The Complex Dynamics of Microplastic Fate in Saltmarshes

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Abstract

Coastal wetlands are characterized by unique biological and physical dynamics that enable them to provide many ecosystem benefits (e.g., coastal protection)—and, potentially, the entrapment of marine microplastics. However, the mechanisms underlying microplastics entrapment and retention are poorly understood. In this thesis, I test the underlying drivers affecting microplastic transport and fate in saltmarshes. Chapter 2 uses a controlled flume experiment to investigate the influence of vegetation structure and microplastic type on microplastic deposition. Following this, Chapter 3 takes an exploratory methods comparison approach to demonstrate the importance of method validation in microplastics research and develop methods for isolating microplastics from saltmarsh sediments and leaf surfaces. Using these optimized methods, Chapter 4 explores the influence of vegetation presence and complexity on microplastic accumulation in saltmarsh sediments in a field site (North Norfolk, UK). Finally, Chapter 5 investigates one route of microplastic export from saltmarshes by quantifying microplastics on detrital leaves of *Atriplex portulacoides* in the Tamar Estuary, UK. My results demonstrate that: (1) microplastic characteristics influence microplastic fate, while vegetation structure does not, in a simulated wetland (Chapter 2); (2) method validation is necessary to increase harmonisation across microplastics research, particularly when dealing with complex matrices (Chapter 3); (3) microplastic accumulation in saltmarsh sediments is not determined by vegetation presence or complexity (Chapter 4); and (4) microplastic adherence to saltmarsh leaf litter is an export mechanism, potentially releasing over 22 million microplastics into the Tamar Estuary with every tide (Chapter 5). Collectively, this thesis shows that, while saltmarshes are a reservoir for microplastics, microplastic deposition and retention are dependent on a myriad of biophysical variables. By increasing our understanding of the transport and fate of microplastics in coastal wetlands, this work contributes to a widening knowledge base on the global cycling of microplastics.

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List of Abbreviations

AONB	Area of Outstanding Natural Beauty
AP	Anthropogenic particle
CNC	Computer Numerical Control
CRM	Certified Reference Material
EPS	Extracellular Polymeric Substances
FPA	Focal Plane Array
FTIR	Fourier Transform Infrared spectroscopy
HDPE	High Density Polyethylene
H_2O_2	Hydrogen Peroxide
ILC	Interlaboratory Comparison
KOH	Potassium Hydroxide
LDPE	Low Density Polyethylene
MP	Microplastic
NaClO	Sodium Hypochlorite
NaBr	Sodium Bromide
NPP	Net Primary Productivity
OEP	Oil Extraction Procedure
PA	Polyamide
PAH	Polycyclic Aromatic Hydrocarbon
PE	Polyethylene
PET	Polyethylene Terephthalate
PP	Polypropylene
PS	Polystyrene
PVC	Polyvinyl Chloride
Py-GC-MS	Pyrolysis Gas Chromatography Mass Spectrometry
RM	Reference Material
SMI	Sediment Microplastic Isolation
SPA	Special Protection Area

SSSI	Site of Special Scientific Interest
$ZnCl_2$	Zinc Chloride

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Chapter One

Introduction

1.1 History of plastic production and pollution

When plastics first hit the commercial market in the 1950s, it was advertised as a revolutionary material, changing the way we would live, even "good for the environment" by replacing ivory and tortoise shell (Altman 2021). Nowadays, plastics are more often associated with images of entangled sea turtles in plastic nets or beached whales full of plastic bags. From the invention of the first fully synthetic plastic, Bakelite in 1907, 115 years have passed to reach the first steps of global action on plastic pollution. In the interim, cumulative plastic production has exceeded 10 billion metric tonnes, half of which was produced after 2010 (Geyer 2020, Liu et al. 2024). The ubiquity of plastics usage is a result of their durability, versatility, flexibility, and perceived low economic cost (Andrady and Neal 2009, Landrigan et al. 2025). Plastics have helped with advancement in medicine, engineering, technology, transport, construction, and agriculture (Andrady and Neal 2009, Geyer 2020). Despite the success of plastics in a multitude of industrial sectors, there are significant negative impacts generated across the full plastics lifecycle, from material extraction to production, use, disposal and release (Landrigan et al. 2025; Figure 1.1). Not only are there direct consequences from plastic pollution, but plastics can also have indirect effects by exacerbating the consequences of breaching other planetary boundaries such as climate change, biodiversity loss, ocean acidification, and land system change (Villarrubia-Gómez et al. 2024). The revolutionary effect of plastics on society is indisputable, however, the continuous increase in plastic production, consumption, and pollution can no longer be ignored. The change in public perception and the pervasive accumulation of plastics in the environment has led to increased pressure on governments and businesses to act on this worldwide pollutant. Ultimately, this has led the United Nations Environment Assembly to adopt a resolution to develop an international legally binding instrument on plastic pollution (UNEP 2022).

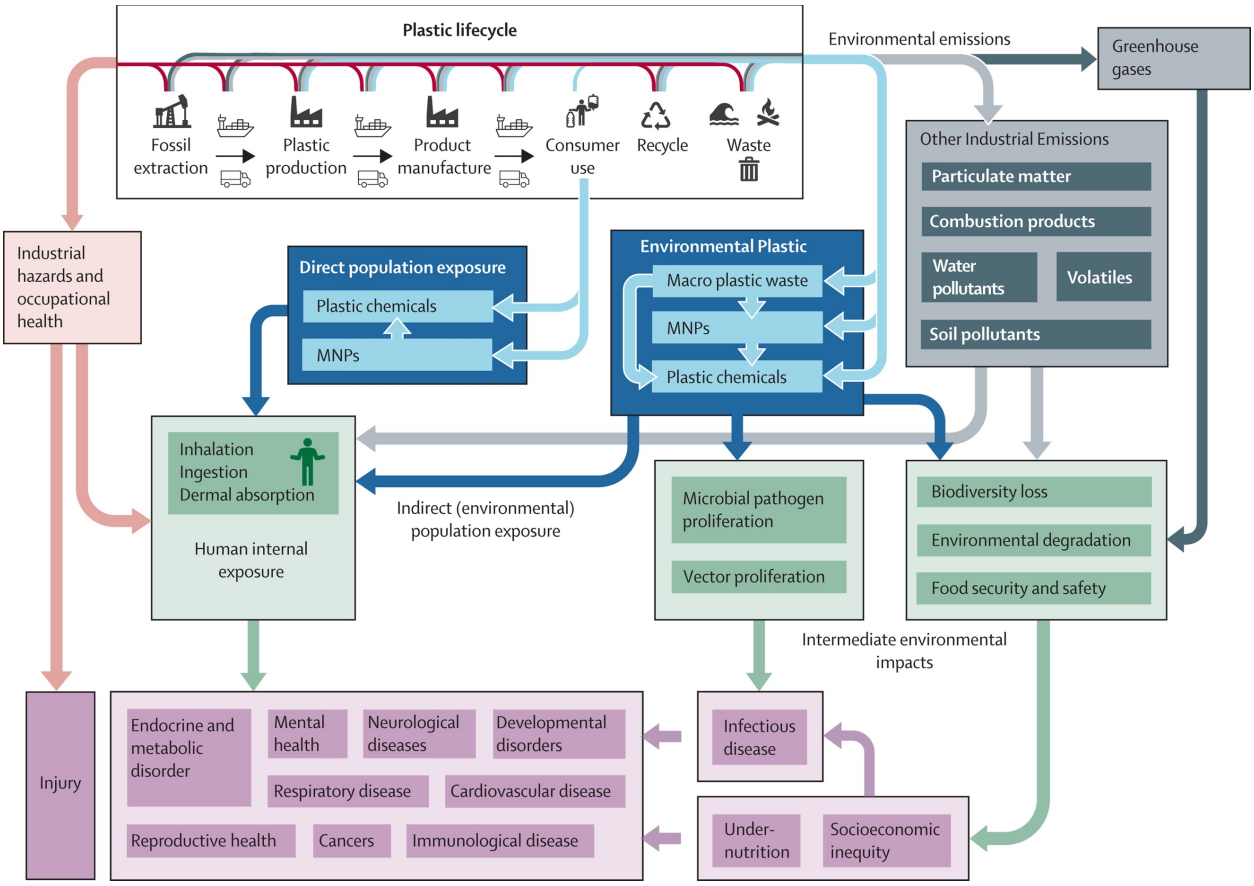

Figure 1.1. The possible human health impacts generated across the full plastics life cycle, from Landrigan et al. (2025).

1.2 The definition and types of plastic

The definition of plastics has many different variations across governing bodies, unions, or Multilateral Environmental Agreements (CIEL 2023). While there is no single definition, it can be summarised as 'materials made wholly or partly of synthetic or semi-synthetic polymers' (Geyer 2020, Sparks et al. 2025). Natural polymers also exist, such as cellulose or DNA. Plastics, however, in addition to the polymer, contain a complex mixture of chemicals consisting of additives, residual processing aids, and non-intentionally added substances (Monclús et al. 2025). Over 16, 000 known chemicals have been identified in plastics, with over 4200 being chemicals of concern, which are persistent, bioaccumulative, mobile, or toxic (Monclús et al. 2025). Plastics can have different properties based on which polymer and associated chemicals are used. For

instance, thermoplastics can be remoulded after heating (e.g., polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, polystyrene, polyamide) whereas thermosets are not able to be remelted and reformed (e.g., polyurethanes, silicone, epoxy and acrylic resins; Geyer, 2020). Additive chemicals are used to customize the plastic, determining properties such as colour, resistance, flexibility, or flammability (Wiesinger et al. 2021, OECD 2022). Over 99% of plastics are produced from fossil-based feedstocks, such as natural gas, oil, and coal (CIEL 2019, Cabernard et al. 2022). The remaining plastics are derived from biomass, such as corn or sugarcane, called biobased plastics (CIEL 2019, European Environment Agency 2020). The largest markets for plastics are packaging, building and construction, and textiles (European Environment Agency 2020, Geyer 2020). The packaging sector produces vast quantities of single-use plastics, usually made from polyethylene, polypropylene, or polyethylene terephthalate, and constitutes almost 40% of total plastic production (Geyer et al. 2017, Chen et al. 2021). The design of single-use packaging and the 'throwaway' culture it promotes means most plastic ends up as waste (Geyer et al. 2017).

1.3 Plastic waste

Global production of plastic has outpaced our ability to deal with plastic waste in a safe and sustainable manner. Over 70% of plastics produced since 1950 has entered landfills or leaked into the environment (OECD 2022, Liu et al. 2024). The remaining plastics have been recycled or incinerated (Liu et al. 2024; OECD, 2022), neither of which are free of environmental nor human health impacts (Tait et al. 2020, Shen et al. 2020, Landrigan et al. 2025). Due to the mismanagement of plastic waste, a cumulative 139 million tonnes of plastic waste have entered aquatic environments (OECD, 2022). The majority of plastic debris originates from land-based activities including mismanaged waste from households (e.g., single-use plastics, packaging), industry (e.g., pre-production pellets, aquaculture gear), and wastewater (e.g., textiles, personal care products; Barnes et al. 2009, Jambeck et al. 2015, Duis and Coors 2016). The remaining proportion comes from maritime activities like fishing and shipping (Andrady 2011, Jambeck et al. 2015). For instance, lost or abandoned fishing gear such as ghost nets, contribute 640 000 tonnes of plastic pollution to the marine environment every year (Richardson et al. 2018). Other entry points of plastics into the environment include runoff from roads, stormwater, agriculture,

and wastewater effluent; wind-blown from land; and atmospheric release from textiles and road dust (Horton et al. 2017a, Rochman 2018, De Falco et al. 2020, Grbić et al. 2020, Evangeliou et al. 2020).

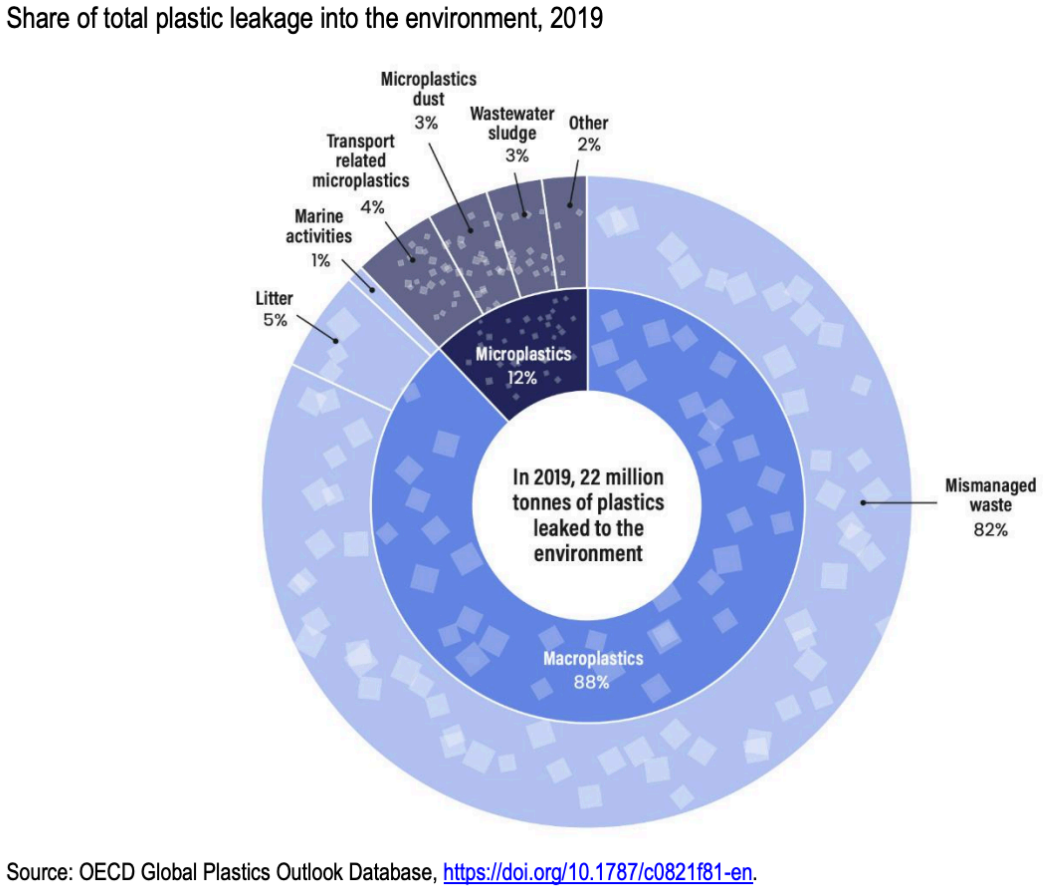

Figure 1.2. Global leakage of microplastics and macroplastics to the environment in 2019. From OECD, 2022. Source: OECD Global Plastics Outlook Database, https://doi.org/10.1787/c0821f81-en.

1.4 Plastic degradation and microplastics

Plastics are resistant to degradation and can remain in the environment for decades or even centuries (Barnes et al. 2009). Throughout this time, they fragment into smaller pieces via exposure to different abiotic and biotic conditions (Andrady 2011, Zhang et al. 2021). Abiotic degradation includes mechanical abrasion or chemical degradation via photo-oxidation and thermo-oxidation (Andrady 2011; Zhang et al. 2021). Biotic degradation can occur via mechanical

break-up by organisms (e.g. chewing), and fragmentation during digestion, or by microorganism degradation (Pathak and Navneet 2017, Dawson et al. 2018, Zhang et al. 2021). As plastics fragment, microplastics are generated. Microplastics are plastic particles and fibres < 5 mm in the longest dimension (Commission Regulation (EU) 2023). The exact size definition of microplastics varies in the literature, with some using an upper limit of 1 mm (Hartmann et al. 2019). The lower size limit is typically defined at 1 µm; particles below this size threshold are subsequently labelled as 'nanoplastics'. Most microplastics are formed from the break-up of larger plastics (i.e., secondary microplastics) but they can also be manufactured, for example, the plastic particles found in personal care products or as pre-production pellets (Cole et al. 2011). There are two categories of secondary microplastics: those that are released during use of the product (e.g., tyre and paint particles, microfibres from clothing and textiles) and those that are fragmented and released after the product has been discarded in the environment (OECD 2022, Thompson et al. 2024). The three largest sources of microplastics to the environment are macroplastics, tyres, and paints (Thompson et al. 2024; Figure 1.3).

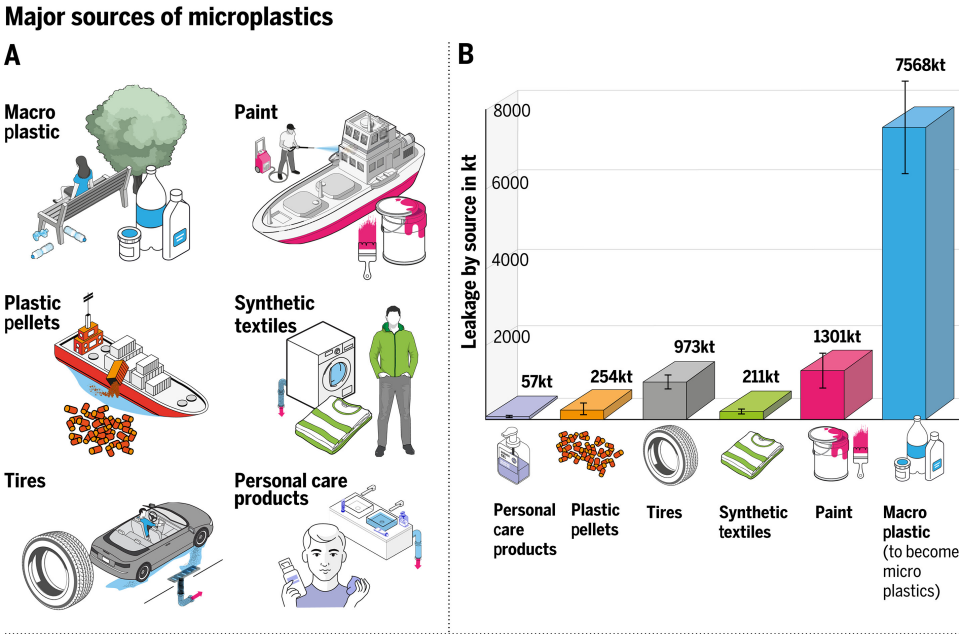

Figure 1.3. Major sources of microplastics to the environment. (A) Six key sources of microplastics. (B) The relative contribution of each source to the marine environment. From Thompson et al. (2024).

