated *in situ* remediation technologies from the beginning. This would ensure that methods are being tailored to the environmental problem they are expected to solve, in this case the highly variable accumulation patterns of microplastics that depend on specific particle characteristics (e.g., density).

Shape has also been found to highly impact microplastic accumulation patterns in sediments (Berezina et al., 2021; Constant et al., 2023; Lee et al., 2022; Liu et al., 2022; Waldschläger & Schüttrumpf, 2019). The more fragmental a particle, the more likely it is to sink quickly from the water column to the top sediment layer, and the more spherical a particle, the more likely it is to travel within the vertical sediment profile (Berezina et al., 2021; Liu et al., 2022; Waldschläger & Schüttrumpf, 2019). If the three polymers used in L378 exhibit different shape characteristics (e.g., because of different responses of the material to the grinding process of plastic sheets into microplastics), their ability to migrate within the sediment profile may be affected. Finally, there may also be other factors, such as gas exchanges and floc formation (Dimante-Deimantovica et al., 2024; Leiser et al., 2021b), driving this downward migration of dense microplastic particles; this area of microplastic research is still largely untouched and requires further studying.

The prominence of medium-sized particles in the sediment of L378 cannot be explained by the pELAstic dosing protocols, as the prevailing size of ground up microplastic particles is above 212 µm for all polymers, specifically 400 µm for PET, 340 µm for PS, and 310 µm for LDPE (see Figures D2-D6 in Appendix D for particle size spectra). Compared to the dosing protocol, this study observed a different distribution of particles between the three size fractions across all polymers (see Table 3.4). For all polymers particles of the 53 µm and 106 µm size fractions were overrepresented in this study, whereas those of the 212 µm size fraction were strongly underrepresented.

Table 3.4 Comparison of this study and the pELAstic dosing protocol considering the distribution of microplastic particles between the three size fractions (212, 106, and 53 µm) across polymers (PET, PS, and LDPE).

Polymer (%) / Size fraction (%)	PET (this study)	PET (dosing protocol)	PS (this study)	PS (dosing protocol)	LDPE (this study)	LDPE (dosing protocol)
212 µm	21.36	80.16	14.76	76.00	5.56	75.29
106 µm	50.81	15.50	48.10	19.10	55.56	19.96
53 µm	27.83	4.50	37.14	4.90	39.89	4.23

Potential explanations for 106 µm being the most commonly detected size fraction in sediments could be the uptake of particles in the food web with particles ≥212

µm accumulating in the digestive tract and potential translocation of particles into tissues (Lehtiniemi et al., 2018; Provencher et al., 2022; Sandgaard et al., 2023; *The pELAstic Project*, 2024; Waldschläger et al., 2022), rendering them unavailable for benthic deposition. In addition, weathering and break-down of those larger particles could be occurring (Alimi et al., 2023; Binda et al., 2024; Dong et al., 2020; Hataley et al., 2022; Sun et al., 2020; *The pELAstic Project*, 2024), transforming them into smaller particles (<106 µm). Bioturbating benthos, while not being confirmed as a microplastic accumulation mechanism by the results of this study, could contribute to both uptake in the food web (small and large particles) and the break-down of larger particles and should thus be a research priority (Frank et al., 2023; Song et al., 2024; Wazne et al., 2023). Generally, particle size poses an interesting case for microplastic concentration in the vertical sediment profile. Dimante-Deimantovica et al. (2024), Leiser et al. (2021b), and Tian et al. (2022) have observed that generally smaller particles penetrate deeper sediment layers than larger particles. Constant et al. (2023) further specify that while the vertical distribution of high-density polymers in sediment is related to sediment grain size, the distribution within each polymer type changes depending on particle size and / or shape, with complex interactions. The interaction between sediment depth and size fraction (within PET and LDPE concentrations, respectively), detected in this study is consistent with these results presented by Constant et al. (2023). Thus, the effects of size (holding density constant) may also be related to sediment composition: In dense sediments, only small particles can migrate into the deeper layers due to their ability to 'slip through'; in less dense sediments larger particles have a gravity-based advantage due to their heavier weight. In this study, particles of the 212 µm size fraction could have penetrated deeper than the lower sampling cut-off (10 cm sampling depth), whereas additional particles of the 53 µm size fraction (and potentially the 106 µm size fraction as well) may have been created by breakdown of larger particles, potentially by benthos. Where these differently sized particles are is highly relevant, as there may be cascading effects through the food web which directly impacts the health of biota (including humans). Since this can only be tested with more data, these complex effects of microplastic characteristics and interactions of predictors on accumulation patterns in

sediment should be investigated further by sampling other media and expanding the lower sampling cut-off for sediment cores.

In summary, the highest microplastic concentrations were detected in the upper sediment layers and a significant negative relationship between sediment depth and microplastic concentration could be demonstrated for all polymers, supporting the gravity-based hypothesis of downward microplastic migration within the sediment profile. Yet, questions regarding the 'missing' PET and the mechanisms driving particle size distribution remain. Coring deeper may help shed light on the question of where the remaining PET has accumulated. Breakdown of larger MP particles is a likely explanation for the unexpected particle size distribution. However, particles of ≥ 212 µm size could also be accumulating within the food web, in which case sampling biota in L378 could aid in solving the question of why the 53 µm and 106 µm size fractions are prominent in the sediment.

3.5.4. Limitations

Sampling occurred mid-season due to limited space at camp and the MES timeline but would have been more informative at the end of the season after all additions and natural mixing of the lake were completed. Late-season sampling would have allowed for more reliable inferences about microplastic distribution patterns as the full yearly load would have been added to the lake. Similarly, using more stations (water depth information points) would potentially have increased the predictive strength of water depth for different polymer concentrations. Future studies could also consider holding water depth constant during sampling, sampling at this depth at different lake areas, to test if location across the lake has a significant impact on polymer concentrations.

In addition, the analysis of duplicate and triplicate samples (which could not be processed due to time and resource constraints) would have been helpful to provide a better estimate of the spatial variation at each station. Given the high variability of the duplicate core processed at centre buoy (see Appendix A), there could be considerable variation of microplastic accumulation within benthic substrates of the same composition, water depth, and rough location in the lake. Whether this is an anomaly or genuine trend can only be determined with additional microplastic data. Local variation

is highly relevant for remediation and must therefore be tested by future studies. Furthermore, the collection of freeze cores would be useful to verify the observed variation and maximum penetration depth of MP particles within the vertical sediment profile; thus, future sediment work targeting MP vertical migration patterns should incorporate this method.

3.6. Conclusions

This study has provided baseline data on sediment composition and data on microplastic accumulation in bottom sediment during the first year of experimental manipulation of L378 at the IISD-ELA by The pELAstic Project. I found that sediments were highly homogenous within sediment zones, and differed in terms of water content, organic content, and mineral content between the littoral and profundal lake areas, resulting in the first classification of L378's erosional, transitional, and depositional sediment zones. These sediment characteristics and depositional processes could potentially affect microplastic accumulation patterns and should thus be included as a standardized measure in all future studies on microplastics in sediments.

While the highest microplastic concentrations were recorded in the depositional zone, water depth was negatively associated with only one of the three polymers, LDPE, and had no significant relationship with the denser polymers (PET and PS). Buoyancy, connecting the predictor density with meteorological factors (e.g., wind or wave action), appears to have a high impact on fate of microplastic accumulation across the lake. While negatively and neutrally buoyant polymers (PET and PS) are less subjected to mixing events and sink to the lake bottom relatively quickly, the positively buoyant polymer in this study (LDPE) was observed to wash up on shore and settle in sediments at a much lower rate (likely related to biofouling). Finally, the lacking standardization of sediment sampling depth and microplastic detection limits complicate comparisons between studies of microplastics in boreal lake sediments.

Sediment depth was a helpful predictor of microplastic concentration across polymers. While microplastic concentrations were highest in the surface sediments, PET and PS particles were detected down to the maximum sampled sediment depth of 10 cm. Generally, 95% of all microplastics were contained in the upper 8 cm of sediment. While

PS was the most prominent polymer in the upper 5 cm of sediment, PET concentrations dominated the lower 5 cm of sediment; little LDPE was detected overall. Less PET than expected was observed. Since this polymer is negatively buoyant, and accumulation outside of sediment is highly unlikely, PET has been theorized to have sunk even deeper into the sediments, or to have formed streaks on the lake bottom that are 'hit or miss' during sampling. Coring to deeper sediment layers and using a ROV during sampling could shed light on this. The size fraction distribution observed in this study did not correspond to that of the dosing protocol, suggesting there may be another mechanism at play. The breakdown of larger particles into the 106 and 53 μm size fractions is a likely explanation, and the food web has been theorized to serve as a temporary reservoir of the 212 μm size fraction. Finally, the sinking speed of microplastics within the sediment is concerning, as it implies a long-term contamination of sediments worldwide (including geologic plastic) that would further challenge remediation technology development.

CHAPTER IV

Conclusion

This study presents findings on the accumulation of microplastics of three common polymer types in sediment of a whole-lake addition experiment (L378) at the IISD-ELA in North-Western Ontario, Canada. The thesis objectives were achieved by analyzing sediment core and sediment grab samples collected at eight Stations of varying benthic substrate across L378. An array of knowledge gaps within microplastic research were illustrated in Chapter 2. Given the variety of microplastic collection, processing, and reporting methods, standardized data and classification systems for microplastic characterization (e.g., according to shape) are still falling short. In addition, aquatic sediments, and more specifically freshwater sediments, remain understudied in their heterogeneity regarding ecosystem characteristics and the resulting different transport pathways and interaction effects of microplastics. Furthermore, experimental research and the development of downstream solutions for microplastic remediation have been neglected. Finally, collaborative efforts with policymakers and SBS to remove roadblocks to microplastic remediation rooted in human behaviour are still falling short. These issues must all be addressed to ensure high quality data, a sufficient understanding of ecosystem mechanisms, and pave the way for removal of microplastics from sediments in aquatic ecosystems.

Chapter 3 provided baseline data on sediment composition and data on microplastic accumulation and distribution patterns in bottom sediment during the first year of experimental manipulation of L378 at the IISD-ELA by The pELAstic Project. Sediment characteristics (water content, organic content, and mineral content) differed strongly between the littoral and profundal stations, resulting in the first characterization of the erosional, transitional, and depositional zones for L378. While water depth was negatively associated with only one of the three polymers (LDPE), sediment depth was a helpful predictor of microplastic concentration across polymers. An interaction of sediment depth and size fraction was detected, leading me to conclude that polymer type as well as size fraction may influence the vertical transport of microplastic particles in

boreal lake sediments. However, vertical transport mechanisms of microplastics in sediments are poorly understood, and comparison between studies is further complicated by a lack of standardization of sampling (especially the lowest sediment horizon during coring) and processing (e.g., detection limits) techniques. Furthermore, the depth at which microplastic particles were detected and the speed of their downward migration is highly concerning, implying that microplastic contamination may be more widespread in sediments than previously thought. Fate may also be highly influenced by the buoyancy of particles which is related to their density / polymer but may change over time as processes such as weathering and biofouling occur. Thus, monitoring and remediation must be able to account for ecosystem-specific transformation and relocation processes that may affect the fate of microplastics. Focusing on locating microplastic hotspots and understanding the mechanisms guiding their formation is a crucial step towards the active removal of microplastics from ecosystems. The findings of this study provide a starting point for more experimental work to uncover microplastic behaviour in aquatic sediments.

4.1. Future work

The pELAstic Project will continue dosing L378 with microplastics for two more years (2024 and 2025) before moving to a long-term monitoring approach. During this time, multiple aspects related to sediments should be prioritized. Firstly, a complete grain size analysis should be conducted to test whether grain size (especially clay and silt content) is correlated with overall microplastic concentration and / or individual polymers and thus a helpful predictor for microplastic accumulation. Secondly, future sediment coring at L378 should sample deeper into the vertical sediment profile, to potentially locate the 'missing' PET and define the sediment depth at which no microplastics of the experimental manipulation can be found; this is highly valuable information for sampling standardization and the development of remediation technologies for microplastic contaminated aquatic sites. Moreover, interactions between sediment depth, particle size, and shape, albeit complex and poorly understood, should be further researched. In addition, the results from biota sampling across levels of biological organization should be compared with those of this study to assess whether the riddle of the size fraction

distribution can be explained by the food web hypothesis. Finally, the potential impacts of bioturbation and the accumulation of microplastics in benthos should be monitored over an extended time to test whether the distribution of polymers and / or particle size in sediment changes over time due to the burrowing and feeding of benthic macroinvertebrates.