1.5 Negative impacts of microplastics

The small size of microplastics makes them of environmental concern owing to their capacity for long-range transport and bioavailability. Microplastics have permeated all environmental compartments: soils and shorelines, freshwater and marine waters, sea ice, the deep sea, the atmosphere, and remote areas (Browne et al. 2011, Cole et al. 2011, Van Cauwenberghe et al. 2013, Obbard et al. 2014, Dris et al. 2016, Horton et al. 2017b, Rochman 2018, Napper et al. 2020). Their global prominence means they have been found in every level of the food web and even in human tissues (Leslie et al. 2022, Jenner et al. 2022). While research into microplastics and human health is still in its infancy, the presence of microplastics in the environment was first documented in the 1970s (Carpenter and Smith 1972). Subsequently, research into the impacts of microplastics began in the early 2000s and has increased exponentially (Thompson et al. 2004, Cole et al. 2011, Zhang et al. 2020). To date, microplastics have been detected in over 1300 aquatic and terrestrial species (Thompson et al. 2024). The presence of microplastics in the environment and wildlife is undeniable. However, there is still uncertainty on whether current levels of microplastics cause harm to the natural environment. There is evidence of effects across all levels of biological organization (Bucci et al. 2020, Kögel et al. 2020). Yet, effects from microplastics are highly variable, with some studies not detecting an effect, and effects differing based on the organism and the type and quantity of microplastics ingested (Bucci et al. 2020, Thornton Hampton et al. 2022). The effects of microplastics on biota can be both physical and chemical in nature. Physical impacts from microplastics include blockage of the gastrointestinal tract, food dilution, and internal abrasion (Wright et al. 2013, Foley et al. 2018, de Ruijter et al. 2020). Microplastics can also leach toxic additives or adsorbed pollutants into tissues of biota (Rochman 2016). For instance, microplastics accumulate persistent organic pollutants (e.g., polycyclic aromatic hydrocarbons, polychlorinated biphenyls) and trace metals, and the presence of these sorbed pollutants on microplastics has been shown to induce lethal and sublethal effects in organisms (Browne et al. 2013, Rochman et al. 2014). Exposure to microplastics has been demonstrated to adversely affect survival, growth, reproduction and ecological function in a wide range of organisms (Gunaalan et al. 2020, Thompson et al. 2024, Boháčková and Cajthaml 2024). The potential for harm from microplastics is evident. If current

levels of plastic production, and consequently waste, are maintained, it is only a matter of time before we see the effects from microplastics significantly erode planetary and human health.

1.6 The complexity of microplastics

Microplastics are a unique contaminant because of the sheer complexity and diversity in their particle characteristics (Rochman et al. 2019). Microplastics vary in physical and chemical properties such as size, shape, colour, density, polymer type, chemical mixtures, and surface chemistry (Lambert et al. 2017, Rochman et al. 2019; Figure 1.4). It is important to recognize this complexity because differences in particle characteristics affects their toxicity, environmental transport, and recovery from environmental samples (Lambert et al. 2017, Rochman et al. 2019, Thornton Hampton et al. 2023). For example, the physical properties of microplastics determines buoyancy which ultimately determines their transport and fate within aquatic systems (Filella 2015). However, these properties can change over time due to environmental exposure, degradation, and interactions with biota (Cole et al. 2016, Lambert et al. 2017, Zhang 2017). Particle density is initially dependent on polymer type, but once in the environment, biofouling from microorganisms, egestion via faecal pellets, or flocculation with other particles can occur, this alters particle density and consequently distribution (Wang et al. 2021, Wu et al. 2024). As such, low-density polymers have been found in benthic sediments (Van Cauwenberghe et al. 2013). Microplastics are sometimes thought of as one contaminant type resulting in simplified experiments or policies, however, recognition of microplastics as a diverse contaminant suite is necessary for a holistic understanding of their sources, transport, fate, and effects (Rochman et al. 2019).

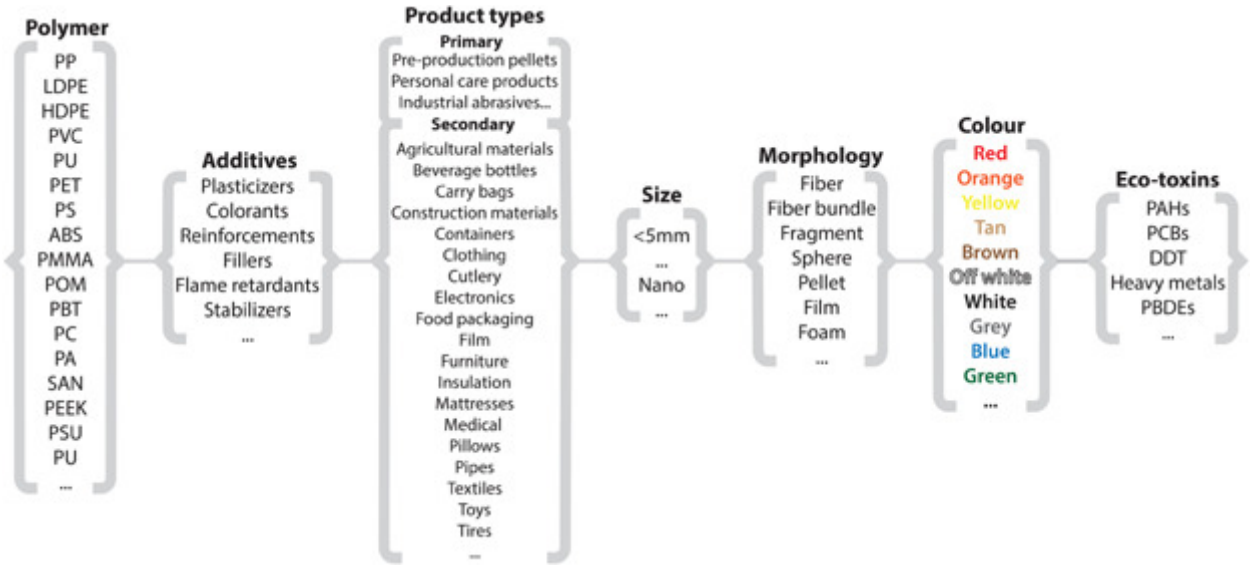

Figure 1.4. The different characteristics of microplastics including polymer type, chemical additives, product type, size, morphology, colour, and sorbed contaminants (eco-toxins). From Rochman et al. (2019).

1.7 Microplastic transport and the plastic cycle

Despite the increase in microplastics research over the last two decades, there are still many unknowns, particularly around the transport and fate of microplastics. More recently, Horton and Dixon (2018); and further built upon by Bank and Hansson (2019) proposed the concept of the 'plastics cycle', wherein plastics transfer through abiotic and biotic compartments similar to the water, nutrient, and carbon cycles (Figure 1.5). Because microplastics research began with a focus on the marine compartment, it was initially thought that oceanic sediments were the final fate for microplastics, with rivers acting as conduits between the land and ocean (Law 2017, Hoellein and Rochman 2021). Attempts to quantify the plastics budget noted that estimates of plastics flowing into the ocean were greater than estimates of plastics at the sea surface, prompting investigations into the 'missing ocean plastics' (Cózar et al. 2014, Law 2017, Weiss et al. 2021, Isobe and Iwasaki 2022). However, these investigations work under the assumption that plastics have a 'final fate' or 'sink', operating in defined environmental compartments (e.g., freshwater, marine, terrestrial, and atmospheric), rather than a dynamic cycle with multiple pools and fluxes across inland, coastal, and oceanic systems (Horton and

Dixon 2018, Hoellein and Rochman 2021). Indeed, microplastics have now been linked to the global dust and water cycles (Bergmann et al. 2019, Brahney et al. 2020). Investigating the transport and fate of microplastics throughout different environmental compartments will help balance the so-called 'plastics budget' (Zhu 2021).

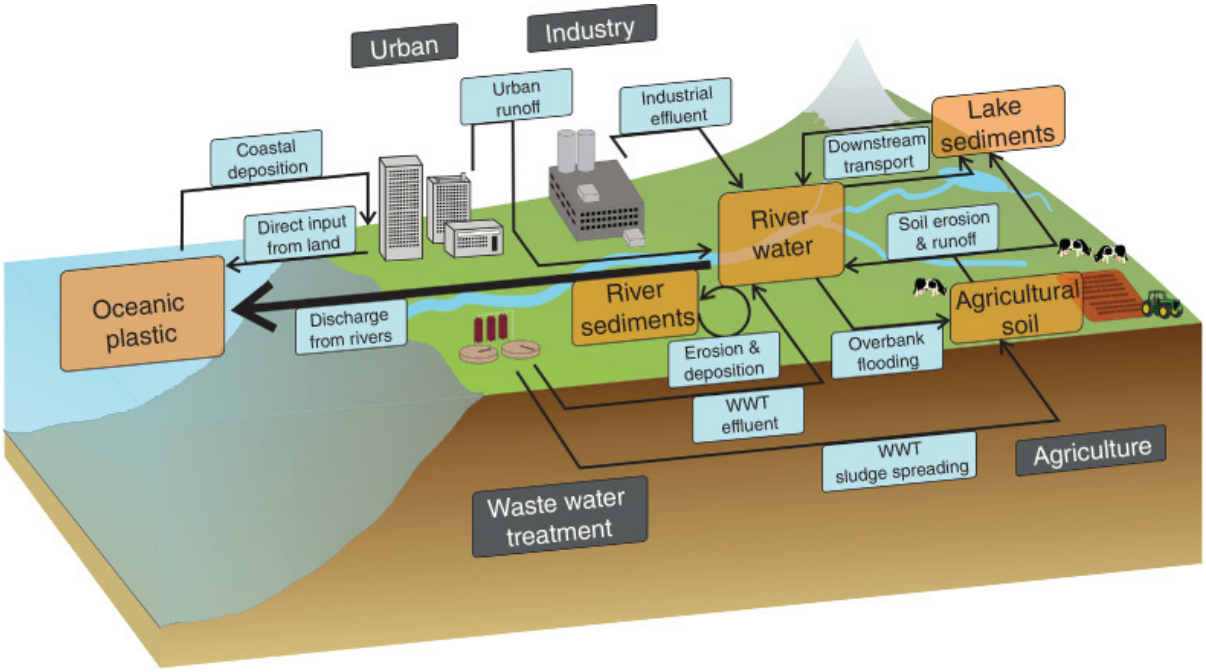

Figure 1.5. First conceptual model of the 'Plastics Cycle' as defined by Horton and Dixon (2018). Orange boxes represent plastic sinks, blue boxes represent transport mechanisms, and arrows represent transport pathways. From Horton & Dixon (2018).

1.8 Microplastics and vegetation

There have been multiple proposed 'sinks' for microplastics such as Arctic sea ice, the deep sea, and biota (Cózar et al. 2014, Woodall et al. 2014), as well as aquatic vegetated areas (Huang et al. 2020, Martin et al. 2020, de Smit et al. 2021, Lloret et al. 2021, Li et al. 2023b). Indeed, microplastics have been quantified in the sediment and canopies of freshwater and coastal macrophytes (Feng et al. 2020, Yin et al. 2021, Hernán et al. 2024). Coastal vegetated habitats (e.g., saltmarshes, mangroves, and seagrasses) are of particular interest because these areas already provide many ecosystem services such as carbon sequestration, storm protection, biodiversity promotion, and water quality enhancement (Barbier et al. 2011, Spalding et al. 2014,

Hagger et al. 2022). The high levels of microplastic accumulation in these habitats has prompted some to suggest this as a novel ecosystem service – the entrapment of microplastics (Sanchez-Vidal et al. 2021, Gallitelli and Scalici 2024, Paduani et al. 2025). Others have argued against this proposition due to the adverse effects plastics can cause to ecosystem functioning. Instead, they contend that plastic entrapment is an unintended consequence of pollution, eventually bringing net negative impacts to these systems (Rangel-Buitrago et al. 2024). Before determining whether the entrapment of plastics in coastal wetlands brings positive or negative outcomes for the planet, another question should be answered first: are these systems truly a sink for microplastics? The presence of microplastics in coastal wetlands is well established – we know they are everywhere. To classify a system as a sink, there must be more microplastics entering a system than exiting (Zhu 2021). Coastal vegetated systems are highly dynamic, experiencing daily and seasonal changes in hydrodynamic energy, biotic communities, and pollution sources (de Deckere et al. 2001, Tolhurst et al. 2002, Möller and Spencer 2002, Lee et al. 2006, Wu et al. 2020, Lloret et al. 2021). Most research in coastal vegetated habitats only quantifies microplastics in one compartment (i.e., sediment) at one time point, and do not compare to relevant unvegetated areas. Because of this discrepancy, it could be that microplastic accumulation in these areas are not due to the presence of vegetation but instead are due to other factors. A few studies have detected increased levels of microplastics in vegetated areas compared to unvegetated ones (Huang et al. 2020, 2023, Jones et al. 2020, Zhao et al. 2022, Pinheiro et al. 2022), but others have not detected a difference (Cozzolino et al. 2020, Unsworth et al. 2021, Wright et al. 2023, Ledet et al. 2024). There are also less than 20 studies quantifying microplastics in saltmarshes to date (Figure 1.6). Therefore, it is unclear if these areas really act as a sink. A more appropriate term may be ‘reservoir’ until we know more about microplastic flux in and out of coastal vegetated systems. A reservoir is a place of storage for a particular substance and could be either a source or a sink of microplastics depending on the level of flux under different conditions and/or at different times (Zhu 2021). Deciphering what could be promoting (or not promoting) microplastics deposition in coastal vegetated systems is necessary to understand the role of vegetation on microplastic behaviour, cycling, and fate.

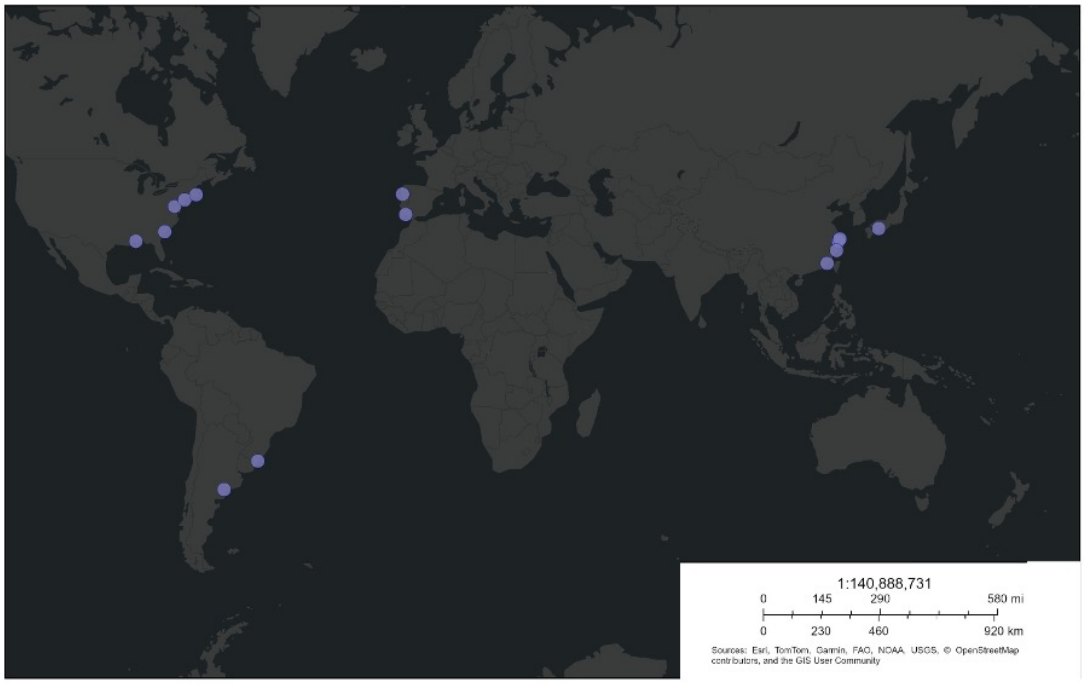

***Figure 1.6*.** Locations of studies investigating microplastics in saltmarsh sediments. Data from Chapter 3 (Table 3.2).

1.9 Aims and objectives

This thesis explores the question "are coastal wetlands a sink for microplastics?" with a focus on saltmarshes. I hypothesize that coastal wetlands are a temporary reservoir of microplastics rather than a sink, and because of the dynamic nature of coastal systems this is dependent on many variables. In pursuit of this goal, I investigate the underlying drivers of microplastic fate in saltmarshes through one laboratory-based study, a methods comparison study, and two field-based studies. Together, my chapters increase our knowledge of the underlying factors affecting microplastic accumulation in coastal wetlands, highlights the transient and dynamic nature of these systems, and contributes to our understanding of coastal wetlands as a microplastic sink:

Chapter 2: Microplastic Shape Influences Fate in Vegetated Wetlands: A Laboratory Investigation

Research Aim: Investigate three factors that could influence microplastic depositional patterns in a simulated coastal wetland: presence of vegetation, structural complexity of vegetation (grassy vs. branched), and microplastic type.

Research Objectives:

i. Use a controlled experimental approach with a flume system to isolate specific variables potentially affecting microplastic deposition.
ii. Quantify microplastics in sediment, on vegetation, and in water across different artificial vegetation scenarios (no vegetation, grassy vegetation, branched vegetation.
iii. Compare microplastic depositional patterns of varying microplastic shapes, sizes, and densities.

Hypothesis: Vegetation type and microplastic characteristics will affect microplastic entrapment and deposition in a vegetated canopy.

Chapter 3: Method Comparisons: Positive Controls with Representative Materials are Essential for the Advancement of Microplastics Research

Research Aim: Demonstrate the importance of method validation tests in microplastics research in an exploratory methods-based study.

Research Objectives:

i. Discuss the value and challenges of method validation tests (i.e., recovery rate tests, spike-recovery tests) in microplastics research.
ii. Use a comparative experimental approach to assess the microplastic recovery rates of different methods for isolating microplastics from saltmarsh sediments and leaf surfaces in the laboratory.
iii. Compare two methods of microplastic characterisation: visual microscopy with micro-FTIR vs. FTIR imaging.

Chapter 4: Exploring Vegetation Complexity as a Driver of Microplastic Accumulation in a North Norfolk, UK Saltmarsh

Research Aim: Explore the influence of vegetation structure and diversity on microplastic deposition in saltmarsh sediments in a field-based investigation.

Research Objectives:

i. Quantify microplastic content in sediments from a North Norfolk, UK saltmarsh across four levels of vegetation diversity: unvegetated, monospecific grass, monospecific branched, and diverse.
ii. Compare the level of microplastic content in saltmarsh sediments between unvegetated and vegetated areas and across areas with increasing vegetation complexity.
iii. Evaluate differences in microplastic characteristics (e.g., shape, size) across saltmarsh sediments with varying types of vegetation cover.

Hypothesis: Vegetation complexity, determined by structural differences and species diversity, will influence underlying sediment microplastic content and composition.

Chapter 5: Saltmarsh Sink: The Role of Saltmarsh Vegetation in Microplastic Flux in the Tamar Estuary, UK

Research Aim: Explore whether microplastics are exported from a saltmarsh by adhering to detrital leaves in a field-based investigation.

Research Objectives:

i. Compare microplastic levels on saltmarsh leaf surfaces across three leaf stages (alive, dead, outflowing detritus) from a saltmarsh in the Tamar Estuary, UK.
ii. Evaluate the relationship between microplastic adherence levels and leaf surface area.
iii. Estimate the possible level of microplastic export via leaf detritus from Tamar Estuary saltmarshes.

Hypothesis: Microplastics can be exported out of vegetated systems via leaf litter because they adhere to vegetation blades.

Chapter Two

Microplastic Shape Influences Fate in Vegetated Wetlands: A Laboratory Investigation

This chapter is a reformatted version of my publication:

McIlwraith, H.K., Lindeque, P.K., Miliou, A., Tolhurst, T.J. and Cole, M., 2024. Microplastic shape influences fate in vegetated wetlands. *Environmental Pollution*, *345*, p.123492. 10.1016/j.envpol.2024.123492

HM conceptualized and carried out the experimental design, investigation and data collection, formal analysis, and writing. MC, TT, PKL, and AM contributed to conceptualization, experimental design, and review and editing.

Coastal areas are prone to plastic accumulation due to their proximity to land based sources. Coastal vegetated habitats (e.g., seagrasses, saltmarshes, mangroves) provide a myriad of ecosystem functions, such as erosion protection, habitat refuge, and carbon storage. The biological and physical factors that underlie these functions may provide an additional benefit: trapping of marine microplastics. While microplastics occurrence in coastal vegetated sediments is well documented, there is conflicting evidence on whether the presence of vegetation enhances microplastics trapping relative to bare sites and the factors that influence microplastic trapping remain understudied. We investigated how vegetation structure and microplastic type influences trapping in a simulated coastal wetland. Through a flume experiment, we measured the efficiency of microplastic trapping in the presence of branched and grassy vegetation and tested an array of microplastics that differ in shape, size, and polymer. We observed that the presence of vegetation did not affect the number of microplastics trapped but did affect location of deposition. Microplastic shape, rather than polymer, was the dominant factor in determining whether microplastics were retained in the sediment or adhered to the vegetation canopy. Across the canopy, microfibre concentrations decreased from the leading edge to the interior which suggests that even on a small-scale, vegetation has a filtering effect. The outcome of this

study enriches our understanding of coastal vegetation as a microplastics sink and that differences among microplastics informs where they are most likely to accumulate within a biogenic canopy.

2.1 Introduction

Plastic debris is found in elevated concentrations in coastal waters, which include highly valued vegetated ecosystems such as mangroves, saltmarshes, and seagrasses (Paduani 2020, Harris et al. 2021, Ouyang et al. 2022). Plastic debris is persistent, harmful, and can break-up into smaller pieces called microplastics (< 5 mm in size), contributing to environmental accumulation and harm (Browne et al. 2007, Cole et al. 2011). Coastal wetlands have many ecosystem functions that benefit both human and wildlife communities, including coastal protection, erosion control, habitat refuge, and carbon storage (Chmura et al. 2003, Barbier et al. 2011, Barbier 2013, Spalding et al. 2014), with vegetation canopies dampening wave action, decreasing turbulence, and promoting sediment deposition (Gacia et al. 1999, Terrados and Duarte 2000, Infantes et al. 2012, Möller et al. 2014). Similarly, coastal vegetation might play a role in the entrapment of macroplastic and microplastic debris (Yao et al. 2019, Waldschläger et al. 2022). Understanding microplastics distribution and interactions in coastal systems is key for protecting these important areas.

Microplastics can enter coastal vegetative habitats from both riverine and marine sources, or by the degradation of trapped macroplastics (Weinstein et al. 2016, Biltcliff-Ward et al. 2022). A number of studies have suggested such habitats may act as a microplastics sink (e.g., Huang et al. 2020, de los Santos et al. 2021, Kreitsberg et al. 2021, Lloret et al. 2021, Navarrete-Fernández et al. 2022), and several field studies have demonstrated vegetated coastal wetlands trap more microplastics than unvegetated sites (Huang et al. 2020, 2023, Jones et al. 2020, Zhao et al. 2022, Pinheiro et al. 2022, Ogbuagu et al. 2022). Yet the mechanisms by which microplastics become entrapped remain poorly elucidated. These studies postulate that because vegetation reduces local turbulence and bed shear stress thereby promoting sediment deposition, the same mechanisms also promote microplastics deposition (Waldschläger et al. 2022). It has also been observed that microplastics can adhere to the vegetation itself (Goss et al. 2018, Jones et al. 2020). However, other field studies have found no difference in microplastic concentration between bare and vegetated sites (Cozzolino et al. 2020, Unsworth et al. 2021, Wright et al. 2023). A meta-analysis by Biltcliff-Ward et al. (2022) found a negligible difference in microplastic concentration between bare and vegetated sites. There are many factors that may influence

whether a difference is observed, such as where samples are collected within a study area. For instance, many studies sample from the dense interior of a canopy (Wu et al. 2020, Cozzolino et al. 2020, Unsworth et al. 2021). It is known, however, that sediment and nonmotile fauna typically accumulate on the edge of a canopy (Leonard et al. 2002, Bologna and Heck 2002). Studies investigating spatial variation of microplastics in coastal vegetation found a similar pattern to sediment deposition – increasing microplastics with proximity to the vegetation edge (Yao et al. 2019, Helcoski et al. 2020, Navarrete-Fernández et al. 2022), although the inverse pattern was observed in one study in Southern Brazil (Pinheiro et al. 2022). Other factors that may influence microplastics accumulation in bare vs. vegetated sites include: nearby population density and proximity to plastic sources; water depth; flow; tidal flux, phase and cycle; bathymetry; sampling and laboratory methodology (Jones et al. 2020, Lloret et al. 2021, Wu et al. 2020).

Given the myriad of drivers that can influence microplastic deposition, some insight can be drawn from well-established sedimentology research (Lofty et al. 2023). There are many additional factors that are known to influence sedimentation that have yet to be tested for microplastics, such as canopy structural complexity, frontal area of vegetation, vegetation species and morphology, local hydrodynamics, and topography (Hendriks et al. 2010, Wilkie et al. 2012, Bouma et al. 2013, Chapman et al. 2015, Chen et al. 2018). Half of all plastic types have a lower density than seawater (Ballent et al. 2013). Due to their low density and slow settling velocity, it is generally assumed that microplastics act most like fine grained sediments (e.g., clay, silt) and organic matter (e.g., leaves, wood, algal debris)(Harris 2020). However, there is a discrepancy in the literature on whether a relationship exists between microplastic abundance and fine sediments and organic matter (Enders et al. 2019). Particle size, shape, and density will affect particle transport and deposition (Waldschläger and Schüttrumpf 2019), and microplastics come in diverse shapes and sizes that vary from sediment grains (e.g., fibres). It is for these reasons that microplastic dynamics are likely different to those for natural sediments (Bridge and Bennett 1992, Horton and Dixon 2018, Mendrik et al. 2023).

Controlled laboratory flume experiments have started to identify the drivers of microplastic entrapment, these include: water flow, plant density, presence of infauna, microplastic polymer type and size, and bed roughness (de los Santos et al. 2021, Ogbuagu et al. 2022, Cozzolino et al. 2022). These studies used live macrophytes taken directly from the environment, which maintains environmental relevance but makes it difficult to isolate individual drivers. For instance, the physical barrier of the plant structure and the epibiont and biofilm coverage on vegetation blades are both factors that could influence microplastics trapping but are difficult to unpack from each other with living plants (de Smit et al. 2021, Ogbuagu et al. 2022). Many of these studies used large industrial pellets as a proxy for microplastics, which are less commonly found in the environment and may act more similarly to fine grained sediments (de los Santos et al. 2021, Harris 2020, Ogbuagu et al. 2022). In contrast, fibres and small particles < 2 mm are more abundant in the environment and are less likely to act like sediment, and so should be incorporated into more studies (Harris 2020, Athey and Erdle 2022).

Using a laboratory flume, we test the hypothesis that the presence and physical structural complexity of vegetation will affect microplastic trapping and that differences in microplastic type will affect their depositional patterns. We predict that more complex vegetation will trap higher loads of microplastics than less complex vegetation. Furthermore, the effect of microplastic shape, size, and polymer type on vegetative interaction and depositional patterns were assessed. The results provide insight on some of the potential variables affecting microplastic trapping in aquatic vegetation canopies and will help to inform potential hotspots of contamination.

2.2 Methods

2.2.1 Flume tank

Experiments were conducted using a closed-loop flume system (Figure 2.1, Armfield Sediment Transport Channel S8 MKII), comprising a linear test section (1.5 m length, 0.08 m width, 0.11 m height). The pump provided a constant mean flow velocity of 14.7 cm/s, consistent with similar flume set-ups (Cozzolino et al. 2022; de Smit et al. 2021). Flow velocities within the flume were measured in triplicate using a flowmeter (Valeport801) at three points in the middle

of the water column: upstream, midstream, and downstream (represented by black dots in Figure 2.1). To ensure microplastics were kept suspended when transporting through the system, two submersible pumps (Boyu FP-350) were inserted into the influent tank and one in the discharge tank (Boyu FP-100; shown in Figure 2.1).

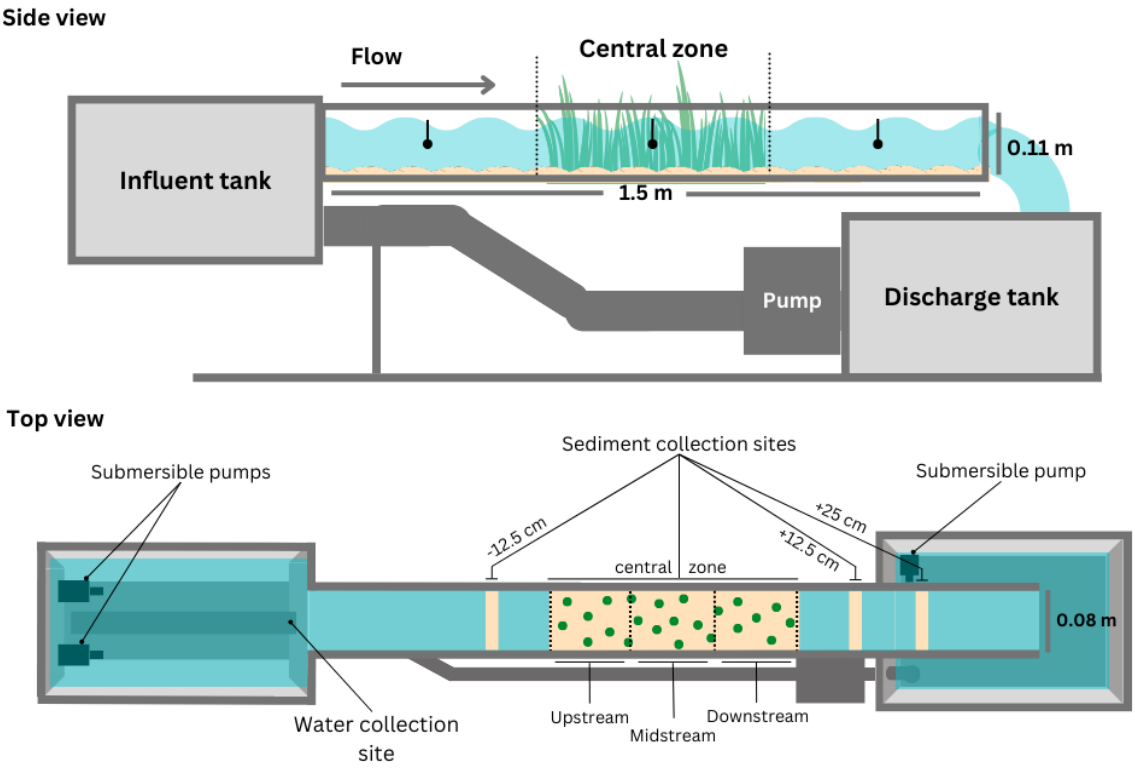

Figure 2.1. Schematic of close-looped flume tank set up with artificial vegetation and sand. Flow velocity was measured at three points represented by black dots in the upper panel (side view). Green dots in lower panel (top view) represent placement of artificial vegetation. Submersible pumps were used to maintain flow of microplastics through the flume tank. Water was sampled throughout the experiment; sediment and vegetation were sampled at the end to quantify microplastic deposition.

2.2.2 Vegetation scenarios

To compare the influence of both the absence and presence of vegetation and the complexity of vegetation on microplastic deposition, three treatments were employed: (1) flat

sand with no vegetation (control, N=3); (2) grassy vegetation (N=3), comprising artificial plants with a 0.2 cm diameter stem and 8 flat blades protruding upwards; and (3) branched vegetation (N=3), comprising artificial plants with a 0.2 cm diameter stem and 6 branches with 12 round leaves each (Figure 2.2, S2.1, S2.2). Artificial plants, constructed from polyethylene, were used in lieu of real vegetation to ensure that all plants were a uniform size and shape and to minimise other biological influences on microplastic behaviour, such as surface biofilms. Surface area of plants was calculated by measuring the length and width of each part of the plant. The stems and branches were assumed to be cylinders, grass blades as rectangles, and rounded leaves as circles. Total surface area was the summation of the stem, branches, and both sides of the leaves and blades. Only the submerged part of each plant was included in the measurements. To maintain the stability of the artificial plants, they were inserted into 2 mm pre-drilled holes within an acrylic sheet (0.08 m long x 0.5 m wide x 3 mm thick), placed on the base of the central area of the flume. The holes were drilled in an irregular pattern but were spread across the length of the acrylic sheet (shown in Figure 2.1). The sheet was placed in the same orientation for each experimental run and 23 plants were inserted for each flume run to achieve a vegetation density of 575 plants m^{-2} which is a density found within 5 m of a saltmarsh edge (Neumeier and Amos, 2006). Acrylic sheets without plant inserts were used during the control runs to account for potential confounds. Prior to each run, 2.4 L of well-sorted sand was added to the flume (mode grain size: 262 µm, measured with a Malvern Mastersizer 2000; Figure 2.3), covering the acrylic sheet to a depth of 2 cm. Only sand was permitted in the flume, rather than silt, because of the nature of the pump set-up. To prevent the accidental introduction of microplastics to the test system, sand was baked at 500°C for a minimum of 4 hours to combust any polymers present.