Generally, the drivers of microplastic accumulation within the vertical sediment profile, e.g., gravity, bioturbation, gas exchanges, and potentially others, remain unclear. More studies are required to understand interactions between different microplastic accumulation predictors and connect them to these vertical drivers. While remediation of aquatic sediments remains difficult, focusing on locating microplastic accumulation hotspots and understanding the mechanisms guiding their formation is a crucial step towards the active removal of microplastics from ecosystems. In addition, more standardization would enable better comparison of findings and make the field of microplastic research more efficient. Future research on the patterns and drivers of microplastic deposition in aquatic sediments and subsequent interaction effects with other ecosystem components is required, and the development of remediation technologies targeting higher-density polymers and sediment as a medium should be advanced. The findings of this study provide a starting point for more experimental work to uncover microplastic behaviour in aquatic sediments.

4.2. Implications and recommendations

The most important recommendations based on the literature review conducted for this study are:

1. **For research: Transport mechanisms of microplastics in sediments via experiments**. Focus within research should be shifted towards the effects and transport mechanisms driving microplastic behaviour in sediments, especially the impacts of bioturbation and abiotic drivers of vertical microplastic transport within sediments. Similarly, increased research efforts are required to better understand and document biotic and abiotic interaction effects, especially in the benthic compartment; ecosystem-wide experiments are invaluable in this context.

Based on this knowledge, adequate microplastic removal technology tailored to sediments (and especially high-density polymers) can then be developed.

2. **For policy: Collaboration with SBS and market-driven microplastic mitigation policy.** Existing roadblocks to microplastic pollution prevention and mitigation rooted in human behaviour must be addressed via collaborative efforts of politics and SBS. In addition, market-driven policies such as making changes towards a circular economy and shifting the costs of microplastic pollution towards producers (e.g., through the EPR principle or taxes, bans, and levies) are urgently required.
3. **Generally: Accelerated standardization of methods.** Existing trends toward the standardization of microplastic collection, processing, and reporting methods must be accelerated to ensure the highest quality and best comparability of data. In addition, the development of standardized microplastic-sediment dating techniques paired with the creation of a global microplastic-sediment archive for the collection of historical and modern microplastic-sediment samples should be prioritized.

Considering the original work on microplastic accumulation and distribution patterns in boreal lake sediments presented in this study, the most important recommendations are:

1. **For L378: The 'missing' PET, the riddle of the size fraction distribution, freeze coring, and remediation method testing.** Locating the 'missing' PET particles via improved sampling methods (e.g., sampling deeper within the sediment and at constant water depth) and solving the riddle of the size fraction distribution via coordination with other project components (different sampling media) is necessary to gain a holistic understanding of microplastic accumulation and distribution patterns in L378. In addition, freeze coring should be used to verify vertical distribution patterns of MPs in sediments. Ultimately, this data could then be used to trial potential remediation methods and / or technologies for sediments, focusing on higher-density polymers such as PET and PS.

2. **For microplastic contaminated aquatic sediments: Development of *in situ* remediation methods tailored to microplastic removal.** Considering the high pollution load in sediments, new *in situ* removal methods for microplastics such as catchment (via filters) and breakdown (potentially via small organisms or biochar) should be further investigated and trialed, with a focus on new method and technology contributions as opposed to traditional remediation methods such as sediment dredging. The novelty of microplastics as a pollutant likely requires a novelty of research approaches and remediation methods.

4.3. Concluding comments

This research has gathered information that supports the polymer-based prediction of microplastic accumulation in boreal lake sediments within the vertical sediment profile, and to an extent along the water depth gradient. Using optical methods, this study found evidence of decreasing microplastic concentration with increasing sediment depth, with a change in polymer ratios (primarily PET and PS) around 5 cm sediment depth; it also uncovered a negative relationship between LDPE and water depth. Furthermore, sediment composition and variation have been analyzed, assigning sediment zones common in sedimentology and limnology and outlining their relevance for microplastics accumulation patterns; these measures should therefore be included as standard measures in future studies.

Ultimately, industry, government, the scientific community, and the public must come together to tackle microplastic pollution. As pollution levels have heavily increased, the benefits plastics once promised – cheapness, durability, and wide-ranging applications – could eventually become outweighed by their potential negative impacts on environmental and human health. Other materials / products exist, and so do remediation options for most environmental matrices; it is now a matter of shifting away from the ‘easy’ way, plastics, towards a more sustainable and healthy future. Microplastic pollution research has the duty and privilege to pave the way for this development by providing high quality data and novel approaches to the remediation of microplastic impacted sites.

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Appendix A – QA/QC

Coefficients of variability: Microplastic counts duplicate at Station 8

Calculation for coefficient of variability: Computing the difference between C13 and C15 calculated for every row; then use formula of Standard Deviation/Mean x 100 to calculate variability.

Variable abbreviations are as follows: 'B' for blue / PET, 'P' for pink / PS, 'Y' for yellow / LDPE, '212' for the 212 µm size fraction, '106' for the 106 µm size fraction, '53' for the 53 µm size fraction.

Table A1. Microplastic counts for the 0-1 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 0-1 cm	MP Counts C15 – 0-1 cm	Difference C13-C15	Coefficient of Variability 0-1 cm
B212	0	6	6	104.1%
P212	2	2	0	
Y212	0	0	0	
B106	5	7	2	
P106	4	10	6	
Y106	0	2	2	
B53	0	2	2	
P53	2	12	10	
Y53	0	1	1	
Total212	2	8	6	
Total106	9	19	10	
Total53	2	15	13	
TotalB	5	15	10	
TotalP	8	24	16	
TotalY	0	3	3	
TotalAll	13	42	29	

Table A2. Microplastic counts for the 1-2 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 1-2 cm	MP Counts C15 – 1-2 cm	Difference C13-C15	Coefficient of Variability 1-2 cm
B212	1	2	1	103.8%
P212	1	2	1	
Y212	0	0	0	
B106	4	5	1	
P106	5	10	5	
Y106	0	1	1	
B53	0	4	4	
P53	1	0	1	
Y53	0	0	0	
Total212	2	4	2	
Total106	9	16	7	
Total53	1	4	3	
TotalB	5	11	6	
TotalP	7	12	5	
TotalY	0	1	1	
TotalAll	12	24	12	

Table A3. Microplastic counts for the 2-3 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 2-3 cm	MP Counts C15 – 2-3 cm	Difference C13-C15	Coefficient of Variability 2-3 cm
B212	2	1	1	88.9%
P212	3	2	1	
Y212	0	0	0	
B106	2	3	1	
P106	0	3	3	
Y106	0	1	1	
B53	0	2	2	
P53	1	5	4	
Y53	1	0	1	
Total212	5	3	2	
Total106	2	7	5	
Total53	2	7	5	
TotalB	4	6	2	
TotalP	4	10	6	
TotalY	1	1	0	
TotalAll	9	17	8	

Table A4. Microplastic counts for the 3-4 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 3-4 cm	MP Counts C15 – 3-4 cm	Difference C13-C15	Coefficient of Variability 3-4 cm
B212	2	1	1	110.3%
P212	1	3	2	
Y212	0	0	0	
B106	2	5	3	
P106	0	0	0	
Y106	1	1	0	
B53	2	2	0	
P53	2	3	1	
Y53	0	0	0	
Total212	3	4	1	
Total106	3	6	2	
Total53	4	5	1	
TotalB	6	8	2	
TotalP	3	6	3	
TotalY	1	1	0	
TotalAll	10	15	5	

Table A5. Microplastic counts for the 4-5 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 4-5 cm	MP Counts C15 – 4-5 cm	Difference C13-C15	Coefficient of Variability 4-5 cm
B212	2	0	2	113.0%
P212	2	0	2	
Y212	0	0	0	
B106	5	1	4	
P106	6	0	6	
Y106	0	0	0	
B53	0	0	0	
P53	5	1	4	
Y53	0	0	0	
Total212	4	0	4	
Total106	11	1	10	
Total53	5	1	4	
TotalB	7	1	6	
TotalP	13	1	12	
TotalY	0	0	0	
TotalAll	20	2	18	

Table A6. Microplastic counts for the 5-6 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 5-6 cm	MP Counts C15 – 5-6 cm	Difference C13-C15	Coefficient of Variability 5-6 cm
B212	0	1	1	91.1%
P212	1	0	1	
Y212	0	0	0	
B106	2	1	1	
P106	0	2	2	
Y106	0	0	0	
B53	1	0	1	
P53	1	0	1	
Y53	0	0	0	
Total212	1	1	0	
Total106	2	3	1	
Total53	2	0	2	
TotalB	3	2	1	
TotalP	2	2	0	
TotalY	0	0	0	
TotalAll	5	4	1	

Table A7. Microplastic counts for the 6-7 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 6-7 cm	MP Counts C15 – 6-7 cm	Difference C13-C15	Coefficient of Variability 6-7 cm
B212	0	0	0	106.5%
P212	1	0	1	
Y212	0	0	0	
B106	1	4	3	
P106	5	0	5	
Y106	1	0	1	
B53	0	0	0	
P53	1	0	1	
Y53	0	0	0	
Total212	1	0	1	
Total106	7	4	3	
Total53	1	0	1	
TotalB	1	4	3	
TotalP	7	0	7	
TotalY	1	0	1	
TotalAll	9	4	5	

Table A8. Microplastic counts for the 7-8 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 7-8 cm	MP Counts C15 – 7-8 cm	Difference C13-C15	Coefficient of Variability 7-8 cm
B212	1	0	1	103.3%
P212	0	1	1	
Y212	0	0	0	
B106	0	0	0	
P106	0	0	0	
Y106	0	1	1	
B53	0	0	0	
P53	1	0	1	
Y53	0	0	0	
Total212	1	1	0	
Total106	0	1	1	
Total53	1	0	1	
TotalB	1	0	1	
TotalP	1	1	0	
TotalY	0	1	1	
TotalAll	2	2	0	

Table A9. Microplastic counts for the 8-9 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 8-9 cm	MP Counts C15 – 8-9 cm	Difference C13-C15	Coefficient of Variability 8-9 cm
B212	0	0	0	150.1%
P212	0	0	0	
Y212	0	0	0	
B106	1	2	1	
P106	3	0	3	
Y106	0	0	0	
B53	0	0	0	
P53	0	0	0	
Y53	0	0	0	
Total212	0	0	0	
Total106	4	2	2	
Total53	0	0	0	
TotalB	1	2	1	
TotalP	3	0	3	
TotalY	0	0	0	
TotalAll	4	2	2	

Table A10. Microplastic counts for the 9-10 cm sediment depth, comparing the variability between two cores (C13 and C15) at Station 8 (centre buoy) in L378.

Count Type / Variable	MP Counts C13 – 9-10 cm	MP Counts C15 – 9-10 cm	Difference C13-C15	Coefficient of Variability 9-10 cm
B212	0	2	2	146.1%
P212	0	0	0	
Y212	0	0	0	
B106	2	1	1	
P106	1	1	0	
Y106	0	0	0	
B53	0	0	0	
P53	1	1	0	
Y53	0	0	0	
Total212	0	2	2	
Total106	3	2	1	
Total53	1	1	0	
TotalB	2	3	1	
TotalP	2	2	0	
TotalY	0	0	0	
TotalAll	4	5	1	

Mean coefficient of variation across all sediment depths between C13 and C15: 96.5%

Matrix spike recovery data

Recovery was assessed via Matrix Spikes by members of Rochman Lab at University of Toronto. The data for Matrix Spikes processes according to the sediment samples protocol, as detailed in this thesis, was provided by Rochman Lab and is available in the tables below.

Table A11. MP particles (by polymer) added to the Matrix Spikes used in this study.

	Added particles								
	PE (212)	PE (106)	PE (53)	PS (212)	PS (106)	PS (53)	PET (212)	PE (106)	PET (212)
2023-MS-01	6	8	7	7	8	8	7	6	5
2023-MS-02	7	6	6	6	8	6	8	8	8
2023-MS-03	6	6	7	8	7	8	5	7	8

Table A12. MP particles (by polymer) recovered from the processed Matrix Spike samples.

	Recovered (# of particles)								
	PE (212)	PE (106)	PE (53)	PS (212)	PS (106)	PS (53)	PET (212)	PE (106)	PET (53)
2023-M	10	3	3	5	10	10	7	6	3
2023-M	13	2	1	6	8	6	8	8	7
2023-M	10	4	2	8	8	8	5	11	5

Table A13. MP recovery rates by polymer and size class for processed Matrix Spike samples.

	Recovery (%)												
	PE (212)	PE (106)	PE (53)	PS (212)	PS (106)	PS (53)	PET (212)	PE (106)	PET (212)	Total PE	Total PS	Total PET	Total
2023-MS-01	166.6667	37.5	42.85714	71.42857	125	125	100	100	60	76.19048	108.6957	88.88889	91.93548
2023-MS-02	185.7143	33.33333	16.66667	100	100	100	100	100	87.5	84.21053	100	95.83333	93.65079
2023-MS-03	166.6667	66.66667	28.57143	100	114.2857	100	100	157.1429	62.5	84.21053	104.3478	105	98.3871

Table A14. Summary of average MP recovery by polymer and in total for processed Matrix Spike samples.

PE	PS	PET	Total
81.53718	104.3478	96.57407	94.65779

Blank correction data

The number of each blank corresponds to the Station of the same number; particles found in the blanks have therefore been deducted from MP counts for the corresponding station.

Table A15. MP count data for each combined field and laboratory blank (total: 8 blanks).

Count Type / Variable	Blank 1	Blank 2	Blank 3	Blank 4	Blank 5	Blank 6	Blank 7	Blank 8
B212	0	0	0	0	0	0	0	0
P212	0	0	0	0	0	0	0	0
Y212	0	0	0	0	0	0	0	0
B106	0	0	0	0	0	0	0	0
P106	0	0	0	0	0	0	0	0
Y106	0	0	0	0	0	0	0	0
B53	0	0	1	0	0	0	0	0
P53	1	0	0	0	0	0	0	1
Y53	0	0	0	0	0	0	0	0
Total212	0	0	0	0	0	0	0	0
Total106	0	0	0	0	0	0	0	0
Total53	1	0	1	0	0	0	0	1
TotalB	0	0	1	0	0	0	0	0
TotalP	1	0	0	0	0	0	0	1
TotalY	0	0	0	0	0	0	0	0
TotalAll	1	0	1	0	0	0	0	1

Sub-sampling during dry-picking

A subsampling method was employed for some MP samples (see section 3.3.3). This method was used for the samples listed below.

Table A16. List of MP samples that were sub-sampled during dry-picking.

Station / slice of sample	Sample ID	# of particles picked	# of particles tallied	Total # of particles recorded
S2 / 0-10 cm	2023-A-2873	46	16	62
S4 / 0-1 cm	2023-A-2673	52	30	82
S5 / 0-1 cm	2023-A-2583	52	14	66
S5 / 2-3 cm	2023-A-2585	47	3	50
S7 / 0-1 cm	2023-A-2833	43	10	53
S8 / 0-1 cm	2023-A-2713	40	2	42

Appendix B – Statistics

Sediment analysis – t-test

Group Statistics

	Near- vs off-shore	N	Mean	Std. Deviation	Std. Error Mean
% of water content	Near-shore stations (<8m water depth)	9	23.06399%	4.624383%	1.541461%
	Off-shore stations (>8m water depth)	150	87.59280%	2.435435%	0.198852%
% of mineral content	Near-shore stations (<8m water depth)	9	76.07966%	4.931619%	1.643873%
	Off-shore stations (>8m water depth)	150	8.70855%	2.122441%	0.173297%
% of organic content	Near-shore stations (<8m water depth)	9	0.85636%	0.361181%	0.120394%
	Off-shore stations (>8m water depth)	150	3.69865%	0.547468%	0.044701%

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means							
						Significance				95% Confidence Interval of the Difference	
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Std. Error Difference	Lower	Upper
water content	Equal variances assumed	11.857	<,001	-72.540	157	<,001	<,001	-64.528813%	0.889564%	-66.285870%	-62.771757%
	Equal variances not assumed			-41.518	8.268	<,001	<,001	-64.528813%	1.554234%	-68.092737%	-60.964890%
mineral content	Equal variances assumed	24.083	<,001	83.597	157	<,001	<,001	67.371108%	0.805906%	65.779291%	68.962925%
	Equal variances not assumed			40.757	8.179	<,001	<,001	67.371108%	1.652982%	63.573777%	71.168440%
organic content	Equal variances assumed	1.358	.246	-15.350	157	<,001	<,001	-2.842295%	0.185161%	-3.208023%	-2.476567%
	Equal variances not assumed			-22.132	10.347	<,001	<,001	-2.842295%	0.128424%	-3.127145%	-2.557444%

Independent Samples Effect Sizes

		Standardizer[a]	Point Estimate	95% Confidence Interval	
				Lower	Upper
water content	Cohen's d	2.592062%	-24.895	-27.723	-22.059
	Hedges' correction	2.604528%	-24.776	-27.590	-21.953
	Glass's delta	2.435435%	-26.496	-29.571	-23.412
mineral content	Cohen's d	2.348296%	28.689	25.445	31.926
	Hedges' correction	2.359589%	28.552	25.323	31.773
	Glass's delta	2.122441%	31.742	28.076	35.400
organic content	Cohen's d	0.539533%	-5.268	-6.153	-4.373
	Hedges' correction	0.542128%	-5.243	-6.123	-4.353
	Glass's delta	0.547468%	-5.192	-6.081	-4.293

Figure B1. Output provided by SPSS Statistics for the t-test comparing group means of near-shore and off-shore stations for sediment measures (water content, organic content, and mineral content).

Microplastic analysis – Linear models

1. PET

Model fitting and assumptions: Simulated residual diagnostics with DHARMa

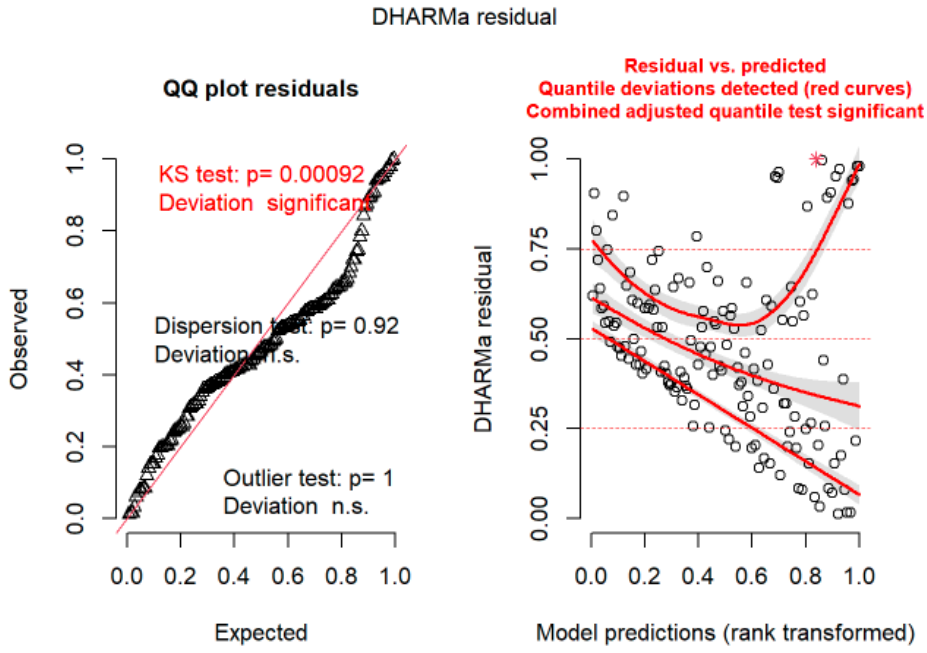

Figure B2. PET analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions under the normal distribution.

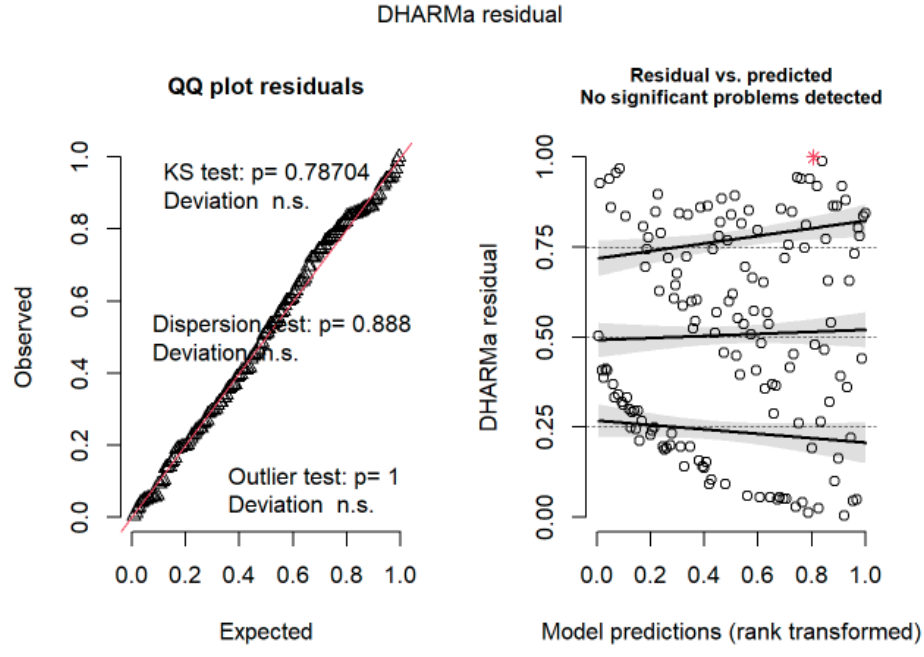

Figure B3. PET analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions with log-transformed MP counts.

Model interpretation: Individual variable effects and interactions

```
## Data: PET
## Models:
## PET_log_mod2: log(concentration + 0.8) ~ station.depth + slice.depth + size.fraction, zi=~0, disp=~1
## PET_log_mod: log(concentration + 0.8) ~ station.depth + slice.depth * size.fraction, zi=~0, disp=~1
##              Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## PET_log_mod2  6 297.57 315.63 -142.78   285.57
## PET_log_mod   8 295.76 319.84 -139.88   279.76 5.8096      2    0.05476 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(PET_log_mod)
```

```
##  Family: gaussian  ( identity )
## Formula:
## log(concentration + 0.8) ~ station.depth + slice.depth * size.fraction
## Data: PET
##
##      AIC      BIC   logLik deviance df.resid
##    295.8    319.8   -139.9    279.8      142
##
##
## Dispersion estimate for gaussian family (sigma^2): 0.378
##
## Conditional model:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                   1.38707    0.26321   5.270 1.37e-07 ***
## station.depth                -0.01683    0.01494  -1.126 0.259976
## slice.depth                  -0.12666    0.03027  -4.184 2.86e-05 ***
## size.fraction106              0.90135    0.26564   3.393 0.000691 ***
## size.fraction212             -0.19401    0.26564  -0.730 0.465160
## slice.depth:size.fraction106 -0.06719    0.04281  -1.570 0.116529
## slice.depth:size.fraction212  0.03537    0.04281   0.826 0.408701
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##  contrast                                                          estimate
##  slice.depth5.5 size.fraction53 - slice.depth5.5 size.fraction106  -0.531788
##  slice.depth5.5 size.fraction53 - slice.depth5.5 size.fraction212  -0.000519
##  slice.depth5.5 size.fraction106 - slice.depth5.5 size.fraction212  0.531269
##     SE  df t.ratio p.value
##  0.123 142  -4.325  0.0001
##  0.123 142  -0.004  1.0000
##  0.123 142   4.320  0.0001
##
## Note: contrasts are still on the log(mu + 0.8) scale
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
## Model has log-transformed response. Back-transforming predictions to
##   original response scale. Standard errors are still on the log-scale.
```

Figure B4. Output provided by R for the PET analysis components.