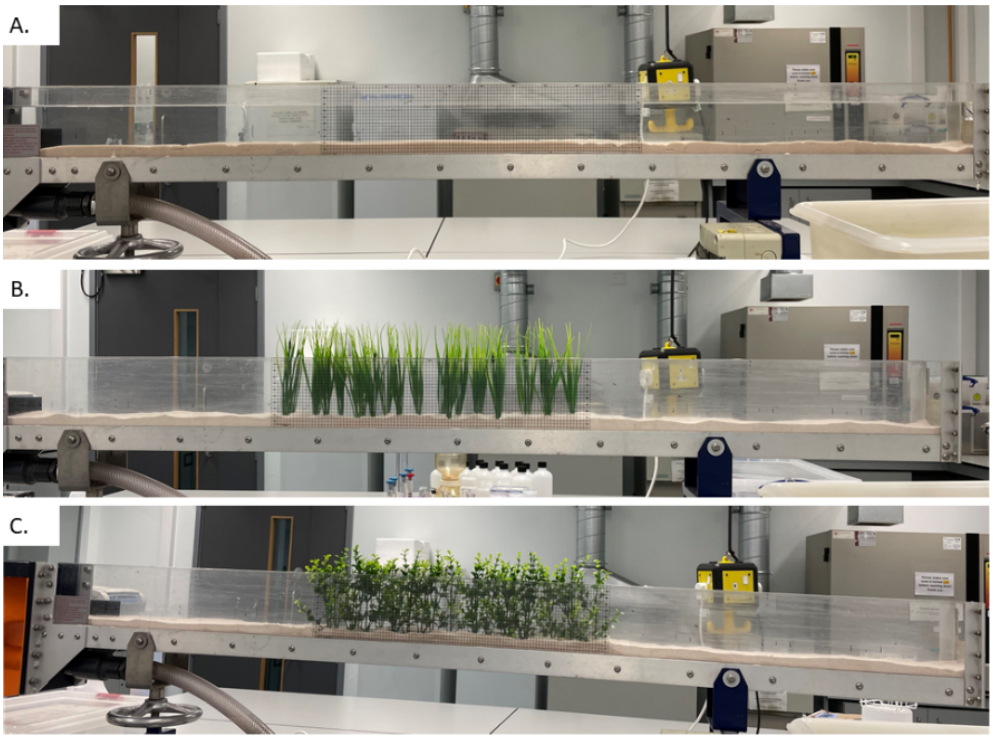

Figure 2.2. Flume tank set-up for control (A), grassy plants (B), and branched plants (C).

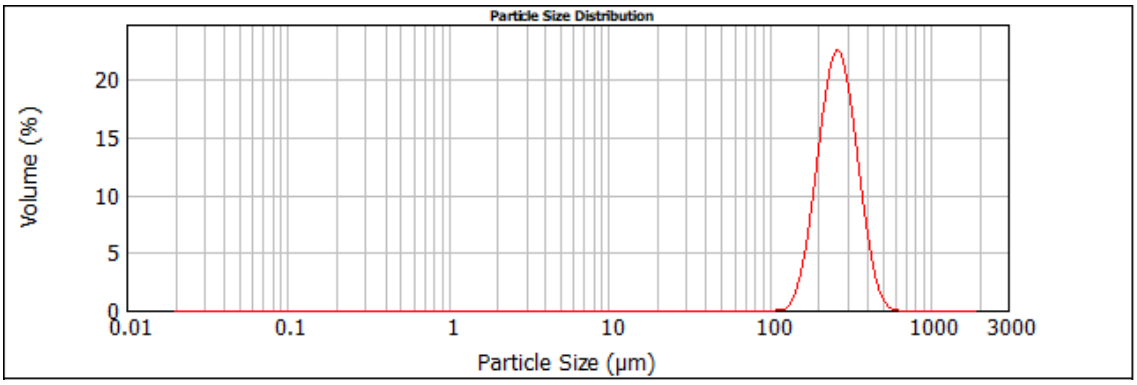

Figure 2.3. Grain size distribution of sand used in the flume, from a Malvern Mastersizer 2000.

2.2.3 Microplastics

To account for the diverse array of microplastics found in environmental samples, the experimental system was spiked with five types of easily identifiable microplastics varying in size, shape, and density (Table 2.1, Figure 2.4). Microplastics were easily identifiable because we chose distinct colours (purple, blue, red, white) that differed from the artificial plants (green). Microplastics were dosed at a concentration of 300 particles L^{-1} for each microplastic type, with

a total microplastic concentration of 1500 particles L^{-1}. While these concentrations exceed environmental concentrations, they ensured that adequate levels of microplastics could be captured within samples.

Table 2.1. Characteristics of microplastics included in the experiment.

Polymer	*Shape*	*Size (µm)*	*Density (g/cm³)*	*Colour*
PVC	Flake	1000	1.38	Purple
PET	Flake	375	1.38	Blue
PET	Fibre	100 – 1000 (median: 610)	1.38	Red
PA6,6	Fibre	100 – 1000 (median: 525)	1.14	Purple
PA6,6	Fragment	1000	1.14	White

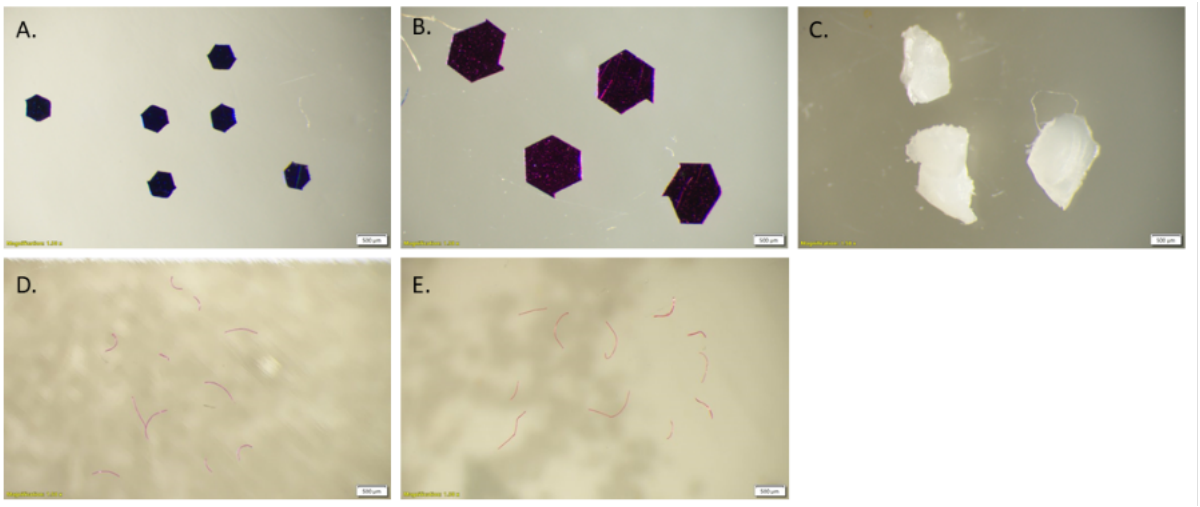

Figure 2.4. Images of microplastics used in the experiment. PET flake (A), PVC flake (B), nylon fragment (C), nylon fibre (D), PET fibre (E).

Polyethylene terephthalate (PET) and nylon (PA6,6) fibres were created by shaving and cutting fleece-like fabrics of each polymer type with a stainless-steel scalpel and scissors. The shavings were rinsed into a glass jar and vacuum filtered through a 1000 µm nylon mesh filter, and the filtrate vacuum filtered through a 100 µm nylon mesh filter. The 100-1000 µm fibres

collected on the mesh filter were rinsed with ultrapure water into a sterile glass jar and stored at room temperature prior to use. Fibre length was ascertained by measuring the length of fibres across five 0.1 mL subsamples of the stock solutions with ImageJ software (version 2.3.0). For PET and nylon (PA6,6) fibres, median length was 610 µm and 525 µm, respectively. Polyvinyl chloride (PVC) and PET flakes and nylon fragments were procured from domestic suppliers, weighed, and added directly to a 300 mL glass jar. Then, aliquots of both fibre types were added to the mixtures.

Pilot tests using microplastics showed particles tended to float, flocculate, and adhere to sides of the flume. Therefore, microplastic mixtures were prepared in advance following a modified protocol from Ramsperger et al. (2020), by incubating them in filtered (2 µm, polycarbonate filter) lake water for 7 days at 30˚C and 100 RPM (VWR Incubating Orbital Mini Shaker) to allow a biofilm to form, though presence of a biofilm was not confirmed. Prior to experimental use, microplastics were filtered out using a 0.45 µm mixed cellulose ester filter, rinsed with ultrapure water, and stored in glass jars with 150 mL of ultrapure water. Following incubation, microplastic readily mixed within the treatment water and flocculation and adherence was visibly reduced.

2.2.4 Experimental runs

For each run, the flume was filled with 30 L of deionized (DI) water and the system turned on for 10 minutes prior to adding any plastics to allow the pump to prime. Microplastics were added to the flume by swirling the 300 mL glass jar containing the particles with 150 mL of DI water and releasing the contents of the jar into the influent tank in the direction of the test section. To track waterborne microplastic concentrations throughout the run, 200 mL water samples were taken five minutes after the addition of microplastics (T_5), with subsequent samples collected every 15 minutes up to one hour (T_{20}, T_{35}, T_{50}, T_{65}). Water samples were collected by using a glass beaker to take dip-samples from the influent tank (adjacent to the entrance of the flume test section; Figure 2.1), which were thrice-rinsed into pre-labelled 300 mL glass jars.

At the end of the run, the pump was turned off and the water was left to drain out for 2 hours, simulating the drainage of an intertidal area on an ebb tide. To determine the deposition of microplastics across the test section, sediment samples were collected: (i) upstream of the central zone (-12.5 cm); (ii) within the central zone; and (iii) at two points downstream of the central zone (+12.5 cm, and +25 cm; Figure 2.1). Samples were collected by scooping-up small sections of the sand (8 cm x 4 cm for upstream and downstream points; 50 cm x 8 cm for the central zone, with the entire depth collected from the sediment surface to the flume base) with a stainless-steel spoon and placing these samples in pre-labelled 300 mL glass jars (Figure S2.3). Due to erosion, transport and deposition during the experiment, depth of sand within and across sections varied from 0 cm – 5 cm depending on location and treatment run. Due to this variation, sediment across the entire central zone was collected. There was less variation in depth in the upstream and downstream points, so smaller sections were collected.

To ascertain whether microplastics were adhering to the surface of the plants, three plants were sampled. For each run, the vegetation section was divided into three equal zones (17 cm x 8 cm with 7 – 8 plants per zone), and a single plant was selected from: (i) the upstream; (ii) midstream; and (iii) downstream areas (Figure 2.1). Each plant was carefully removed from the acrylic base and rinsed thoroughly with DI water over a pre-labelled 300 mL glass jar to collect any adhered microplastics.

Following each run, the flume was thoroughly cleaned by twice flushing the system out with DI water. A negative control test was run three times at random points throughout the study to ensure there was no contamination from previous runs of the flume and to ensure there was no airborne contamination of similar microplastics throughout collection and processing. For these tests, the flume was run without any plastics, sediments, or vegetation and two water samples were taken at 5 and 10 minutes after priming the pump. Samples from the blank runs contained 1 – 3 PA fibres and 0 – 1 PET fibres, and no flakes or fragments were present. Sample processing is described in Supplementary Information.

2.2.5 Sample processing

Water and plant-adherence samples were vacuum filtered across 10 µm nylon mesh filters. Using a modified Computer Numerical Control (CNC) machine fitted with a camera, 24 images of each filter were captured and stitched together using ImageJ software (version 2.3.0). Microplastics were quantified and their longest dimension measured on ImageJ.

Sediment samples were dried at 50˚C overnight and then weighed. A density separation protocol, using a sediment-microplastic isolation (SMI) unit and $ZnCl_2$ (r = 1.45 – 1.5 g/cm^3), was conducted per the method of Coppock et al. 2017. In brief: for each sediment sample, 30 g of dried sediment was collected in a glass beaker and added to the SMI unit. 750 mL of $ZnCl_2$ was added, and the sample mixed with a magnetic stir bar for five minutes, and then the solution left for 1 hour. The top layer of supernatant was vacuum filtered onto 10 µm nylon filters. Filters were imaged with CellSens software and an Olympus SZX16 microscope. Fibres were counted manually under the microscope and all other microplastics were quantified with ImageJ software.

2.2.6 Data analysis

Data was assessed for normality (Shapiro-Wilks Test) and homogeneity of variances (Bartlett's test). When assumptions were met, ANOVA tests with post-hoc Tukey test were used. Alternatively, a Kruskal-Wallis test was used for non-parametric data. We tested whether there was a relationship between vegetation treatment and flow velocity. To compare differences in flow velocity across treatments and flume sections, individual tests were run for each section of the flume tank (i.e., upstream, midstream, downstream). To measure the rate of microplastic deposition across each vegetation treatment, we calculated the microplastic loss rate from the water column by the equation:

$$\frac{Final\ MP\ concentration\ (\mathrm{T}_{65}) - Initial\ MP\ concentration\ (T_5)}{Total\ Time}$$

We tested whether there were effects on the microplastic loss rate from vegetation treatment or microplastic type. Only microfibres were included in the statistical tests because they were consistently present in the water samples, whereas the other microplastic types were not. Microplastics found on the vegetation were reported as microplastics per cm^2. We compared microplastic concentrations across vegetation treatment and across vegetation zone. We also tested a two-way ANOVA with both vegetation treatment and zone as factors. However, Akaike Information Criterion (AIC) model selection distinguished the one-way ANOVA with vegetation zone as a factor was the best fit model. Microplastics in sediment were standardized by microplastics per cm^2 and data was log transformed to maintain normality. We tested the relationship between vegetation treatment, sediment section, and microplastic concentration. We also calculated the percent relative abundance of each microplastic type across matrices and treatments by dividing the sum of each microplastic type by the total number of microplastics within matrix and treatment, multiplied by 100. Data was analysed using R statistical software (version 4.2.1) in an RStudio environment (version 2022.02.0+443), using significance level $p < 0.05$.

2.3 Results

2.3.1 Vegetation effects on water flow velocity

All treatments were characterised by a turbulent flow regime (Table 2.2). The grass treatment showed a decrease in flow velocity within the vegetation patch, whereas the branched treatment showed an increase (Figure S2.4). There was no significant difference in overall flow velocity across treatments ($x^2 = 3.1$, $p = 0.2$, df = 2). However, the flow velocity across treatments measured within the vegetation patch was significantly different between the grass treatment with the control and branched treatments ($F_{2,6} = 55$, $p = 0.0001$; Figure S2.4). Flow velocities across treatments in the upstream ($x^2 = 5.6$, $p = 0.06$, df = 2) and downstream ($x^2 = 2.5$, $p = 0.3$, df = 2) sections of the flume were not significantly different (Figure S2.4).

Table 2.2. Mean flow velocity, Reynolds number, and flow regime for each experimental treatment. Reynolds number was calculated using the equation: $Re = \frac{\rho u L}{\mu}$, where ρ is the density of the fluid, u is flow speed, L is the water depth (6 cm), and μ is the dynamic viscosity of the liquid.

Experimental test	***Mean flow***	***Reynolds number***	***Turbulent or laminar flow***
Control	0.15	6879	Turbulent
Grass	0.14	6420	Turbulent
Branched	0.15	6879	Turbulent

2.3.2 Microplastic concentrations decreased in the water column over time.

Combined PET fibre and PA fibre concentrations decreased over the course of the flume run in all treatments (Figure 2.5; Figure 2.6). The mean ± standard deviation (s.d.) rate of microfibre loss in particles min^{-1} from the water column was 5.5 ± 1.5 for control, 6.2 ± 1.3 for grass, and 5.0 ± 0.9 for branched treatments. There was no significant interaction between the effects of treatment and microfibre type ($F_{2, 12}$ = 0.1, p = 0.9). Furthermore, there was no significant difference in microfibre rate of loss across treatments (p = 0.4) or between microfibre types (p = 0.3; Figure 2.5). The other microplastic types were generally found in low abundance in the water column, and the concentration of flakes in the branched treatment had a large variation at t20 and t35, but no notable pattern was discerned (Figure 2.6). Notably, flakes and fragments were observed to settle out of the water column quickly and travel along the sediment bed by saltation, rolling, and sliding.

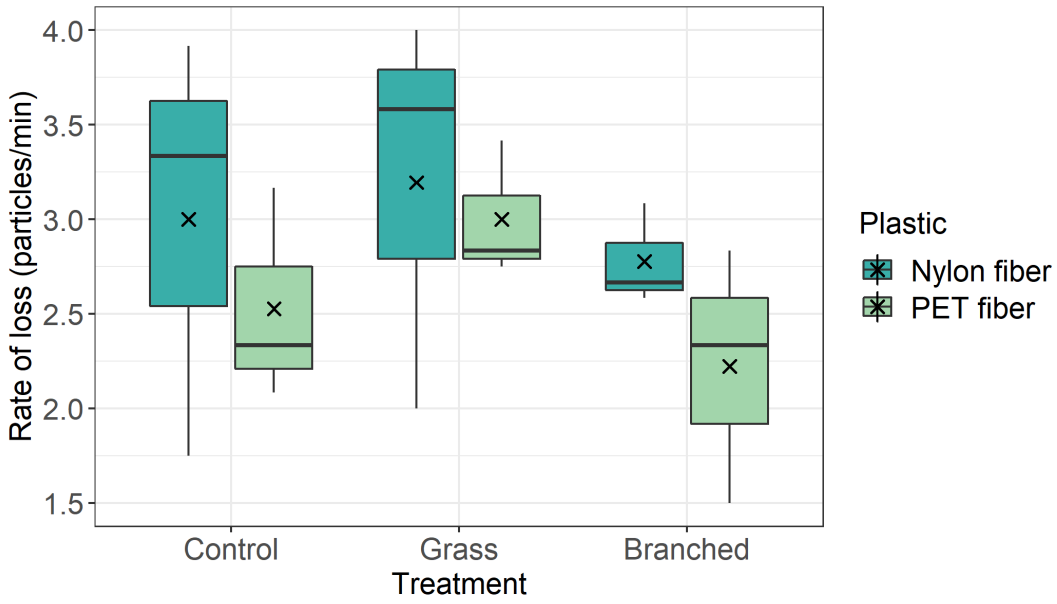

Figure 2.5. Rate of microfibre loss from the water column over time, for each vegetation treatment. Boxes represent the interquartile range (IQR; 25th -75th percentile), horizontal line indicates the median, and whiskers extend to the maximum and minimum values within 1.5x the IQR. X indicates the mean.

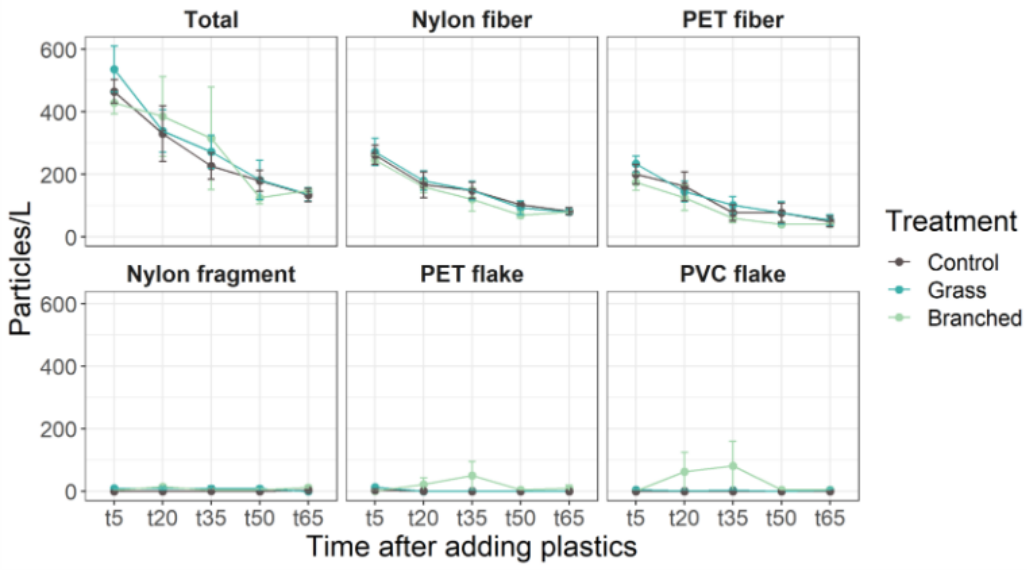

Figure 2.6. Number of microplastics in the water column over the course of the flume run for each treatment and plastic type.

2.3.3 Microplastics were trapped on vegetation blades.

The number of microplastics adhered to the artificial plants did not differ significantly between the two vegetation treatments (Figure 2.7a). Grass and branched plants had a mean ± s.d. of 3.1 ± 1.8 and 3.3 ± 1.7 microplastics cm^{-2}, respectively ($F_{1,16}$ = 0.05, p = 0.8). However, variance of the microplastic concentration was best explained by vegetation zone. There was a significant difference between plants positioned upstream than those positioned downstream (ANOVA: $F_{2,15}$ = 4.7, p = 0.03; Tukey: p = 0.03). Plants positioned in the middle of the patch were not significantly different from the other groups (p > 0.05). However, when both treatment and vegetation zone were included as interacting parameters, neither the interaction ($F_{2,12}$ = 0.3, p = 0.8) nor treatment (p = 0.8) was significant, and vegetation zone (p = 0.05) was borderline significant. The relative abundance of different microplastic types was similar across the two vegetation treatments, but the two types of flakes show a gradual decrease across vegetation zone for the branched treatment, and the grass treatment show an increase of both types of flakes in the midstream section (Figure 2.7b).

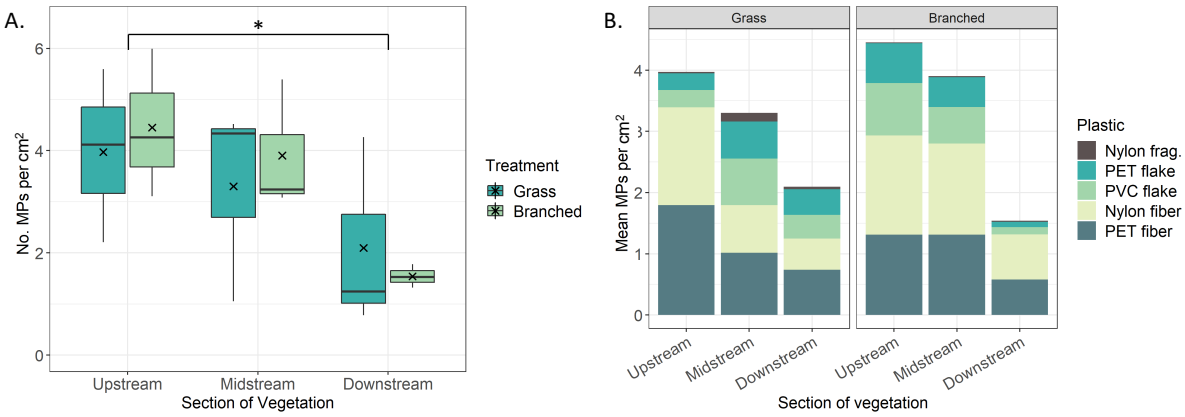

Figure 2.7. (A) Number of microplastics (MPs) per cm^2 adhered to vegetation for each treatment and vegetation section. X represents the mean. Microplastic concentrations differed significantly between the upstream and downstream sections ($F_{2,15}$ = 4.7, p = 0.03), but not by treatment. (B) Mean number of microplastic types found at each vegetation zone and for each treatment.

2.3.4 Depositional patterns of microplastics in sediment varied.

Total microplastic concentration in the sediment was not significantly different across treatments (p = 0.5), although there was more variability in the grass and branched treatments compared to the control (Figure 2.8a). The sediment section where microplastics deposited was significantly different (p = 0.009) and the interaction between treatment and sediment section was significant ($F_{4,18}$ = 5.6, p = 0.001). For the grass treatment, microplastic concentrations between the vegetated central section and sections -12.5 cm upstream and +25 cm downstream of the central sections was significantly different; significant differences were not observed between upstream and either downstream sections, or between the central section and the +12.5 cm downstream section (Figure 2.8a; Table S2.1). In the branched treatment, microplastic concentrations were significantly different between the central section and the +12.5 cm downstream section (Figure 2.8a; Table S2.1). With no vegetation (control), there was a gradual increase in microplastic deposition across the sediment bed with the peak accumulation zone at +12.5 cm downstream and then a decrease at +25 cm downstream, but these differences were not significant (Figure 2.8a).

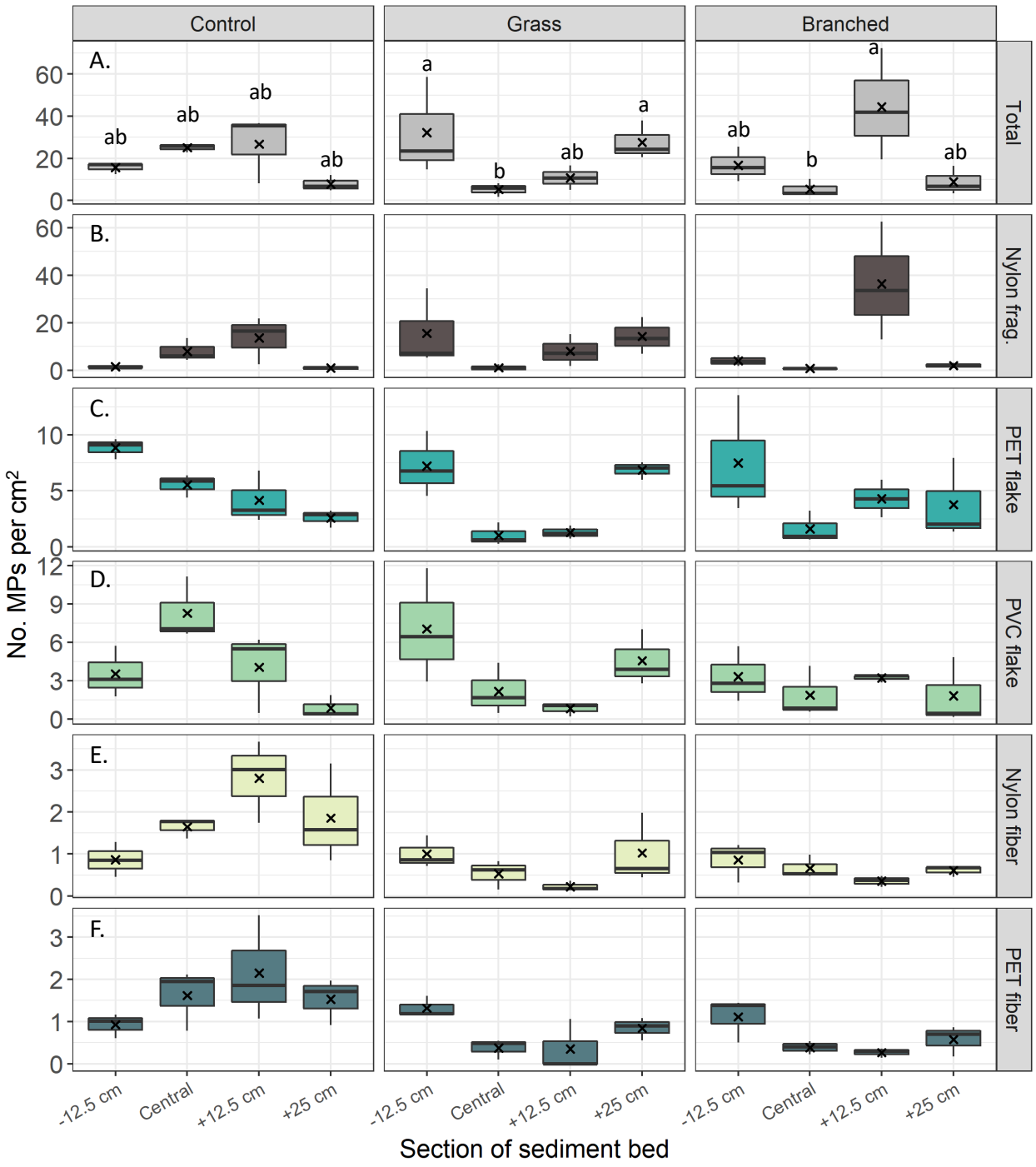

Figure 2.8. Number of microplastics (MPs) per cm^2 in sediment for each vegetation treatment and sediment section, separated by (A) total microplastic and (B-F) individual microplastic type. X denotes the mean and letters denote significance groups based on post-hoc Tukey test.

When microplastic concentrations in sediment are split across microplastic type, the patterns within and across treatments vary (Figure 2.8b-f). Within the control treatment, microplastic fibre and fragment concentrations gradually increased and PET flakes decreased along the sediment bed, while PVC flakes were most abundant in the central section (Figure 2.8). The patterns observed in the grass treatment are largely the same across plastic types. All show a decrease in abundance along the sediment bed until they accumulate again at +25 cm downstream (Figure 2.8). Additionally, the two types of fibres show the same pattern in the branched treatment as in the grass treatment and oppose the pattern observed in the control treatment, whereas the flakes and nylon fragments show different depositional patterns to the grass treatment. All show a decrease in the central section and an uptick in the +12.5 cm downstream position, which is most noticeable with the nylon fragments (Figure 2.8). This increase in abundance in the downstream sections of the vegetation patch was observed during the grass and branched flume runs, where nylon fragments and some PVC and PET flakes were buried within a bedform just after the vegetation (Figure S2.5).

2.3.5 Relative abundance of each microplastic type differed across matrices.

Microplastic concentrations in water, adhered to vegetation, or deposited in the sediment varied depending on the type of microplastic. There was a higher proportion of fibres in the water and adhered to the plants than in sediment. The two types of flakes were mainly found on the vegetation and in sediment and nylon fragments were primarily found in the sediment (Figure 2.9a). Generally, these differences did not change between treatments, though the branched plants did have a higher proportion of the two flakes in the water column than the other treatments (Figure 2.9b). These patterns are grouped mainly by shape (e.g., fibre vs. flake) rather than polymer type (e.g., PET vs. Nylon).

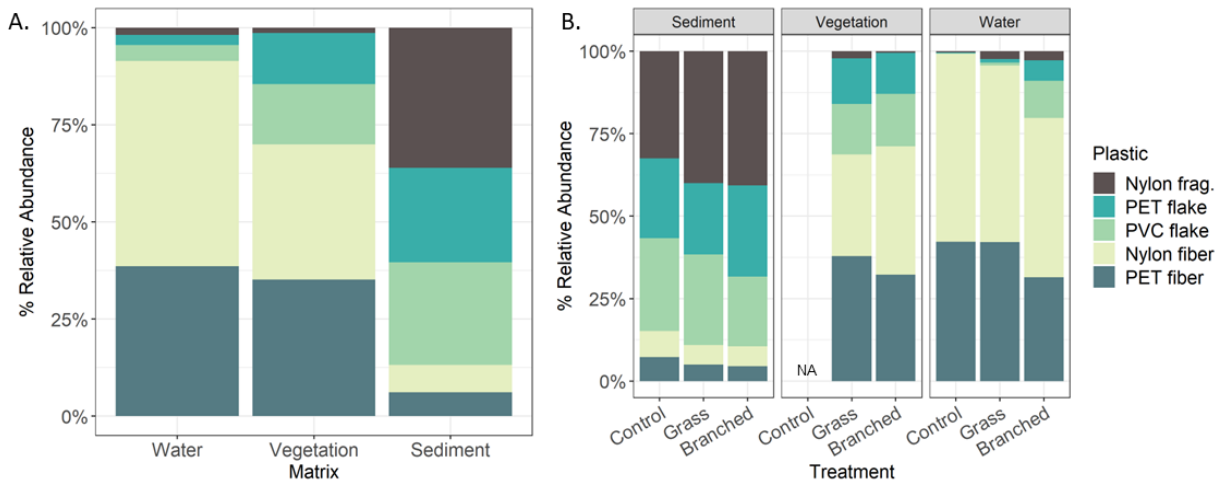

Figure 2.9. Percent relative abundance of microplastic types across (A) matrices and (B) across vegetation treatments.

2.4 Discussion

In this experimental flume study, we observed that microplastic shape was an influencing factor in determining microplastic fate. For instance, a higher proportion of fibres were caught on the above ground canopy than deposited directly in sediment, while the opposite was true for flakes and fragments. Moreover, microfibre concentrations decreased from the leading edge of the vegetation canopy to the downstream end, indicating a filtering effect. Vegetation presence and complexity affected location of microplastic deposition. Burial of microplastics downstream of the vegetation patch was observed in both vegetation treatments but not the control and suggests that accumulation may occur over longer periods of time because of a potential for reduced resuspension.

2.4.1 Vegetated habitats as a microplastic sink.

Microfibres settled out of the water column consistently over time and this decline was unaffected by the vegetation (Figure 2.5). Several studies have considered that plants act to reduce hydrodynamic flow causing microplastics to settle out (Cozzolino et al. 2022, de los Santos et al. 2021, de Smit et al. 2021, Ogbuagu et al. 2022, Waldschläger et al. 2022). In this study, the grass treatment did show a reduction in flow within the canopy, however it may not have been

sufficient to promote higher rates of microplastic settlement as compared to the control treatment. This suggests that increased trapping of microplastics requires other factors in addition to purely vegetation presence. This may include canopy size, canopy density, presence of infauna and epibiont coverage on plant blades, and bed roughness (Cozzolino et al. 2020, de los Santos et al. 2021, Jones et al. 2020, Ogbuagu et al. 2022, Zhao et al. 2022).

While microplastic abundance in the sediment bed did not vary across vegetation treatments, the sites of highest microplastic deposition varied. Typically, highest microplastic concentrations were found prior to or after the vegetated canopies, as compared with the vegetated area itself. Obstacles in flowing water locally alter velocity and generate turbulence, which can cause increased sediment scour (increased erosion) at low obstacle density and skimming flow (less erosion) at high obstacle density (Mayaud et al. 2016). In the flume, we hypothesise that microplastics did not readily settle within the vegetation patches because both types of artificial plant increased flow velocity within the vegetation, and this was observed through increased scour at the base of the plants. At higher plant densities we hypothesize that skimming flow might result in a greater settling of microplastics within vegetation patches. The increased sediment scour is typical for the marsh grass *Spartina alterniflora*, where emergent stems with a narrow base followed by upper branching often create a maximum flow velocity near the bed (Leonard and Luther 1995, Leonard and Croft 2006, Nepf 2012). As we measured flow velocity at a single point in the middle of the canopy and these changes in velocity are small, this maximum velocity would not have been measured. Downstream of the vegetation patch, water velocity decreases in the absence of obstructions, thereby creating a deposition zone outside of the vegetation (Figure S2.5) (Chen et al. 2012, Follett and Nepf 2012). On a larger scale, this phenomenon occurs with grasses aiding the creation of sand dunes (Olson 1958). While the concentration of microplastics in the downstream sections were not significantly different between the control and vegetative treatments, this downstream deposition zone caused by the vegetation resulted in the burial of microplastics. This burial indicates that vegetation could help prevent resuspension of microplastics rather than solely promoting deposition (Figure S2.5). Over time, this may enhance the accumulation of microplastics on the edges of vegetated sediments. However, the experimental flume system used here was limited by using sand and these patterns

could have changed if other sediment types were used, such as clay and silt. For instance, this burial will be different for more muddy sediments which have different properties from sand, such as cohesion, reduced mobility and propensity for flocculation processes that promote microplastic adherence (Murray 1977, Grabowski et al. 2011). Nevertheless, in a field study, Xu et al. (2023b) also found that the reduction in sediment erosion by mangroves was the primary determinant of microplastic abundance rather than sediment accretion. The upstream accumulation of microplastics may be explained by a decrease in turbulent energy as water begins to flow through the vegetation, thereby causing sediments (and microplastics) to settle, and has been observed for sediments along tidal marsh creekbanks and edges (Neubauer et al. 2002, Leonard et al. 2002).