Plotting: Slice depth and size fraction, and water depth and size fraction

```
##   slice.depth predicted std.error  conf.low conf.high size.fraction
## 1           1  2.065389 0.1616080 1.2818053  3.143910            53
## 2           1  5.798605 0.1616080 3.9941182  8.282294           106
## 3           1  1.645036 0.1616080 0.9764041  2.565337           212
## 4           2  1.724500 0.1370621 1.1253293  2.510134            53
## 5           2  4.635790 0.1370621 3.3456472  6.327430           106
## 6           2  1.431710 0.1370621 0.9020311  2.126228           212
```

Figure B5. Output provided by R for plotting the two-way interaction between slice depth and size fraction in the PET analysis.

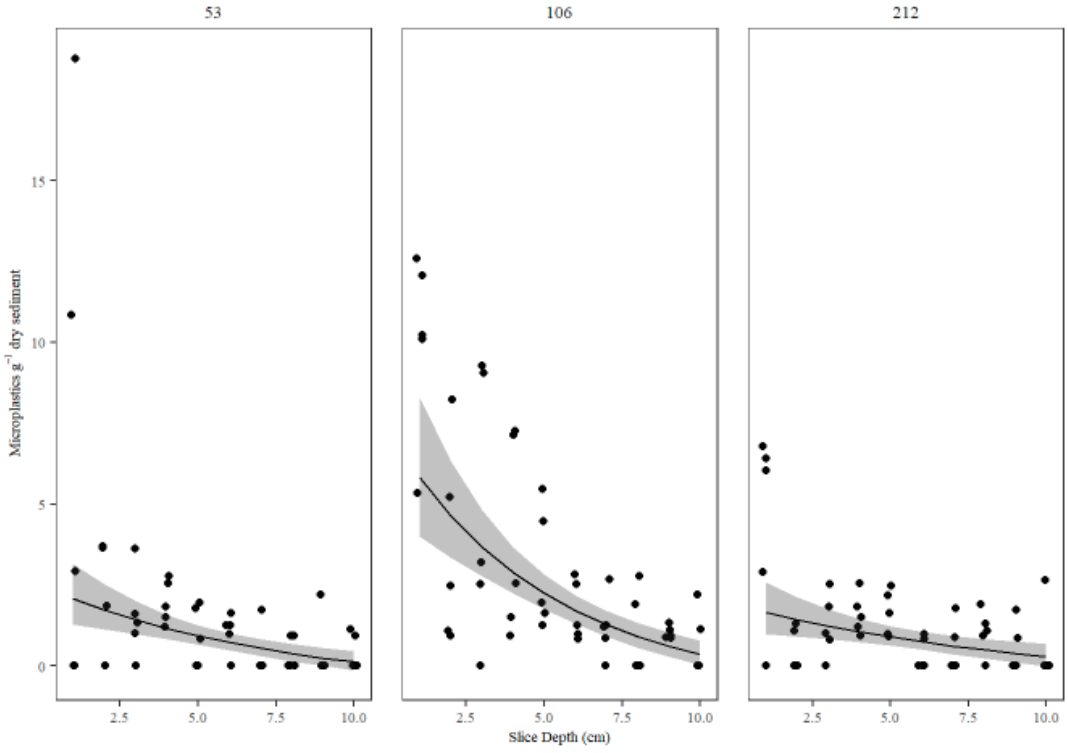

Figure B6. Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for PET concentration. The 106 µm size fraction differs highly significantly from the 212 µm and 53 µm size fractions (which do not differ significantly from each other), although primarily in the shallower sediment depths. The black line shows the predicted relationship between sediment depth and PET concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##   station.depth predicted std.error  conf.low conf.high size.fraction
## 1           7.1 0.9699108 0.1192969 0.5981218  1.440566           53
## 2           7.1 2.2123425 0.1192969 1.5795673  3.013385          106
## 3           7.1 0.9708304 0.1192969 0.5988483  1.441730          212
## 4          10.6 0.8686525 0.0925284 0.5897545  1.203520           53
## 5          10.6 2.0400036 0.0925284 1.5653263  2.609940          106
## 6          10.6 0.8695196 0.0925284 0.5904766  1.204561          212
```

Figure B7. Output provided by R for plotting the relationship between water depth and size fraction in the PET analysis.

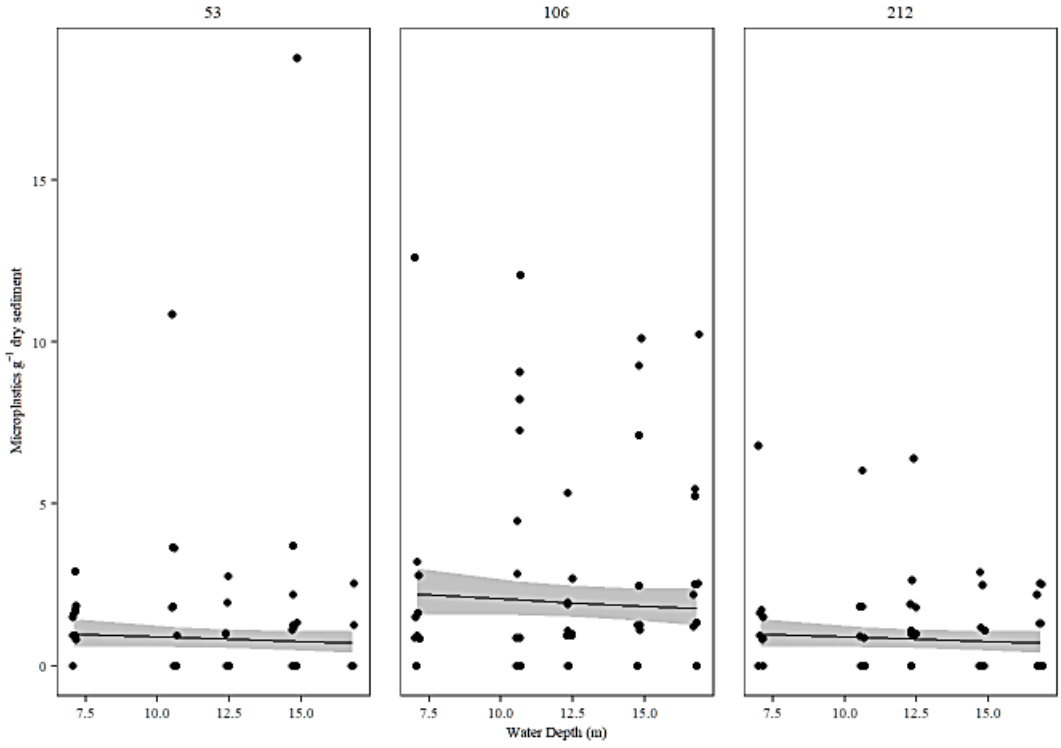

Figure B8. Visualization of the relationship between microplastic concentration and water depth for PET. There are no significant differences between size fractions at different water depths. The black line shows the predicted relationship between water depth and PET concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##     size.fraction cutoff
## 53             53      8
## 106           106      8
## 212           212      8
```

Figure B9. Output provided by R for the 95% sediment depth cutoff of PET concentrations for each size fraction.

2. PS

Model fitting and assumptions: Simulated residual diagnostics with DHARMa

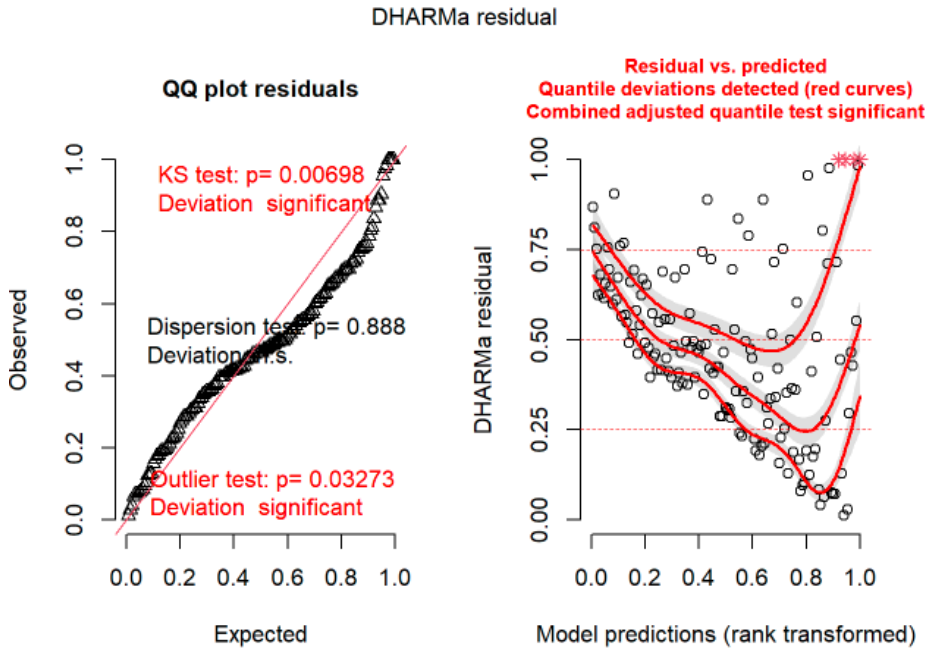

Figure B10. PS analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions under the normal distribution.

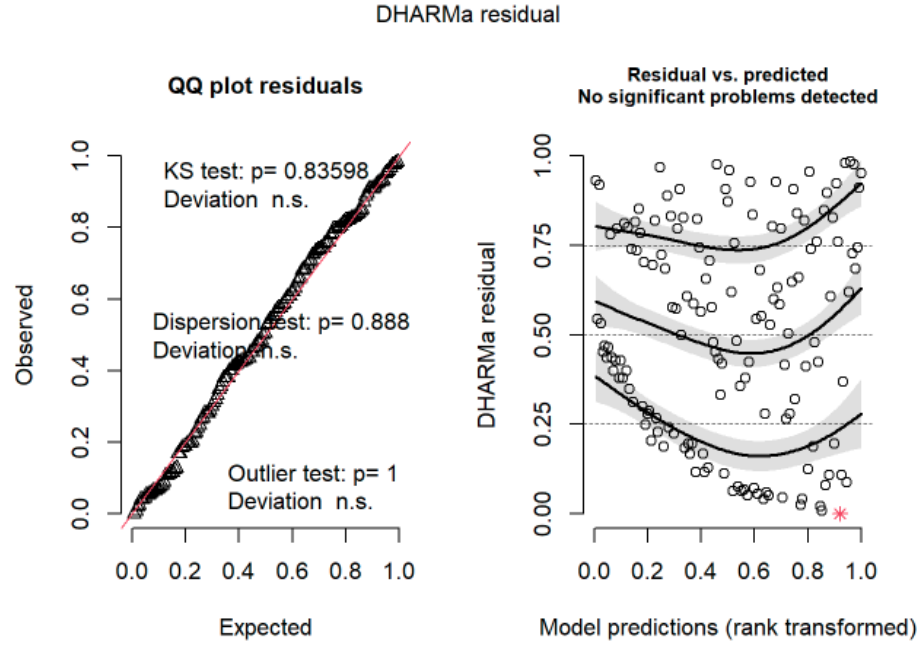

Figure B11. PS analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions with log-transformed MP counts.