The accumulation of microplastics at the edges of vegetated canopies has been observed in tidal wetlands and mangrove forests (Yao et al. 2019, Helcoski et al. 2020, Duan et al. 2021) and is in accordance with fine sediment behaviour (Soler et al. 2020). However, flow conditions in the field are more complex and multidirectional (e.g., waves), whereas flow within a flume is restricted to unidirectional flow (Tinoco et al. 2020); and the flume used in this study is smaller than previous studies and may be more affected by constraints from flume width (Williams 1970). While flumes are useful for determining specific drivers of microplastic trapping on a fine scale, it is only representative of the parameters used. Across larger spatial and temporal scales, other driving forces may have a greater influence on microplastic trapping than those observed here. For instance, water depth and velocity change across tidal periods creating variable flow patterns and much more dynamic conditions (Neumeier and Ciavola 2004, Tinoco et al. 2020). Sediment composition can also influence microplastic retention (Greenshields et al. 2025). This work is a first step at isolating specific variables (i.e., physical structure of vegetation and microplastic type) that could be driving microplastics trapping in vegetated canopies, though there are many other drivers that can and have been explored (de los Santos et al. 2021, de Smit et al. 2021, Ogbuagu et al. 2022, Cozzolino et al. 2022).

2.4.2 Adherence to vegetation

Branched and grassy artificial plants both had microplastics adhered to their surface, with higher concentrations of microplastics adhered to plants on the leading edge of the canopy than at the downstream end (Figure 2.7). This filtering pattern has been observed in the field with both macroplastics and microplastics (Yao et al. 2019, Stead et al. 2020, Navarrete-Fernández et al. 2022), where some have suggested that these coastal wetlands act as an estuarine 'filter' that mitigates plastic transport into the open marine environment (Biltcliff-Ward et al. 2022). Fibres were the predominant type of microplastic found attached to both types of plants. This is in alignment with what is found in the field, with a review showing microplastics have been identified on 10-100% of aquatic plant canopies and macroalgae sampled, with 76% of the microplastics identified being fibrous (Huang et al. 2023). Additionally, we observed some sand grains adhered to the surface of the artificial plants, usually at the node of where two blades connected or at leaf-stem nodes, suggesting there was a physical interaction and blockage by the artificial plants and the adherence of microplastics to plants was not just due to electrostatic attraction. Physical blockage, entanglement or hydrophobic attraction were likely the primary drivers of trapping in this simulated system, particularly as artificial plants were used. A limitation of this study is that the electrostatic attraction between the microplastics and artificial plants could have increased this adherence, despite attempts to create a biofilm on the microplastic particles themselves. In living plants, microplastics adherence could further stem from entrapment on epibionts (e.g., algae, hydroids, bryozoans) that increase surface area and roughness, microbial biofilms that secrete extracellular polymeric substances (EPS), algal mucus layers with polysaccharide compounds, and root systems that can affect vertical migration of microplastics (Gutow et al. 2016, Sundbæk et al. 2018, Jones et al. 2020, Sfriso et al. 2021, Zhao et al. 2022, Li et al. 2023b). Further work is needed to explore how biofilm coverage on vegetation influences microplastic adherence to vegetation blades. One study investigating road dust particle capture on submerged artificial vegetation found that particle capture increased with particle size, stem density, and biofilm presence (Fauria et al. 2015). Others have found that the submergence level, surface roughness, rigidity and flexibility, and complexity of aquatic canopies can affect trapping (Cozzolino et al. 2020, de Smit et al. 2021). As we used rigid and emergent

stems, the microplastics were 'forced' to flow through and interact with the plants. In contrast, flexible and submerged vegetation may provide more movement and potentially fewer microplastic-plant interactions. If silk, wooden, or live plants were used instead of the synthetic ones used here, potentially fewer microplastics could have adhered due to reduced electrostatic attraction or changes in stem flexibility. Alternatively, more microplastics could have adhered because of increased surface roughness on live plants. This is an area that requires further exploration. Still, the adherence of microplastics to vegetated canopies suggests that only quantifying microplastics in the sediment bed may not tell the full story of microplastic fate and field studies that collect more than one sample type (e.g., sediment, vegetation, water) would be beneficial for comparisons within a geographic area.

2.4.3 Microplastic pathways

Here, we observed differences in microplastic fate primarily based on their shape (Figure 2.9). In the environment, Helcoski et al. (2020) found a higher proportion of fragments in the sediment at the vegetation edge of a tidal wetland as compared to the interior of the vegetation. However, Huang et al. (2020) did not find a difference between microplastic shape in sediment inside and outside a seagrass canopy. A limitation of many existing field studies is that samples are taken from one area of a vegetated bed, so it is difficult to compare whether there are differences in concentrations of microplastic type across a bed. From flume studies, others have found differences based on polymer, noting that polymers with higher densities (PET and Nylon) are more likely to become trapped in a seagrass patch, but they did not compare differences among shapes (de los Santos et al. 2021). In sediments, grain shape does not differ as greatly as microplastic shape (e.g., fragments, fibres, flakes, spheres) and the differences between densities of commonly employed polymer particles (0.9 – 1.5 g/cm^3) is narrower compared to sediment and organic matter particle densities (0.9 – 3.0 g/cm^3; Harris 2020). Due to this variability, microplastic shape may have a more meaningful influence on transport. However, Waldschläger and Schüttrumpf (2019) did not observe a large effect from microplastic shape on erosion thresholds as compared to microplastic densities, but did note that while not statistically tested, spheres and fibres showed particularly different erosion behaviour from other shapes. Another study investigating microplastic dispersion across a German estuary noted that high density

fibres had similar dispersion patterns to non-fibrous low-density polymers (Enders et al. 2019). Salinity is another factor that could have influenced microplastic depositional patterns. Here, freshwater was used rather than salt water, which is more representative of areas higher upstream in an estuary rather than at the mouth of the estuary. Salinity can affect the surface charge and buoyancy of microplastics. For instance, Mendrik et al. (2023) found that salinity increased the settling velocity for higher density microplastics but lowered it for low density particles like polystyrene. These results were also shape specific, with no impact from salinity on the settling velocity of fibres (Mendrik et al. 2023). On a field study scale, Wu et al. (2020) did not find an effect from salinity on microplastic abundance in a vegetated tidal flat. Future studies could investigate how different salinity levels affect microplastic adherence to vegetation and whether there are changes in microplastic depositional patterns within a vegetated canopy.

2.4.4 Conclusions

Shape was the primary factor determining the fate of microplastics in a modelled coastal vegetative system. Presence of vegetation and differences in vegetation structure affected which area of the sediment bed microplastics deposited. Fibres were found in greatest abundance in the water and adhered to plants, whereas flakes were observed adhered to plants and deposited in sediment, and fragments were primarily found in the sediment. This pattern is indicative of how each microplastic shape travelled in this system: fibres were largely transported as suspended load, flakes travelled as suspended load and bedload, and nylon fragments were primarily transported as bedload. Here, we show that microplastic transport and deposition should not be generalised across all plastics, but rather differences in microplastic characteristics will affect where they accumulate and whether they are retained within a vegetated bed.

Chapter Two: Supplementary Information

Table S2.1. Post-hoc Tukey test comparing microplastic concentration in sediment across treatment and section of the sediment bed.

		DIFF	LWR	UPR	P ADJ.
TREATMENT	Grass-Branched	0.1194063	-0.5092415	0.7480541	0.8839559
	Sand-Branched	0.3035107	-0.3251371	0.9321585	0.4613809
	Sand-Grass	0.184104390	-0.444543400	0.8127521 8	0.7475274 1
POSITION	12.5Downstream-Upstream	0.07838488	-0.72347	0.880245	0.992958
	25Downstream-Upstream	-0.51943649	-1.3213	0.282423	0.303811
	Within-Upstream	-0.87997216	-1.68183	-0.07811	**0.02777**
	25Downstream-12.5Downstream	-0.59782136	-1.39968	0.204038	0.196007
	Within-12.5Downstream	-0.95835703	-1.76022	-0.1565	**0.014998**
	Within-25Downstream	-0.36053567	-1.1624	0.441324	0.608176

TREATMENT:POSITION	Grass:Upstream – Branched:Upstream	0.56839	-1.24692	2.383697	0.989851
	Sand:Upstream – Branched:Upstream	0.007395	-1.80791	1.822702	1
	Branched:12.5Downstream – Branched:Upstream	0.927192	-0.88811	2.742499	0.780931
	Grass:12.5Downstream – Branched:Upstream	-0.47372	-2.28902	1.341591	0.997733
	Sand:12.5Downstream – Branched:Upstream	0.357463	-1.45784	2.172769	0.999827
	Branched:25Downstream – Branched:Upstream	-0.77607	-2.59138	1.039237	0.913507
	Grass:25Downstream – Branched:Upstream	0.548294	-1.26701	2.363601	0.992358
	Sand:25Downstream – Branched:Upstream	-0.75475	-2.57006	1.060557	0.92676
	Branched:Within – Branched:Upstream	-1.26832	-3.08363	0.546984	0.374447
	Grass:Within – Branched:Upstream	-1.28254	-3.09785	0.532763	0.359302
	Sand:Within – Branched:Upstream	0.486734	-1.32857	2.302041	0.99714
	Sand:Upstream – Grass:Upstream	-0.561	-2.3763	1.254311	0.99084
	Branched:12.5Downstream – Grass:Upstream	0.358802	-1.4565	2.174109	0.99982
	Grass:12.5Downstream – Grass:Upstream	-1.04211	-2.85741	0.773201	0.646262
	Sand:12.5Downstream – Grass:Upstream	-0.21093	-2.02623	1.604379	0.999999

Branched:25Downstream – Grass:Upstream	-1.34446	-3.15977	0.470847	0.297631
Grass:25Downstream – Grass:Upstream	-0.0201	-1.8354	1.795211	1
Sand:25Downstream – Grass:Upstream	-1.32314	-3.13845	0.492167	0.318057
Branched:Within – Grass:Upstream	-1.83671	-3.65202	-0.02141	**0.045609**
Grass:Within – Grass:Upstream	-1.85093	-3.66624	-0.03563	**0.042891**
Sand:Within – Grass:Upstream	-0.08166	-1.89696	1.733651	1
Branched:12.5Downstream – Sand:Upstream	0.919797	-0.89551	2.735104	0.788828
Grass:12.5Downstream – Sand:Upstream	-0.48111	-2.29642	1.334196	0.99741
Sand:12.5Downstream – Sand:Upstream	0.350068	-1.46524	2.165375	0.999858
Branched:25Downstream – Sand:Upstream	-0.78346	-2.59877	1.031842	0.908581
Grass:25Downstream – Sand:Upstream	0.540899	-1.27441	2.356206	0.993145
Sand:25Downstream – Sand:Upstream	-0.76214	-2.57745	1.053162	0.922322
Branched:Within – Sand:Upstream	-1.27572	-3.09102	0.539589	0.366528
Grass:Within – Sand:Upstream	-1.28994	-3.10525	0.525368	0.351566
Sand:Within – Sand:Upstream	0.479339	-1.33597	2.294646	0.997491
Grass:12.5Downstream –	-1.40091	-3.21621	0.414399	0.247832

Branched:12.5Downstream				
Sand:12.5Downstream – Branched:12.5Downstream	-0.56973	-2.38504	1.245577	0.989664
Branched:25Downstream – Branched:12.5Downstream	-1.70326	-3.51857	0.112045	0.079923
Grass:25Downstream – Branched:12.5Downstream	-0.3789	-2.1942	1.436409	0.999699
Sand:25Downstream – Branched:12.5Downstream	-1.68194	-3.49725	0.133365	0.087163
Branched:Within – Branched:12.5Downstream	-2.19551	-4.01082	-0.38021	**0.009027**
Grass:Within – Branched:12.5Downstream	-2.20974	-4.02504	-0.39443	**0.008447**
Sand:Within – Branched:12.5Downstream	-0.44046	-2.25576	1.374849	0.998803
Sand:12.5Downstream – Grass:12.5Downstream	0.831178	-0.98413	2.646485	0.87275
Branched:25Downstream – Grass:12.5Downstream	-0.30235	-2.11766	1.512952	0.999966
Grass:25Downstream – Grass:12.5Downstream	1.02201	-0.7933	2.837316	0.670998
Sand:25Downstream – Grass:12.5Downstream	-0.28103	-2.09634	1.534273	0.999984
Branched:Within – Grass:12.5Downstream	-0.79461	-2.60991	1.0207	0.90084

Grass:Within – Grass:12.5Downstream	-0.80883	-2.62413	1.006478	0.890402
Sand:Within – Grass:12.5Downstream	0.96045	-0.85486	2.775756	0.744027
Branched:25Downstream – Sand:12.5Downstream	-1.13353	-2.94884	0.681774	0.532215
Grass:25Downstream – Sand:12.5Downstream	0.190831	-1.62448	2.006138	1
Sand:25Downstream – Sand:12.5Downstream	-1.11221	-2.92752	0.703094	0.558733
Branched:Within – Sand:12.5Downstream	-1.62579	-3.44109	0.189521	0.109049
Grass:Within – Sand:12.5Downstream	-1.64001	-3.45531	0.1753	0.103099
Sand:Within – Sand:12.5Downstream	0.129271	-1.68604	1.944578	1
Grass:25Downstream – Branched:25Downstream	1.324364	-0.49094	3.13967	0.31686
Sand:25Downstream – Branched:25Downstream	0.02132	-1.79399	1.836627	1
Branched:Within – Branched:25Downstream	-0.49225	-2.30756	1.323054	0.996852
Grass:Within – Branched:25Downstream	-0.50647	-2.32178	1.308833	0.996001
Sand:Within – Branched:25Downstream	1.262804	-0.5525	3.07811	0.380416
Sand:25Downstream – Grass:25Downstream	-1.30304	-3.11835	0.512263	0.338097

Branched:Within – Grass:25Downstream	-1.81662	-3.63192	-0.00131	**0.049721**
Grass:Within – Grass:25Downstream	-1.83084	-3.64614	-0.01553	**0.046777**
Sand:Within – Grass:25Downstream	-0.06156	-1.87687	1.753747	1
Branched:Within – Sand:25Downstream	-0.51357	-2.32888	1.301734	0.99551
Grass:Within – Sand:25Downstream	-0.52779	-2.3431	1.287512	0.99438
Sand:Within – Sand:25Downstream	1.241484	-0.57382	3.05679	0.403944
Grass:Within – Branched:Within	-0.01422	-1.82953	1.801085	1
Sand:Within – Branched:Within	1.755057	-0.06025	3.570363	0.064515
Sand:Within – Grass:Within	1.769278	-0.04603	3.584585	0.060781

Figure S2.1. Examples of branched (left) and grassy (right) individual shoots.

Figure S2.2. Comparison of artificial set-up vs. live plants from a saltmarsh on the North Norfolk coast.

Figure S2.3. Example where a sand sample was collected from the flume.

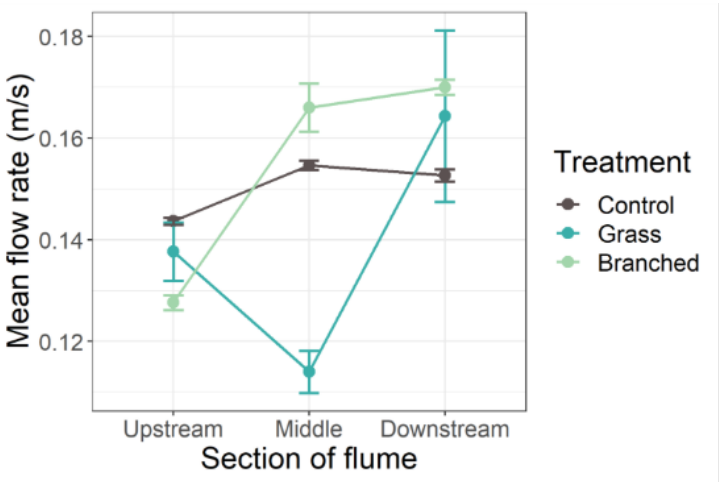

***Figure S2.4*.** Mean with standard error of flow velocities across the flume tank for each treatment.

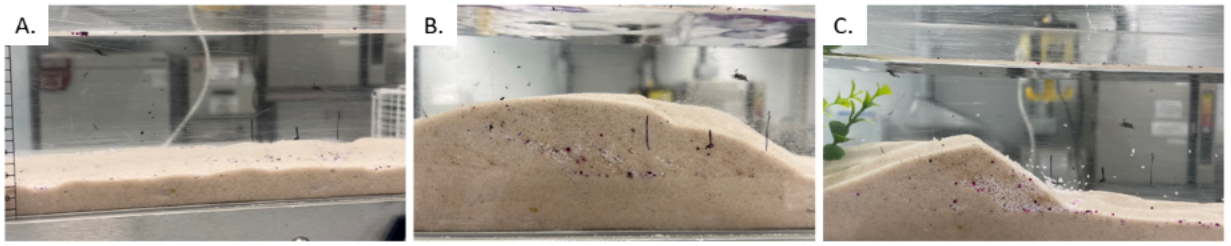

***Figure S2.5*.** Microplastic burial in (A) control (B) grass and (C) branched treatments.

Chapter Three

Method Comparisons: Positive Controls with Representative Materials are Essential for the Advancement of Microplastics Research

This chapter is a reformatted version of my publication: **McIlwraith, H.K.,** Lindeque, P.K., Tolhurst, T.J. and Cole, M., 2025. Positive controls with representative materials are essential for the advancement of microplastics research. *Microplastics and Nanoplastics*, *5*(1), p.9. 10.1186/s43591-025-00115-y

HM conceptualized and carried out the experimental design, investigation and data collection, formal analysis, and writing. MC, PKL, and TJT contributed to conceptualization, experimental design, and review and editing.