Model interpretation: Individual variable effects and interactions

```
## Data: PS
## Models:
## PS_log_mod2: log(concentration + 0.75) ~ station.depth + slice.depth + size.fraction, zi=~0, disp=~1
## PS_log_mod: log(concentration + 0.75) ~ station.depth + slice.depth * size.fraction, zi=~0, disp=~1
##             Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## PS_log_mod2  6 345.01 363.08 -166.51   333.01
## PS_log_mod   8 347.55 371.64 -165.78   331.55 1.4621      2     0.4814
```

```
summary(PS_log_mod)
```

```
##  Family: gaussian  ( identity )
## Formula:
## log(concentration + 0.75) ~ station.depth + slice.depth * size.fraction
## Data: PS
##
##      AIC      BIC   logLik deviance df.resid
##    347.6    371.6   -165.8    331.6      142
##
##
## Dispersion estimate for gaussian family (sigma^2): 0.534
##
## Conditional model:
##                              Estimate Std. Error z value Pr(>|z|)
## (Intercept)                   2.09190    0.31282   6.687 2.27e-11 ***
## station.depth                -0.02445    0.01776  -1.377   0.1686
## slice.depth                  -0.20025    0.03598  -5.566 2.61e-08 ***
## size.fraction106              0.22197    0.31569   0.703   0.4820
## size.fraction212             -0.58185    0.31569  -1.843   0.0653 .
## slice.depth:size.fraction106  0.01680    0.05088   0.330   0.7413
## slice.depth:size.fraction212  0.05979    0.05088   1.175   0.2399
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##  contrast                           estimate    SE  df t.ratio p.value
##  size.fraction53 - size.fraction106    -0.314 0.147 144  -2.141  0.0853
##  size.fraction53 - size.fraction212     0.253 0.147 144   1.723  0.2001
##  size.fraction106 - size.fraction212    0.567 0.147 144   3.864  0.0005
##
## Note: contrasts are still on the log(mu + 0.75) scale
## P value adjustment: tukey method for comparing a family of 3 estimates
```

```
## Model has log-transformed response. Back-transforming predictions to
##   original response scale. Standard errors are still on the log-scale.
```

Figure B12. Output provided by R for the PS analysis components.

Plotting: Slice depth and size fraction, and water depth and size fraction

```
##   slice.depth predicted std.error conf.low conf.high size.fraction
## 1           1  3.621331 0.1400176 2.564518  5.015102            53
## 2           1  5.235995 0.1400176 3.788821  7.144592           106
## 3           1  2.644149 0.1400176 1.823580  3.726351           212
## 4           2  2.920571 0.1269630 2.105924  3.967595            53
## 5           2  4.276392 0.1269630 3.160833  5.710162           106
## 6           2  2.100039 0.1269630 1.467501  2.913008           212
```

Figure B13. Output provided by R for plotting the two-way interaction between slice depth and size fraction in the PS analysis.

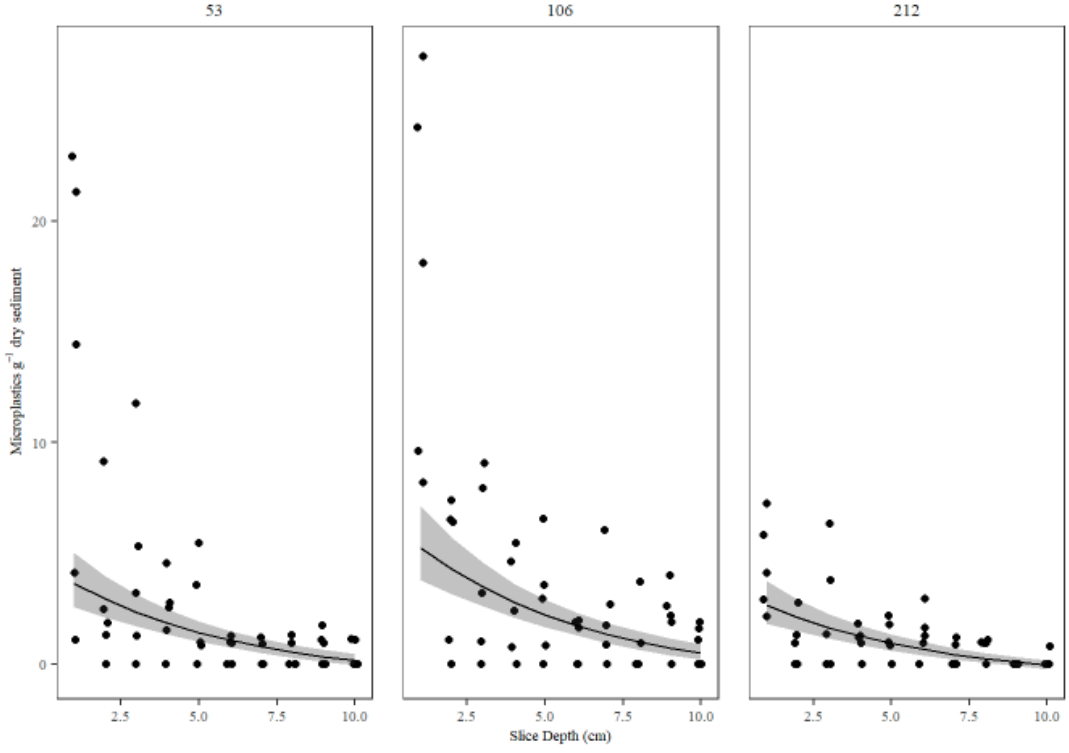

Figure B14. Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for PS concentration. The 53 µm and 106 µm size fractions differ marginally, the 53 µm and 212 µm size fractions do not differ, and the 106 µm and 212 µm size fractions differ highly. These effects are most prominent in the shallower sediment depths. The black line shows the predicted relationship between sediment depth and PS concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##   station.depth predicted std.error  conf.low conf.high size.fraction
## 1           7.1 1.5135710 0.1397383 0.9672780  2.233648           53
## 2           7.1 2.3496797 0.1397383 1.6015992  3.335736          106
## 3           7.1 1.0075647 0.1397383 0.5833919  1.566674          212
## 4          10.6 1.3279237 0.1083830 0.9272274  1.824348           53
## 5          10.6 2.0954588 0.1083830 1.5467549  2.775250          106
## 6          10.6 0.8634177 0.1083830 0.5522944  1.248870          212
```

Figure B15. Output provided by R for plotting the relationship between water depth and size fraction in the PS analysis.

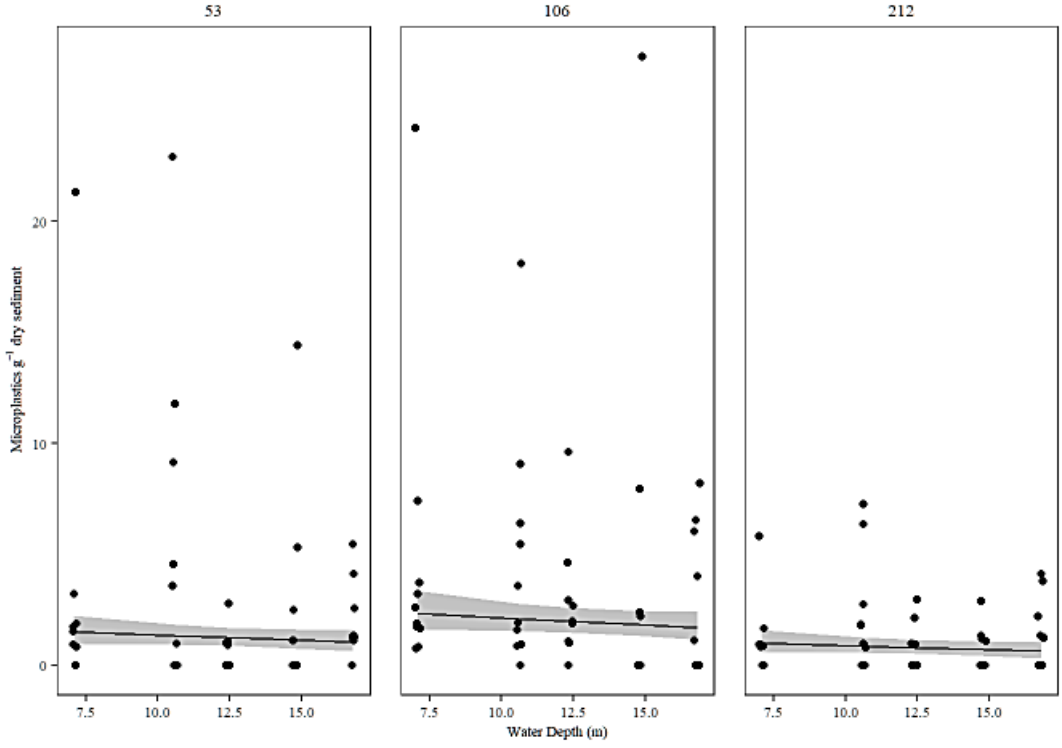

Figure B16. Visualization of the relationship between microplastic concentration and water depth for PS. There are no significant differences between size fractions at different water depths. The black line shows the predicted relationship between water depth and PS concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##     size.fraction cutoff
## 53             53      7
## 106           106      8
## 212           212      7
```

Figure B17. Output provided by R for the 95% sediment depth cutoff of PS concentrations for each size fraction.

3. LDPE

Model fitting and assumptions: Simulated residual diagnostics with DHARMa

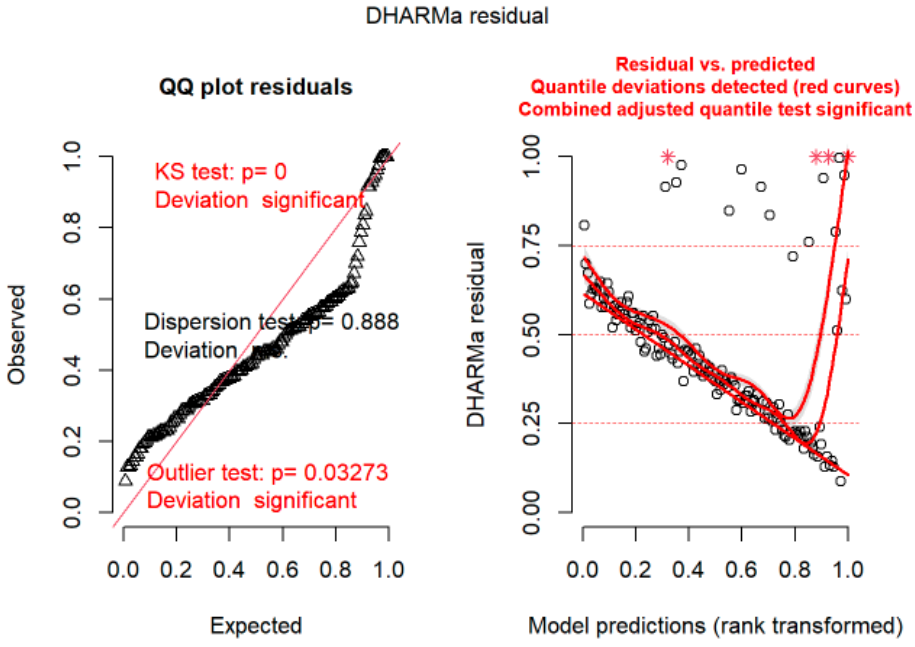

Figure B18. LDPE analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions under the normal distribution.

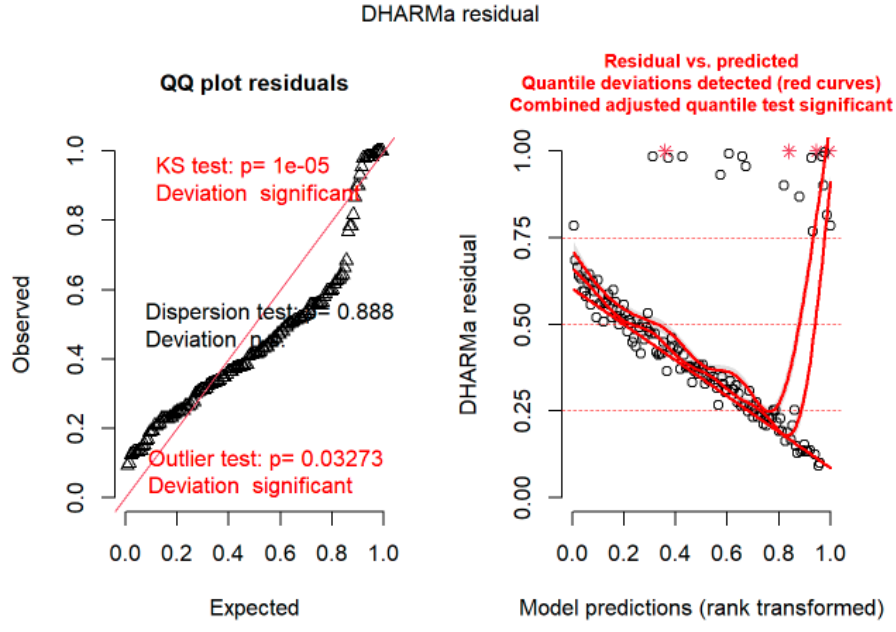

Figure B19. LDPE analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions with log-transformed MP counts.