Reporting accurate microplastics concentrations across environmental matrices is imperative for robust monitoring and regulation. However, recovering microplastics from complex matrices, such as soils and sediments, is hugely challenging. Numerous methods have been published to facilitate microplastics extraction from such matrices, but these protocols typically lack validation of microplastic recovery efficiency. We argue that environmentally realistic microplastic recovery rate experiments must be utilized consistently to increase the validity of microplastics pollution research, particularly for studies focused on complex matrices. Here, we outline the importance of harmonized recovery rate tests and demonstrate this experimentally using saltmarsh sediments and leaf surfaces as a case study. Building-upon established protocols, an iterative approach was used to test the recovery of four types of environmentally relevant microplastics across 24 protocols (Part 1). Microplastics included polypropylene (PP) fragments, polyethylene (PE) films, polyamide (PA) fibres and polyester (PET) fibres ranging in size from 180 – 1060 µm (min. 5 of each added per sample). Two protocols attaining >50% microplastic recovery were then optimized to attain maximal recovery of all plastic types and replicated (n = 3) to determine precision. They were compared to a well-used microplastic isolation method (n = 3; Part 2). We also compared three recovery rate tests for microplastics on saltmarsh leaf surfaces using traditional micro-FTIR methods vs. semi-

automated FTIR imaging (n = 3; Part 3). Most methods demonstrated efficient removal of organic and inorganic materials with reasonable recovery rates for fragments and films, but many methods failed to sufficiently recover fibres. For sediments, recovery ranged from 40 – 100% for fragments, 20 – 100% for films, and 0 – 120% for fibres. On leaf surfaces, recovery ranged from 75 – 366% for fragments, 100 – 183% for films, and 0 – 100% for fibres. The overestimated recovery rates were from the FTIR imaging method. This underscores the need for environmentally representative reference microplastics for method validation. Owing to the differences and complexities across environmental matrices, the standardization of microplastic extraction methods is unlikely. Therefore, recovery rate experiments with representative reference microplastics should be a requirement to increase quality, harmonization, and comparability.

3.1 Introduction

Microplastics pollution has garnered widespread attention in the public and governmental sectors, with their presence reported across all environmental compartments, food webs, and in human tissues (Horton and Dixon 2018, Carbery et al. 2018, Kutralam-Muniasamy et al. 2023). This level of concern has resulted in a United Nations resolution to end plastic pollution (UNEA resolution 5/14), initiating intergovernmental negotiations to create the first legally binding instrument on plastic pollution (UNEP 2022). As governments discuss and implement these policies, it will be necessary to have continual monitoring of microplastic contamination across environmental compartments. This will aid assessments of risk to ecosystems and evaluate the efficacy of management programs (Lusher et al. 2021, McIlwraith et al. 2023). Scientifically robust analytical methods are essential for ensuring quality of reporting and to maintain trust and reliability of the microplastics research field.

The harmonization of methods for microplastics analysis is an ongoing task. Interlaboratory comparison (ILC) studies comparing the performance and replicability of methods have found large variability in microplastic recovery across laboratories (Isobe et al. 2019, Müller et al. 2020, Cadiou et al. 2020, van Mourik et al. 2021, De Frond et al. 2022, Martínez-Francés et al. 2023, Thornton Hampton et al. 2023). Notably, these tests almost exclusively focus upon laboratory methodology, however errors can be introduced at any point in the microplastics quantification procedure: environmental sampling, sample processing, enumeration, and identification (Brander et al. 2020, Lusher et al. 2020a, Weber and Kerpen 2023). Due to the variation and complexity of differing environmental matrices researchers may not be accurately reporting the number and types of microplastics present in the environment. This is particularly problematic for more complex matrices, such as sewage sludge, soil, fine estuarine sediment, or sediments with high organic content, that require multiple processing steps (Lusher et al. 2020a). Increased processing steps and exposure time to equipment and to airborne microplastics in the laboratory and field may result in a higher risk of procedural contamination and of microplastic loss (Isobe et al. 2019, Prata et al. 2021, Dimante-Deimantovica et al. 2022). Weber and Kerpen (2022) highlight how data extrapolation from minor inaccuracies arising during processing can lead to inaccuracy by under- and over

representation in reported results (Weber and Kerpen 2023). As such, it is imperative that studies are transparent in reporting their methodologies and consider quality control and assurance criteria (Brander et al. 2020, Cowger et al. 2020a, Lusher et al. 2020a, 2021).

Many efforts have been made to limit the level of overestimation of microplastics contamination, through negative controls (i.e., procedural blanks), appropriate washing and handling procedures, and operating in clean laboratories (Brander et al. 2020, Prata et al. 2021, Munno et al. 2023). However, positive controls (i.e., recovery rate or spike recovery experiments) used to evaluate method accuracy are not regularly reported across the literature, despite many proposing this as a necessary requirement (e.g., Quinn et al. 2017, Lares et al. 2019, Möller et al. 2020, Brander et al. 2020, Cowger et al. 2020a, Provencher et al. 2020, Primpke et al. 2023, Munno et al. 2023). Historically, studies comparing laboratory methods have predominantly focused on evaluating mass reduction of samples via effective removal of organic and inorganic materials and on minimal adverse impacts on polymers rather than microplastic recovery (Möller et al. 2020, Brander et al. 2020, Lusher et al. 2020a, Primpke et al. 2023). Without appropriate measures to evaluate microplastic recovery, such as positive controls, microplastic concentrations can be considerably underestimated (Way et al. 2022, Prata et al. 2024). Moreover, biases in the recovery rates of different types of particles may result in an underestimation and misrepresentation of the particle shapes, polymers or sizes present in environmental samples. For example, environmental microplastic concentrations increase as microplastic size decreases, but small microplastics (<1 mm) have highly variable recovery rates in ILCs (Isobe et al. 2019, Lindeque et al. 2020, De Frond et al. 2022, Thornton Hampton et al. 2023). The variation in microplastics processing and recovery makes across-study comparisons difficult and thus it is harder to evaluate the level of microplastic contamination in the environment.

In this perspective, we argue that recovery rate experiments with harmonized reference materials are necessary for comparability and validation of microplastics studies, particularly when dealing with complex matrices. Using saltmarsh sediments as a case study, we highlight the difficulty in relying on protocols suitable for other types of sediments, and the importance of

validating select methodologies by using a diverse array of representative microplastics in a spike-recovery experiment. We also compare the recovery efficiencies of three methods for isolating, quantifying, and characterising microplastics from leaf surfaces, an emerging matrix of interest but with minimal established laboratory procedures.

3.2 Principle of recovery rate experiments

3.2.1 Value of recovery rate experiments

A spike recovery experiment consists of dosing or 'spiking' a sample matrix with known amounts and types of analyte (e.g., microplastics of varying shapes, sizes, and polymers). The sample matrix is then processed using the protocol of interest and comparisons are made between the amount of analyte recovered and the original amount of analyte added. This test evaluates the efficiency of a protocol in recovering the analyte of interest. Use of spike recovery tests can help overcome four key challenges:

(1) Methodological inconsistency: Despite many studies using common methods to extract microplastics in the laboratory, not all methods can be used for every sample type due to differences in sample complexity (Lusher et al. 2020a). Some sample types require more steps than others, different digestion solutions based on the type of organic matter (e.g., KOH for fatty tissues vs. H_2O_2 for vegetal matter), different density separation set-ups, or different sample sizes (Möller et al. 2020, Lusher et al. 2020a). Moreover, there will always be variations in equipment or materials used, with accessibility, cost and resources all being key factors. Researchers may slightly adjust methods to fit with what equipment or materials they have available. Even slight adjustments, such as vacuum filtration vs. sieving, may affect recovery rates of microplastics (Dimante-Deimantovica et al. 2022). Differences in personnel expertise may also affect recovery rates (Piccardo et al. 2022).

(2) Sample inconsistency: Individual sample properties can vary within a matrix. For example, sediments have large variability in grain size and organic content, and this can differ within one sampling area (Bläsing and Amelung 2018, Möller et al. 2020). These variations can affect microplastic recovery efficiency (Cashman et al. 2020, Radford et al.

2021, Monteiro and Pinto da Costa 2022, Weber and Kerpen 2023). Thus, even if the exact same extraction methods with the same materials, analyst and equipment were used for one sample type (e.g., sediments), recovery rates may still differ due to individual sample properties (Way et al. 2022). By including a recovery test of one method across sediment types, researchers can inform how efficient the method works across each one. With this information, results could be corrected based on that efficiency if needed, while maintaining comparability with harmonized extraction methods.

(3) Mass reduction does not equate to microplastic recovery: Studies quantifying microplastics in the environment often use an extraction method from a previously validated study, where the original research had high recoveries of microplastics. However, many studies rarely repeat the recovery rate test on their own samples, often finding that the validated method works well at reducing their own sample mass and is therefore appropriate for their work. As we highlight in our case study, mass reduction does not always correlate with recovery rates. One issue is that fibres and small particles have similar sizes and densities to fine sediment and organic matter so methods that reduce all fine sediment/organic matter may result in microplastic underestimation (Kane and Clare 2019, Harris 2020). There is a balance that must be met between sediment reduction – to be able to visualize the microplastics - and microplastic recovery within the sample.

(4) Comparative studies: A wide range of laboratories across the globe have contributed to microplastics research, developing an array of methods that are affordable and attainable to a given laboratory. Harmonized recovery rate experiments would ensure these diverse methodologies can all contribute comparable data for environmental microplastic monitoring and research. By including recovery rate tests as part of a harmonized protocol, we can maintain accessibility and inclusivity to microplastics research while allowing for comparability across method variations.

3.2.2 Challenges of recovery rate experiments

Despite their value, a meta-analysis of microplastic literature by Way et al. (2022) found only 8% of studies included recovery rate tests, and many failed to include representative microplastics or describe their methods in a replicable manner (Way et al. 2022). The challenges associated with conducting these validation steps largely relate to using reference microplastics that are representative of those found in the environment. Microplastics are a diverse contaminant suite varying in polymer type, shape, size, colour, and additive content (Rochman et al. 2019). These properties can affect microplastics recovery. For example, microplastics <212 µm have been found to be difficult to extract from complex matrices, likely due to microplastic loss from multiple processing steps and difficulty visualizing particles in dirty samples (Thornton Hampton et al. 2023). Weathering of microplastics in the environment can also affect their identification in samples because of changes in colour and surface properties (ter Halle et al. 2017).

Reference microplastics can be difficult to source and researchers often create their own microplastics, each choosing different polymers, shapes, and sizes. One proposed solution is to use pre-made soda tablets or capsules with known amounts and types of microplastics that researchers can use to spike their sample matrix (Dehaut et al. 2023, Martínez-Francés et al. 2023), but even these come with their own challenges. For instance, in an ILC study comparing candidate reference materials (RMs), gelatin capsules did not dissolve completely when added to different matrices, the creation of tablets containing fibres was time consuming, and the relative standard error of tablets often increased as the size of added particles decreased (Martínez-Francés et al. 2023).

Another difficulty of recovery rate tests is discriminating between the spiked microplastics and environmentally present microplastics in the sample matrix. One option is to remove environmental microplastics from substrates, via density separation, combustion, or chemical digestion, before spiking with reference microplastics. However, these processes are highly labour intensive and might impact the results by changing sediment properties that influence microplastics recovery (e.g., organic matter content (Radford et al. 2021)). Another

option is to cite a previous study that used a simplified but non-representative matrix of the main study (e.g., sand) or perform their own recovery tests on this simplified matrix and apply the recovery rate to a more complex sample type (e.g., mud). There is scope for this type of approach (it makes sense to use the same method as a previous study for comparability), however, without recovery validation in the 'new' or different matrix, it is likely that microplastics extraction efficacies will differ significantly among sample types.

3.3 A case study of saltmarsh sediments and leaf surfaces

To demonstrate the necessity of representative recovery rate experiments we conducted a case study of microplastics recovery from saltmarsh sediments (Part 1 and 2). Saltmarsh sediments are complex matrices comprising a high proportion of clays and silts, with a high organic content and small particulate size. Extraction of microplastics from such complex sediments is challenging, typically requiring multiple processing steps that can lead to higher rates of contamination and microplastic loss (Lusher et al. 2020a, Prata et al. 2021, Dimante-Deimantovica et al. 2022). Therefore, it is important that studies validate the laboratory methods used to ensure the results are representative of microplastics in the environment.

We also tested recovery rates of microplastics from leaf surfaces (Part 3), a matrix that has largely been overlooked. Microplastics on vegetation surfaces is an emerging topic, and as such, there are no standard practices for isolating adhered microplastics. Studies on coastal vegetation have primarily determined microplastic presence on vegetation blades by observing the plant surface under a microscope and picking out putative microplastics directly off the plant (Cozzolino et al., 2020, Jones et al., 2020). Other studies on terrestrial plants have soaked leaves in ultrapure water followed by ultrasonic cleaning (Perera et al. 2024). After isolating microplastics from a sample matrix, microplastics must be identified and chemically characterised, which can also differ across studies. The most common techniques employed are visual identification with optical microscopy followed by FTIR (Cowger et al. 2020b). However, there are also semi-automated methods that exist to identify and characterise microplastics via FTIR imaging (Primpke et al. 2017). FTIR imaging scans the whole sample which avoids operator bias and the time-consuming step of manual sorting (Primpke et al., 2017). This method has not

yet become commonly employed across microplastics research, and this is likely due to the low amount of sample that can be processed, often requiring sub-sampling and relatively 'clean' filters to avoid particles obscuring others underneath (Lusher et al., 2020). Whichever method is used, a method validation test must be employed to estimate the recovery efficiency of a protocol. This allows for better comparison across studies and is an important step in the quality assurance and quality control of the research.

3.4 Methods

3.4.1 Literature review methods

We searched the peer-reviewed literature for studies focused on microplastics in saltmarsh sediments to evaluate whether and how method validation tests were used. Using Web of Science (all databases) and Google Scholar, we used the search terms "microplastic* AND "tidal marsh" OR "salt marsh" OR "saltmarsh". Only studies that sampled and quantified microplastics from within the sediment of a vegetated tidal marsh were included in this assessment. In total, 15 studies were recorded.

3.4.2 Part 1: Initial test protocols for sediment

We tested 27 protocols to isolate microplastics from saltmarsh sediments. Tests #1-24 were part of an iterative approach (Part 1) to find a method that recovered over 70% of a given microplastic type and were often only tested once, if recovery was low (Table S3.1). Tests #25-27 were used to determine the efficacy and precision of optimized protocols and are highlighted in the 'Method comparison for sediments' section (Part 2). A description of each tested protocol is available in Table S3.1. In brief, protocols included variations on methods described in the literature (e.g., Crichton et al. 2017, Quinn et al. 2017, Coppock et al. 2017, Pfeiffer and Fischer 2020, Bellasi et al. 2021, Monteiro and Pinto da Costa 2022). This includes density-separations or oleophilic extractions, using sediment-microplastic isolation (SMI) units or centrifugation with $ZnCl_2$, NaBr or rapeseed oil to separate microplastics from inorganic sediment. SMI units are custom-built apparatuses made of PVC piping and a ball valve designed to isolate microplastics in a single step, developed by Coppock et al. (2017) (Figure 3.1). In addition, chemical digestions

using NaClO, Fenton's and H_2O_2, were tested to remove organic material from the sample. Protocols were adjusted with the aim of achieving both sample mass-reduction, to facilitate visualization of microplastics, and high recovery rates for all microplastic types. All tests were conducted in a positive-pressure laboratory under a laminar flow hood to minimize contamination.

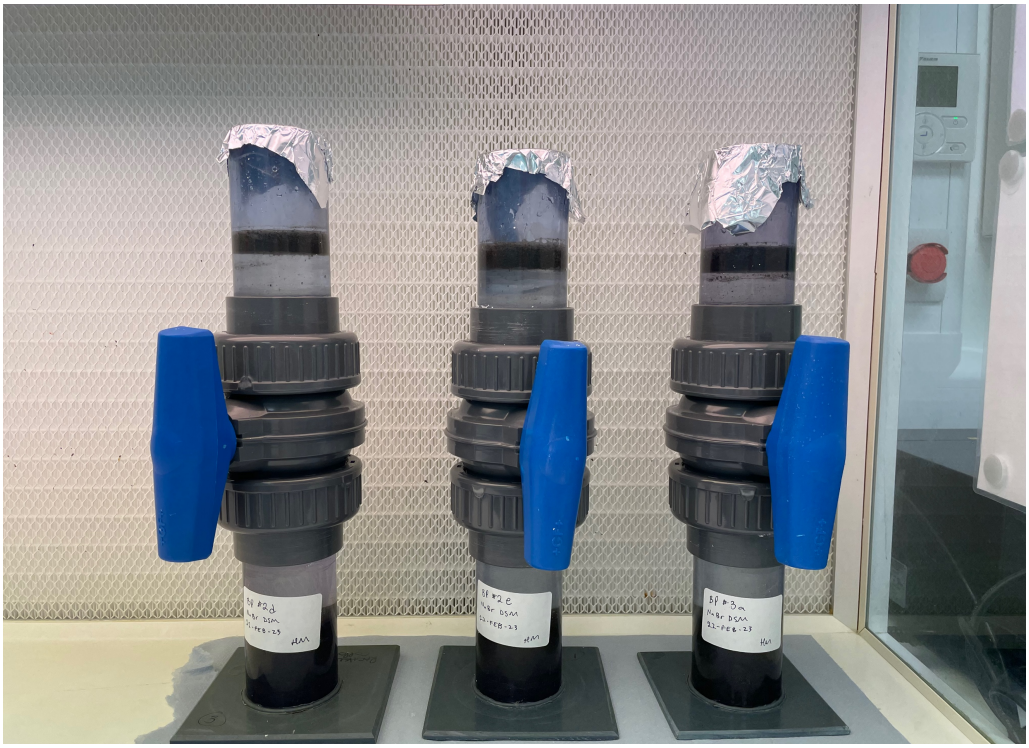

Figure 3.1. NaBr density separation of saltmarsh sediments using a Sediment-Microplastic Isolation unit (Table S3.1; test #2).