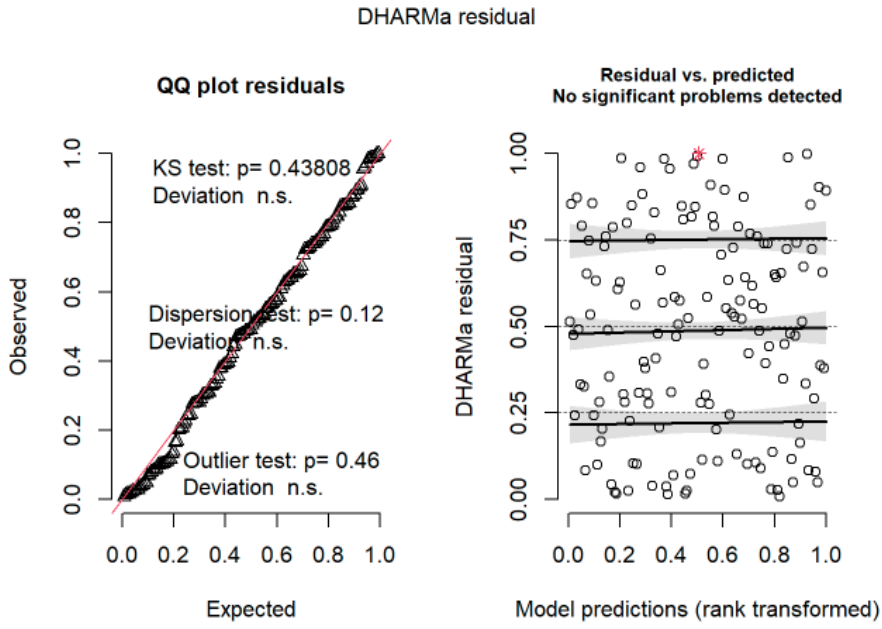

Figure B20. LDPE analysis DHARMa plot with residuals and predicted values to test the normality and homogeneity of variance assumptions under the Poisson distribution.

Model interpretation: Individual variable effects and interactions

```
## Data: PE
## Models:
## PE_pois_mod2: count ~ station.depth + slice.depth + size.fraction + offset(log.dry.weight), zi=~0, disp=~1
## PE_pois_mod: count ~ station.depth + slice.depth * size.fraction + offset(log.dry.weight), zi=~0, disp=~1
##              Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
## PE_pois_mod2  5 150.19 165.24 -70.095   140.19
## PE_pois_mod   7 148.36 169.44 -67.180   134.36 5.8295      2    0.05422 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
summary(PE_pois_mod)
```

```
##  Family: poisson  ( log )
## Formula:
## count ~ station.depth + slice.depth * size.fraction + offset(log.dry.weight)
## Data: PE
##
##      AIC      BIC   logLik deviance df.resid
##    148.4    169.4    -67.2    134.4      143
##
##
```

```
## Conditional model:
##                               Estimate Std. Error z value Pr(>|z|)
## (Intercept)                    2.80329    0.73723   3.802 0.000143 ***
## station.depth                 -0.17166    0.05586  -3.073 0.002117 **
## slice.depth                   -0.63169    0.18030  -3.504 0.000459 ***
## size.fraction106              -0.92658    0.65041  -1.425 0.154274
## size.fraction212              -2.47793    1.32976  -1.863 0.062401 .
## slice.depth:size.fraction106   0.43454    0.20080   2.164 0.030465 *
## slice.depth:size.fraction212   0.23529    0.38328   0.614 0.539297
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
##  contrast                              ratio    SE  df null z.ratio p.value
##  size.fraction53 / size.fraction106  0.684 0.246 Inf    1  -1.054  0.5426
##  size.fraction53 / size.fraction212  6.500 4.937 Inf    1   2.464  0.0366
##  size.fraction106 / size.fraction212 9.500 7.062 Inf    1   3.028  0.0069
##
## P value adjustment: tukey method for comparing a family of 3 estimates
## Tests are performed on the log scale
```

Figure B21. Output provided by R for the LDPE analysis components.

Plotting: Slice depth and size fraction, and water depth and size fraction

```
##   slice.depth  predicted std.error   conf.low conf.high size.fraction
## 1           1 0.60636332 0.3553568 0.30217095 1.2167830            53
## 2           1 0.88622349 0.3193539 0.47392402 1.6572109           106
## 3           1 0.09328664 0.7411852 0.02182335 0.3987653           212
## 4           2 0.43661545 0.3242022 0.23127978 0.8242530            53
## 5           2 0.63813040 0.2842808 0.36553518 1.1140116           106
## 6           2 0.06717159 0.7267628 0.01616457 0.2791305           212
```

Figure B22. Output provided by R for plotting the two-way interaction between slice depth and size fraction in the LDPE analysis.

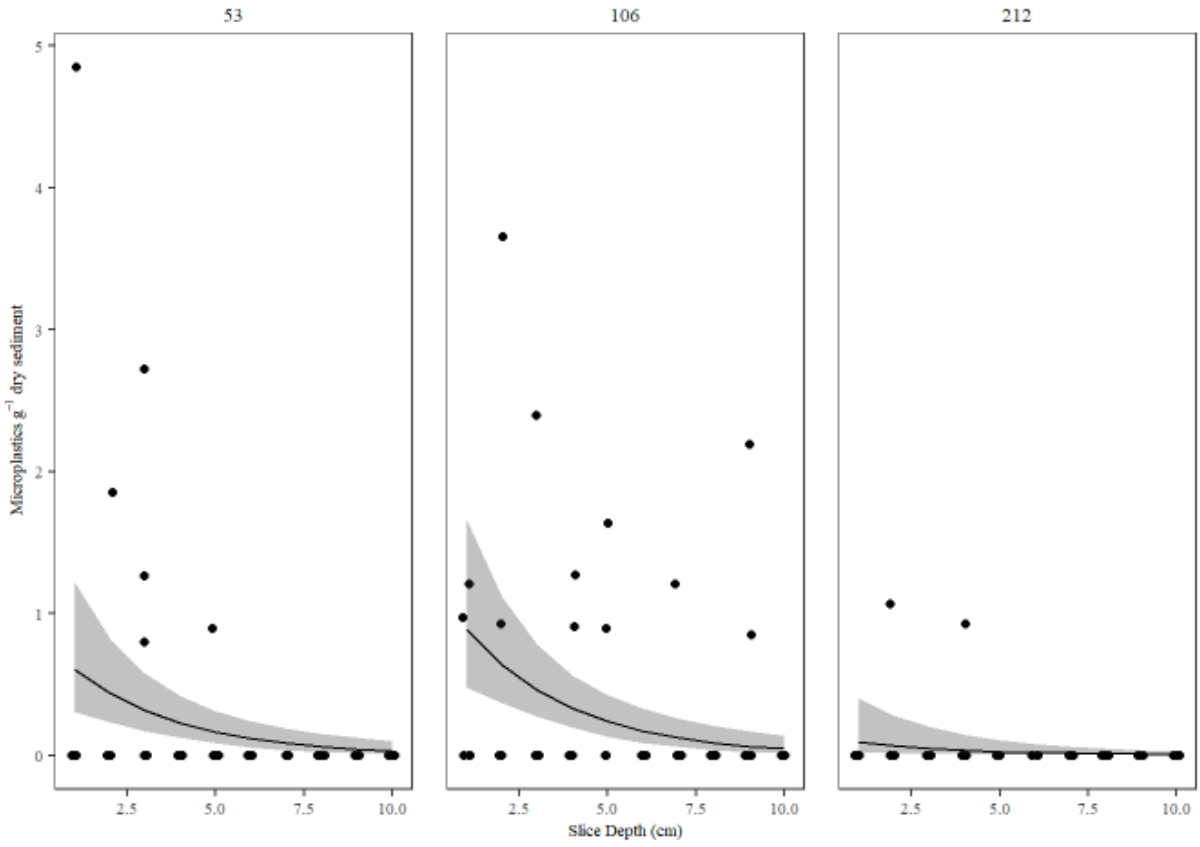

Figure B23. Visualization of the interaction between the predictors sediment depth (i.e., slice depth) and size fraction for LDPE concentration. The 53 µm size fraction does not differ significantly from the 106 µm size fraction. The 53 µm and 212 µm size fractions differ marginally, and the 106 µm and 212 µm size fractions differ highly significantly, although primarily in the shallower sediment depths. The black line shows the predicted relationship between sediment depth and LDPE concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##   station.depth  predicted std.error    conf.low conf.high size.fraction
## 1           7.1 0.33962315 0.3609877 0.167387878 0.6890815            53
## 2           7.1 0.49637240 0.3256081 0.262210174 0.9396491           106
## 3           7.1 0.05224970 0.7439013 0.012158329 0.2245400           212
## 4          10.6 0.18639028 0.3240595 0.098760503 0.3517736            53
## 5          10.6 0.27241661 0.2841180 0.156096034 0.4754177           106
## 6          10.6 0.02867542 0.7266991 0.006901482 0.1191454           212
```

Figure B24. Output provided by R for plotting the relationship between water depth and size fraction in the LDPE analysis.

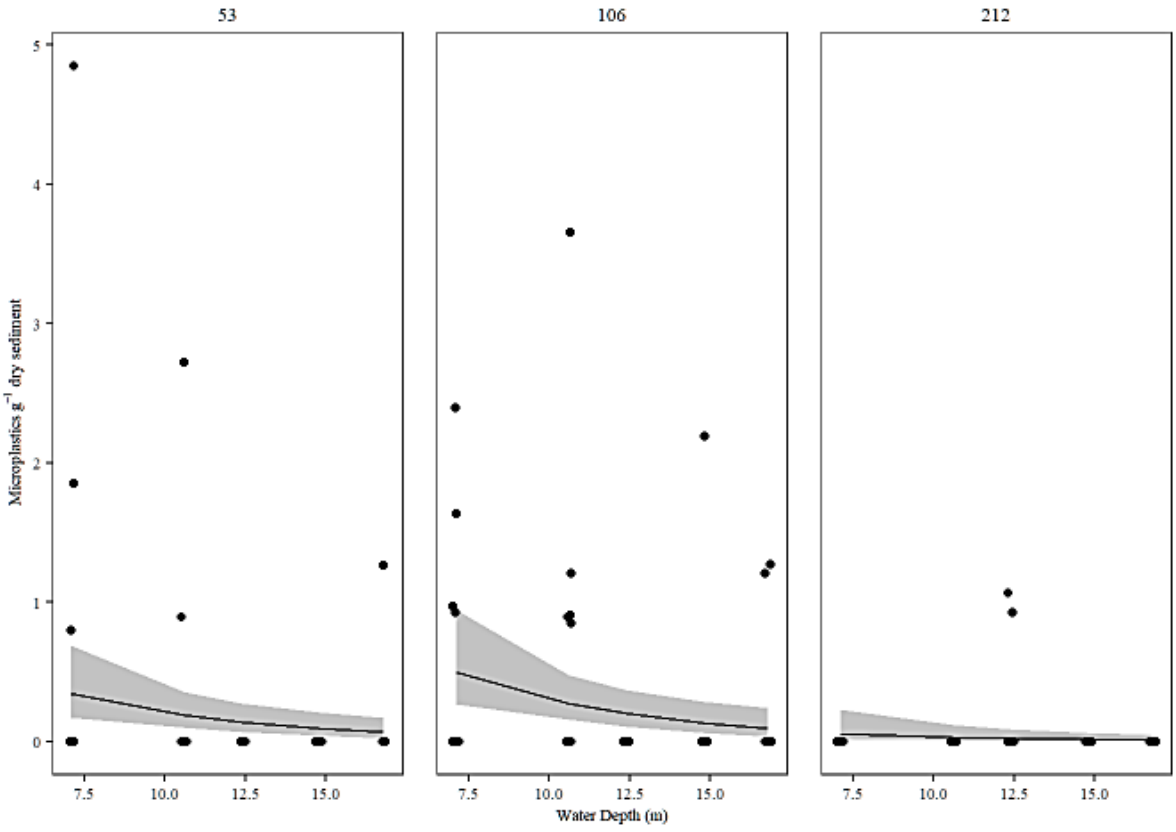

Figure B25. Visualization of the relationship between microplastic concentration and water depth for LDPE. There is a slight negative relationship between LDPE concentration in the sediment (averaged across sediment depth) and water depth. The interaction between slice depth and size fraction is marginally significant, altering the slopes for LDPE concentration of different size fractions. Generally, LDPE concentrations are very low for the 212 µm size fraction. The black line shows the predicted relationship between water depth and LDPE concentration, the grey ribbons around the line represent the 95% confidence intervals for the estimated marginal means.

```
##     size.fraction cutoff
## 53             53      4
## 106           106      8
## 212           212      3
```

Figure B26. Output provided by R for the 95% sediment depth cutoff of LDPE concentrations for each size fraction.

Appendix C – Supplementary Tables

Table C1. Mean microplastic concentrations (particles / g of dry sediment) and SDs for all water depths according to polymer (across size fractions and slice depth).

Water depth (m)	Mean PET	SD PET	Mean PS	SD PS	Mean LDPE	SD LDPE	Mean TOTAL Particles	SD TOTAL Particles
0.6	0.00	/	1.62	/	0.00	/	1.62	/
0.8	3.82	/	6.32	/	0.17	/	10.31	/
3.6	2.70	/	1.26	/	0.18	/	4.14	/
7.1	5.33	6.10	9.02	15.09	1.34	2.01	15.69	22.78
10.6	7.96	9.05	12.33	15.36	1.11	1.27	21.41	24.99
12.4	4.01	3.00	4.12	4.00	0.20	0.42	8.34	6.71
14.8	7.20	9.23	7.08	13.91	0.22	0.69	14.50	22.93
16.8	4.68	3.22	6.69	5.21	0.37	0.60	11.745	7.80

Table C2. Mean microplastic concentrations (particles / g of dry sediment) and SDs in the depositional zone for slice depth from 0 to 10 cm, according to polymer (across size fractions and water depth).

Slice depth (cm)	Mean PET	SD PET	Mean PS	SD PS	Mean LDPE	SD LDPE	Mean Total concentration of particles	SD Total concentration of particles
0-1	20.98	9.77	34.69	18.56	1.40	2.52	57.08	28.81
1-2	5.90	3.88	8.22	6.89	1.50	1.65	15.62	11.27
2-3	7.55	4.96	10.84	10.38	1.43	1.49	19.82	15.88
3-4	7.42	2.86	5.94	3.99	0.62	0.58	13.98	6.06
4-5	5.49	1.79	6.09	5.60	0.68	0.94	12.26	7.39
5-6	3.06	0.48	3.09	2.13	0.00	0.00	6.15	2.28
6-7	2.07	1.49	3.09	3.30	0.24	0.54	5.40	4.11
7-8	2.35	1.74	2.15	1.94	0.00	0.00	4.50	3.33
8-9	1.97	0.96	2.88	1.72	0.61	0.96	5.45	2.47
9-10	1.60	1.10	1.51	0.97	0.00	0.00	3.11	0.79

Appendix D – Supplementary Figures

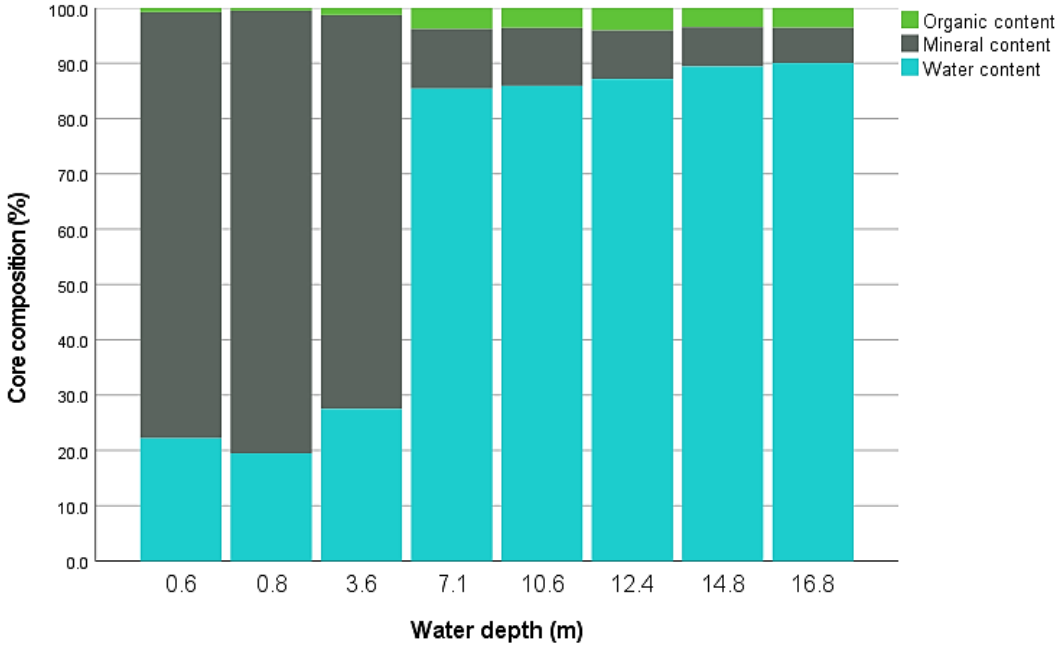

Figure D1. Mean core composition by organic content, mineral content, and water content across the water depth gradient (N = 159).

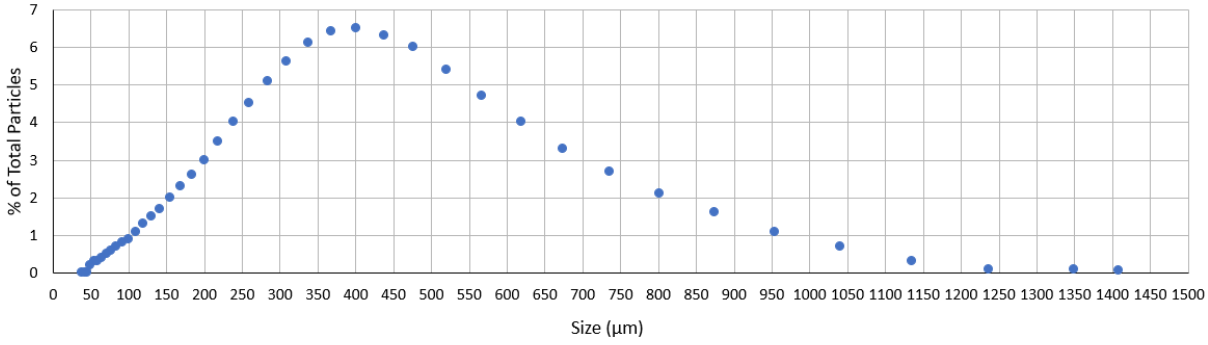

Figure D2. Particle size spectrum of PET (post-grinding, pre-addition; *The pELAstic Project*, 2024).

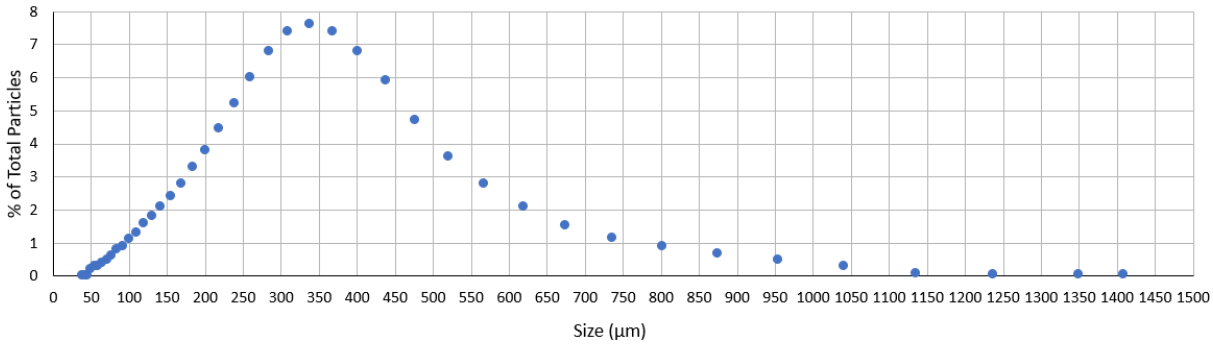

Figure D3. Particle size spectrum of PS (post-grinding, pre-addition; *The pELAstic Project*, 2024).

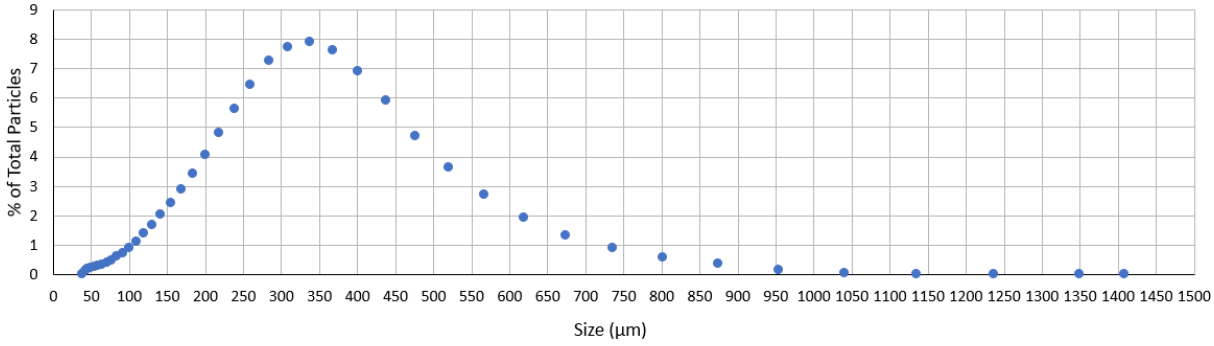

Figure D4. Particle size spectrum of LDPE – drum 1 (post-grinding, pre-addition; *The pELAstic Project*, 2024).

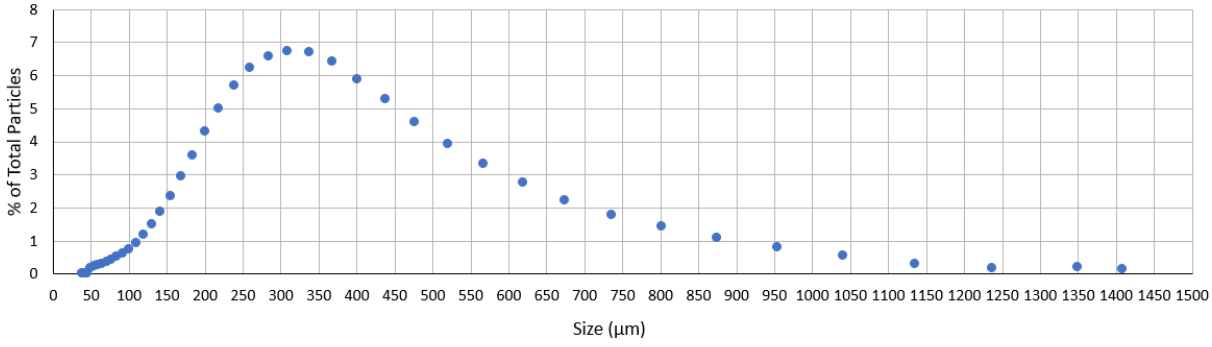

Figure D5. Particle size spectrum of LDPE – drum 2 (post-grinding, pre-addition; *The pELAstic Project*, 2024).

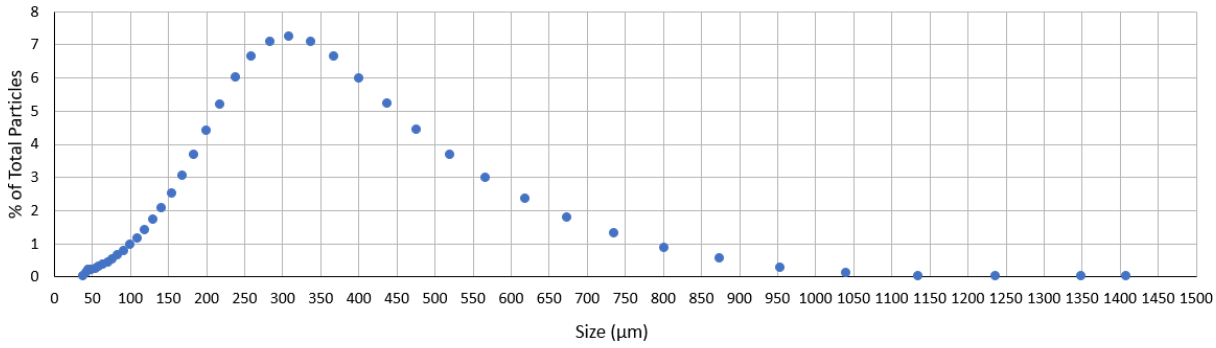

Figure D6. Particle size spectrum of LDPE – drum 3 (post-grinding, pre-addition; *The pELAstic Project*, 2024).

Appendix E – Microplastics Count Data

The microplastic count data provided here is the original data before blank correction. Microplastic counts in blanks can be found in Appendix A.

Sample ID	Unique Sample ID (ELA ID)	Start Date Quantification	B212	P212	Y212	B106	P106	Y106	B53	P53	Y53	Total212	Total106	Total53	TotalB	TotalP	TotalY	TotalAll	Comments	End Date Quantification
C3-S1-10,6m-1cm-	2023-A-2583	11/03/2024	5	6	0	10	15	1	9	20	0	11	26	29	24	41	1	66	/	11/03/2024
C3-S1-10,6m-2cm-	2023-A-2584	08/03/2024	0	3	0	9	7	4	4	11	0	3	20	15	13	21	4	38	/	11/03/2024
C3-S1-10,6m-3cm-	2023-A-2585	08/03/2024	2	7	0	10	10	0	4	14	3	9	20	21	16	31	3	50	/	08/03/2024
C3-S1-10,6m-4cm-	2023-A-2586	08/03/2024	2	2	0	8	6	1	2	6	0	4	15	8	12	14	1	27	/	08/03/2024
C3-S1-10,6m-5cm-	2023-A-2587	08/03/2024	1	2	0	5	4	1	2	5	1	3	10	8	8	11	2	21	/	08/03/2024
C3-S1-10,6m-6cm-	2023-A-2588	06/03/2024	0	1	0	3	2	0	0	2	0	1	5	2	3	5	0	8	/	06/03/2024
C3-S1-10,6m-7cm-	2023-A-2589	06/03/2024	0	0	0	1	1	0	0	1	0	0	2	1	1	2	0	3	/	06/03/2024
C3-S1-10,6m-8cm-	2023-A-2590	06/03/2024	0	1	0	0	1	0	1	1	0	1	1	2	1	3	0	4	/	06/03/2024
C3-S1-10,6m-9cm-	2023-A-2591	06/03/2024	1	0	0	1	0	1	0	0	0	1	2	0	2	0	1	3	/	06/03/2024
C3-S1-10,6m-10cm	2023-A-2592	06/03/2024	0	1	0	0	2	0	0	0	0	1	2	0	0	3	0	3	/	06/03/2024
C11-S2-7,1m-1cm-	2023-A-2673	15/03/2024	7	6	0	13	25	1	3	22	5	13	39	30	23	53	6	82	/	15/03/2024
C11-S2-7,1m-2cm-	2023-A-2674	15/03/2024	0	1	0	1	8	1	2	2	2	1	10	6	3	11	3	17	/	15/03/2024
C11-S2-7,1m-3cm-	2023-A-2675	15/03/2024	1	0	0	4	4	3	2	4	1	1	11	7	7	8	4	19	/	15/03/2024
C11-S2-7,1m-4cm-	2023-A-2676	15/03/2024	2	0	0	2	1	0	2	2	0	2	3	4	6	3	0	9	/	15/03/2024
C11-S2-7,1m-5cm-	2023-A-2677	15/03/2024	2	1	0	2	1	2	1	1	0	3	5	2	5	3	2	10	/	15/03/2024
C11-S2-7,1m-6cm-	2023-A-2678	13/03/2024	1	2	0	1	2	0	2	0	0	3	3	2	4	4	0	8	/	13/03/2024
C11-S2-7,1m-7cm-	2023-A-2679	13/03/2024	1	1	0	0	2	0	2	0	0	2	2	2	3	3	0	6	/	13/03/2024
C11-S2-7,1m-8cm-	2023-A-2680	13/03/2024	1	1	0	3	4	0	1	1	0	2	7	2	5	6	0	11	/	13/03/2024
C11-S2-7,1m-9cm-	2023-A-2681	13/03/2024	2	0	0	1	3	0	0	2	0	2	4	2	3	5	0	8	/	13/03/2024
C11-S2-7,1m-10cm	2023-A-2682	13/03/2024	0	0	0	0	2	0	1	0	0	0	2	1	1	2	0	3	/	13/03/2024
C13-S3-16,8m-1cm	2023-A-2693	23/02/2024	0	2	0	5	4	0	1	2	0	2	9	3	6	8	0	14	/	23/02/2024
C13-S3-16,8m-2cm	2023-A-2694	23/02/2024	1	1	0	4	5	0	0	1	0	2	9	1	5	7	0	12	/	23/02/2024
C13-S3-16,8m-3cm	2023-A-2695	23/02/2024	2	3	0	2	0	0	0	1	1	5	2	2	4	4	1	9	/	23/02/2024
C13-S3-16,8m-4cm	2023-A-2696	23/02/2024	2	1	0	2	0	1	3	2	0	3	3	5	7	3	1	11	/	23/02/2024
C13-S3-16,8m-5cm	2023-A-2697	23/02/2024	2	2	0	5	6	0	0	5	0	4	11	5	7	13	0	20	/	23/02/2024
C13-S3-16,8m-6cm	2023-A-2698	23/02/2023	0	1	0	2	0	0	2	1	0	1	2	3	4	2	0	6	/	23/02/2024
C13-S3-16,8m-7cm	2023-A-2699	23/02/2023	0	1	0	1	5	1	0	1	0	1	7	1	1	7	1	9	/	23/02/2024
C13-S3-16,8m-8cm	2023-A-2700	21/02/2024	1	0	0	0	0	0	0	1	0	1	0	1	1	1	0	2	/	21/02/2024
C13-S3-16,8m-9cm	2023-A-2701	21/02/2024	0	0	0	1	3	0	0	0	0	0	4	0	1	3	0	4	/	21/02/2024
C13-S3-16,8m-10cr	2023-A-2702	21/02/2024	0	0	0	2	1	0	1	1	0	0	3	2	3	2	0	5	/	21/02/2024

C15-S3-16,8m-1cm	2023-A-2713	27/03/2024	6	2	0	7	10	2	3	12	1	8	19	16	16	24	3	43	/	27/03/2024
C15-S3-16,8m-2cm	2023-A-2714	27/03/2024	2	2	0	5	10	1	5	0	0	4	16	5	12	12	1	25	/	27/03/2024
C15-S3-16,8m-3cm	2023-A-2715	27/03/2024	1	2	0	3	3	1	3	5	0	3	7	8	7	10	1	18	/	27/03/2024
C15-S3-16,8m-4cm	2023-A-2716	27/03/2024	1	3	0	5	0	1	3	3	0	4	6	6	9	6	1	16	/	27/03/2024
C15-S3-16,8m-5cm	2023-A-2717	27/03/2024	0	0	0	1	0	0	0	1	0	0	1	1	1	1	0	2	/	27/03/2024
C15-S3-16,8m-6cm	2023-A-2718	22/03/2024	1	0	0	1	2	0	1	0	0	1	3	1	3	2	0	5	/	22/03/2024
C15-S3-16,8m-7cm	2023-A-2719	22/03/2024	0	0	0	4	0	0	0	0	0	0	4	0	4	0	0	4	/	22/03/2024
C15-S3-16,8m-8cm	2023-A-2720	22/03/2024	0	1	0	0	0	1	0	0	0	1	1	0	0	1	1	2	/	22/03/2024
C15-S3-16,8m-9cm	2023-A-2721	22/03/2024	0	0	0	2	0	0	0	0	0	0	2	0	2	0	0	2	/	22/03/2024
C15-S3-16,8m-10cr	2023-A-2722	22/03/2024	2	0	0	1	1	0	0	1	0	2	2	1	3	2	0	5	/	22/03/2024
C19-S4-12,4m-1cm	2023-A-2753	19/02/2024	6	2	0	5	9	0	0	1	0	8	14	1	11	12	0	23	/	19/02/2024
C19-S4-12,4m-2cm	2023-A-2754	19/02/2024	1	0	1	1	1	0	0	0	0	2	2	0	2	1	1	4	/	19/02/2024
C19-S4-12,4m-3cm	2023-A-2755	19/02/2024	1	0	0	0	1	0	1	0	0	1	1	1	2	1	0	3	/	19/02/2024
C19-S4-12,4m-4cm	2023-A-2756	19/02/2024	1	1	1	1	5	0	3	3	0	3	6	6	5	9	1	15	/	19/02/2024
C19-S4-12,4m-5cm	2023-A-2757	19/02/2024	1	1	0	2	3	0	2	1	0	2	5	3	5	5	0	10	/	19/02/2024
C19-S4-12,4m-6cm	2023-A-2758	19/02/2024	1	3	0	1	2	0	1	1	0	4	3	2	3	6	0	9	/	19/02/2024
C19-S4-12,4m-7cm	2023-A-2759	19/02/2024	2	0	0	3	3	0	0	1	0	2	6	1	5	4	0	9	/	19/02/2024
C19-S4-12,4m-8cm	2023-A-2760	19/02/2024	2	1	0	2	0	0	0	0	0	3	2	0	4	1	0	5	/	19/02/2024
C19-S4-12,4m-9cm	2023-A-2761	19/02/2024	0	0	0	1	2	0	0	1	0	0	3	1	1	3	0	4	/	19/02/2024
C19-S4-12,4m-10cr	2023-A-2762	19/02/2024	3	0	0	0	0	0	0	0	0	3	0	0	3	0	0	3	/	19/02/2024
C27-S5-14,8m-1cm	2023-A-2833	01/03/2024	2	2	0	7	19	0	13	10	0	4	26	23	22	31	0	53	/	01/03/2024
C27-S5-14,8m-2cm	2023-A-2834	01/03/2024	0	0	0	2	0	0	3	2	0	0	2	5	5	2	0	7	/	01/03/2024
C27-S5-14,8m-3cm	2023-A-2835	01/03/2024	0	1	0	7	6	0	1	4	0	1	13	5	8	11	0	19	/	01/03/2024
C27-S5-14,8m-4cm	2023-A-2836	01/03/2024	1	1	0	6	2	0	1	0	0	2	8	1	8	3	0	11	/	01/03/2024
C27-S5-14,8m-5cm	2023-A-2837	28/02/2024	2	0	0	1	0	0	0	0	0	2	1	0	3	0	0	3	/	28/02/2024
C27-S5-14,8m-6cm	2023-A-2838	28/02/2024	0	0	0	1	0	0	1	0	0	0	1	1	2	0	0	2	/	28/02/2024
C27-S5-14,8m-7cm	2023-A-2839	28/02/2024	0	0	0	1	0	0	0	0	0	0	1	0	1	0	0	1	/	28/02/2024
C27-S5-14,8m-8cm	2023-A-2840	28/02/2024	1	1	0	0	0	0	0	0	0	2	0	0	1	1	0	2	/	28/02/2024
C27-S5-14,8m-9cm	2023-A-2841	28/02/2024	0	0	0	1	2	2	2	1	0	0	5	3	3	3	2	8	/	28/02/2024
C27-S5-14,8m-10cr	2023-A-2842	28/02/2024	0	0	0	1	0	0	1	1	0	0	1	2	2	1	0	3	/	28/02/2024
C31-S6-0,8m-1-10c	2023-A-2873	20/03/2024	5	3	0	11	13	1	7	22	0	8	25	29	23	38	1	62	/	20/03/2024
C38-S7-0,6m-1-10c	2023-A-2880	20/03/2024	0	0	0	0	6	0	0	4	0	0	6	4	0	10	0	10	/	20/03/2024
C43/G1-S8-3,6m-B	2023-A-2907	20/03/2024	1	0	0	5	4	0	9	4	1	1	9	14	15	8	1	24	/	20/03/2024