Test sediment was collected from two sites: tests #1-8 used sediment from a saltmarsh located on the north Norfolk coast, United Kingdom (52°57'34.7"N 1°01'04.8"E) and the remaining tests used sediment from a saltmarsh in the Tamar estuary, United Kingdom (50°23'18.6"N 4°18'30.0"W). Properties of sediment from each marsh were similar, with mean grain size of 11.4 µm ± 0.29 SE and total organic matter content of 11.6% ± 0.19 SE for the north Norfolk coast site and mean grain size of 11.3 µm ± 0.46 SE and organic matter content of 13.1% ± 1.2 SE for the Tamar estuary site (Figure 3.2). Grain size was measured using a particle size analyzer (Beckman Coulter LS230) and organic matter content was determined using a loss-on-

ignition protocol (Smeaton et al. 2022). All sediment was frozen and stored at -20°C. Prior to testing, sediment was prepared by freeze drying in a ModulyoD freeze dryer (Thermo Electron Corporation) for a minimum of two days. Once dried, sediment was stored in sealed glass jars at room temperature. For all tests, sediment was sieved with a 1 mm stainless-steel sieve to remove microplastic >1 mm and thereby focus testing on the smaller size fraction of microplastics.

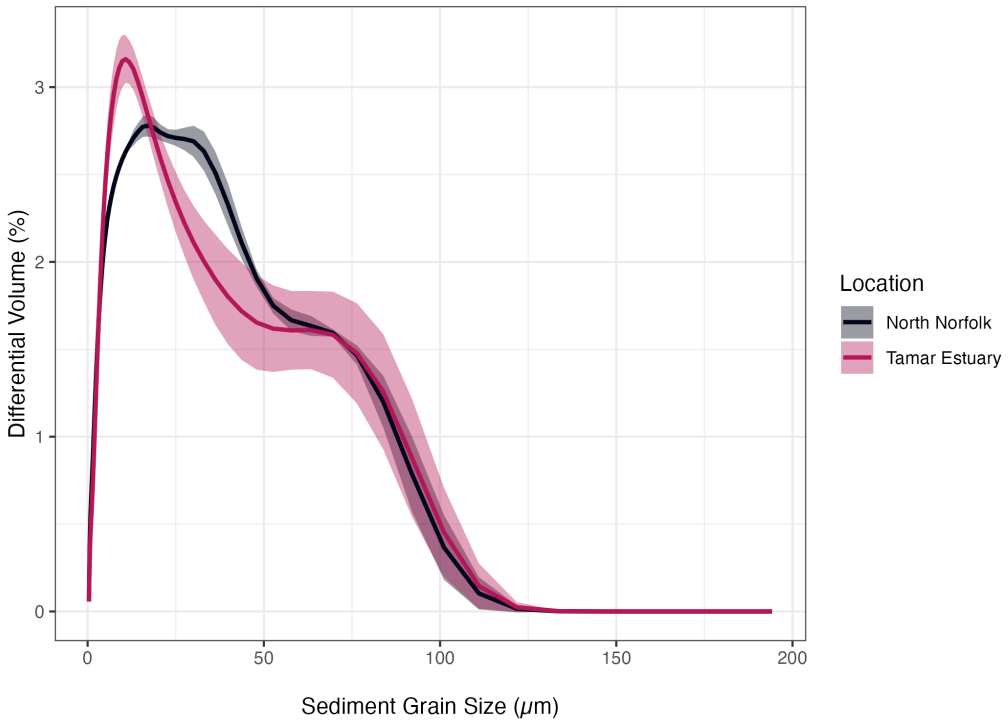

Figure 3.2. Sediment grain size distributions for each location of test sediment. Solid line indicates the mean percent differential volume. Shaded areas represent the standard deviation.

Recovery rate tests used four types of microplastics: polypropylene (PP) fragments, polyethylene (PE) films, polyamide (PA) fibres, and polyester (PET) fibres (Figure S3.1). PP fragments were created by filing a yellow PP box with a flat steel file, PE films were cut from an orange plastic bag, and both types of fibres were shaved with a metal scalpel from two types of fleece-like fabrics (purple PA and red PET). All microplastics were separately vacuum filtered onto mesh sizes between 10 and 1000 µm. Microplastics were added to dried sediment (pre-sieved to < 1 mm) and mixed with a stainless-steel spoon prior to each tested protocol. The exception was

tests #14-17, where microplastics were added after the digestion step and prior to density separation to elucidate in which step microplastic loss was occurring.

For tests #25-27, which compared optimized protocols, a random number generator was used to determine the number of each microplastic type per sample to ensure a blind count for the analyst. Numbers were generated between 5-10 for each polymer and shape of microplastic, and total microplastics added ranged between 29 and 38 (Table S3.2). The longest dimension for each plastic type ranged from 412 to 945 µm (mean ± standard deviation (s.d.): 612 ± 136 µm) for PP fragments, 265 to 959 µm (mean ± sd: 500 ± 142 µm) for PE films, 338 to 1063 µm (mean ± sd: 708 ± 176 µm) for PA fibres, and 187 to 981 µm (mean ± sd: 657 ± 207 µm) for PET fibres (Figure 3.4).

Mass reduction of sediments was determined by subtracting the post-processing dry weight from the original dry weight and reporting as a percentage of weight removed. Samples were air dried on nylon filters until they maintained a constant weight (Mettler AE 200). Microplastic recovery rates were determined by visualizing filters under an Olympus SZX16 microscope and enumerating and measuring reference putative microplastics using CellSens imaging software. Because of the distinct colours of added microplastics they were easily distinguishable from possible equipment contamination, such as from the SMI unit (grey PVC). Putative microplastics were also characterized using micro-FTIR to confirm polymer type (Perkin Elmer Spotlight 400; reflectance mode; 4000-600 cm^{-1}; 4 scans).

3.4.3 Part 2: Method comparison for sediment

Optimized protocols from Part 1 were repeated and compared to test efficacy and precision (tests #25-27; n = 3). A procedural blank was run for each method (N = 3) following the same procedure as spiked samples but without sediment or reference microplastics. No contamination was observed on the filters. These tests used the same sample mass (40 g), digestion solution (1% NaClO), and density separation solution (NaBr, d = 1.48 g/mL) but differed in the density separation set up:

Protocol #25 and #26

Samples were processed with an organic digestion followed by a density separation. Each 40 g sample was split into four 10 g subsamples at the start of the process to ensure the sample would fit and was evenly split across the 50 mL Falcon tubes for the density separation step. First, the 10 g subsamples were digested overnight with 50 mL each of 1% NaClO (50°C, 120 RPM) in an orbital incubator (Sanyo). Each subsample was sieved onto a 20 µm stainless-steel sieve (Fisherbrand, ISO 3310/1), triple rinsed with ultrapure water, rinsed once with NaBr, then rinsed into 50 mL falcon tubes and topped with NaBr to the 50 mL mark. Contents were mixed for 5 minutes by shaking falcon tubes manually and with a vortex. Samples were centrifuged at 1750 RPM for 5 minutes with 5 brake speed. The supernatant was vacuum filtered onto 20 µm nylon mesh and triple rinsed with ultrapure water. For protocol #26, samples were centrifuged a second time, by adding 35 mL NaBr to the remaining sediment in the falcon tubes after the first supernatant was decanted, homogenizing the sample by hand and with a vortex, centrifuging, and filtering as previously described.

Protocol #27

Spiked sediment was incubated overnight with 200 mL of 1% NaClO at 50°C and 120 RPM in an orbital incubator (Sanyo). Digested sediment was sieved onto a 20 µm stainless steel sieve (Fisherbrand, ISO 3310/1), triple rinsed with ultrapure water, followed by one rinse with NaBr, and then rinsed with NaBr into a 250 mL glass beaker. Sediment-Microplastic Isolation (SMI) units were prepared by purging the ball valve with NaBr and left to settle for 5 minutes before filtering off any potential contamination in the unit, as per Coppock et al. (2017). The sample, a magnetic stir bar, and additional NaBr was then added to the SMI unit. The glass beaker was rinsed with NaBr into the SMI to ensure all sample contents transferred. Each sample was mixed for 5 minutes on a stir plate, then left to settle in the SMI unit for 24 hours. The next day, the supernatant above the headspace was vacuum filtered onto a 20 µm nylon mesh filter.

3.4.4 Part 3: Method comparison for leaf surfaces

We tested three methods (n = 3) for isolating, quantifying and characterising microplastics from leaf surfaces; two of which used optical microscopy to identify microplastics and the third used FTIR imaging. All methods were trialled using saltmarsh leaves (Sea Purslane, *Atriplex portulacoides*) collected from the Tamar estuary (detailed location specified in Chapter 5). For all tests, five leaves were spiked with four types of identifiable microplastics: yellow polypropylene fragments, orange polyethylene films, purple polyamide fibres, and red polyester fibres, as described for sediment tests. A random number generator was used to determine the number (5-10) of each microplastic type added to samples. The number of microplastics added ranged from 25 to 33 microplastics per sample (Table S3.3). The longest dimension for all microplastics added ranged from 195 to 1372 µm (mean ± standard deviation (s.d.): 610 ± 209 µm). The average particle length for each plastic type is listed in Table 3.1. Spiked microplastics were placed on leaf surfaces using fine-tipped tweezers and observed under an Olympus SZX16 microscope. Recovery rates were calculated by dividing the number of microplastics recovered by the number of microplastics added and multiplying by 100.

Table 3.1. Size of microplastics added to leaf surface samples for method validation tests.

Microplastic	**Minimum length (µm)**	**Maximum length (µm)**	**Mean ± standard deviation (µm)**
PP Fragment	195	1254	574 ± 202
PE Film	247	1299	658 ± 252
PA Fibre	217	1372	601 ± 188
PET Fibre	243	1044	615 ± 193

Method 1: Wash, no density separation, optical microscopy

The first tested method used a solution of NaClO and Tween80 to wash microplastics off the surface of the leaves. Leaves (five per sample) were placed in 720 mL glass jars with 100 mL of a 1% NaClO (bleach) and 0.5% Tween80 surfactant solution and placed in an orbital shaker (Cole-Palmer SI600) for 30 minutes at 180 RPM. Leaves were then removed from solution, and the outside of the leaves were rinsed with ultrapure water into the jar. The solution was filtered onto 20 µm nylon mesh filters with vacuum filtration and triple rinsed with ultrapure water. Microplastic recovery rates were determined by visual sorting under an Olympus SZX16 microscope and any particles that resembled spiked microplastics were picked out of the sample. Each particle was measured and imaged with CellSens software (version 2.1) and then placed onto a glass slide wrapped in aluminium foil to provide a reflective background for spectroscopy. For each sample, the first three particles of each microplastic type were characterized using micro-FTIR in reflectance mode (n = 36; Perkin Elmer Spotlight 400; 4000-700 cm^{-1}; 4-8 scans, 4 resolution).

Method 2: Wash, density separation, optical microscopy

The second tested method used the same initial step as Method 1, by washing the leaves with 100 mL of 1% NaClO and 0.5% Tween80. Following this step, the solid residue on the 20 µm filters was rinsed into a 150 mL glass beaker with NaBr (d = 1.46 g/mL) for a density separation. The NaBr solution was transferred to 15 mL centrifuge tubes, rinsing the sides of the beaker with additional NaBr. Prior to centrifugation, tubes were shaken for 1 minute. Samples were then centrifuged at 3000 RPM for 5 minutes and 5 brake speed (Eppendorf Centrifuge 5810 R; 17.3 cm radius). The supernatant was vacuum filtered onto 20 µm nylon mesh and triple rinsed with ultrapure water. Filters were visually sorted to pick out particles resembling spiked microplastics and placed on glass slides wrapped in aluminium foil. Consistent with Method 1, the first three particles of each microplastic type were characterized using reflectance micro-FTIR (n = 36; 4000-700 cm^{-1}; 4-8 scans, 4 resolution).

Method 3: Wash, density separation, FTIR imaging

The third method used the same sample processing steps as Method 2. However, after the density separation with NaBr, the supernatant was filtered onto 5 µm silver membrane filters (13 mm, Sterlitech). Method 3 used FTIR spectral imaging to identify microplastics (Perkin Elmer Spotlight 400 with MCT detector). For each sample, a background spectrum was collected at a spectral resolution of 8 cm^{-1}, 15 scans per pixel, interferometer speed of 2.2 cm s^{-1}, and 25 µm pixel size. A visual image survey was collected of each filter using PerkinElmer SpectrumIMAGE software followed by spectral imaging of the whole filter using the same parameters as the background scan but at 4 scans per pixel. All files were atmospheric corrected in SpectrumIMAGE software to remove spectral interference from atmospheric water vapor and carbon dioxide. Sample spectra were compared to a reference polymer database using the free software program, siMPle (https://simple-plastics.eu/). Files were first converted to file formats that are compatible with the siMPle software. Spectra were matched to the reference database using a Pearson's correlation coefficient threshold of 0.65. The software compares the Pearson correlation factors of untreated spectra as well as the first and second derivative spectra to the reference database. A spectral match is considered when the untreated spectra and the first derivative are assigned to the same polymer entry (Primpke et al. 2017, Primpke et al. 2020).

3.5 Results and Discussion

3.5.1 Literature review of microplastic extraction from saltmarsh sediments

From our analysis of relevant literature, we established that only three of 15 studies that sampled microplastics from tidal marsh or saltmarsh sediments performed their own validation tests (Helcoski et al. 2020, Li et al. 2022b, Ertel et al. 2023) (Table 3.2), while four papers referenced a previous study's recovery rates (Fraser et al. 2020, Lloret et al. 2021, Almeida et al. 2023, Vermeiren et al. 2023). Only two studies included fibres in their recovery tests (Almeida et al. 2023, Ertel et al. 2023), while most used fragments or beads (Helcoski et al. 2020, Fraser et al. 2020, Lloret et al. 2021, Li et al. 2022b, Vermeiren et al. 2023). Particles tested included polyethylene, polypropylene, polyamide, polyvinyl chloride, polystyrene, polyethylene terephthalate, and crumb rubber, ranging from 0.16 to >5 mm (Table 3.2); most studies tested

between one and three different polymer types and one tested six different polymers. It is clear that recovery rate tests are not used consistently for complex matrices.

Table 3.2. Literature review of recovery rate tests for microplastic extraction from saltmarsh sediments.

Ref #	Author	Year	Sediment type of study	Validation test?	Sediment type for validation test	Reference plastics	Size of plastics	No. of each plastic type	No. replicate tests	% recovered
1	Helcoski et al.	2020	Tidal marsh, mudflat	Yes	Mixture of soil, plant material, and water	Polypropylene squares	3 x 3 cm	5	1	100%
2	Li et al.	2022	Saltmarsh, mangrove, beach, forest, paddy field	Yes	Not stated	PE particle, PP fragment	<0.5 mm, 0.5 - 1mm, 1-2mm, 2-3mm, 3-4mm, 4-5mm)	Two of each type and size class (N = 24)	Not stated	100% ± 0%
3	Ertel et al.	2023	Saltmarsh	Yes	Not stated	PE beads, crumb rubber, PA fiber	Not stated	Not stated	5	87%
4	Fraser et al.	2020	Saltmarsh, river	Cites another study (Zhang et al., 2020)	Glass beads	Polypropylene and polyethylene beads	0.5 – 1.5 mm	Unclear	Unclear	97% to 98%

5	Lloret et al.	2021	Saltmarsh	Cites another study (Zobkov & Esiukova, 2017)	Bottom sediments (very fine, fine, medium, coarse-grained)	PET squares	0.90 ± 0.39 mm	40	14	97.1% ± 2.6% (range: 85% - 100%)
6	Almeida et al.	2023	Saltmarsh	Cites another study (Revoira et al., 2020)	Sediment with 1% and 5% organic matter content	LDPE film, HDPE fragment, PET particles, PA fibers, HDPE spheres	< 5 mm	10 mg of each type	n = 3 per sediment type	66% to 100%
7	Vermeiren et al.	2023	Saltmarsh, mudflat	Cites another study (Vemeiren et al., 2020)	Mudflat (combusted to remove MPs)	PE, PP, PVC, PS, PA, PET fragments	0.16 - 6.89 mm; average 1 mm or 0.3 mm	30 to 84	1 (once for each plastic type)	90.7% for MPs >0.5 mm; 95% for MPs < 0.5 mm
8	Khan & Prezant	2018	Saltmarsh	No						
9	Yao et al.	2019	Saltmarsh, mudflat	No						

10	Cozzolino et al.	2020	Salt marsh, seagrass, bare	No							
11	Li et al.	2020	Saltmarsh	No							
12	Wu et al.	2020	Saltmarsh, mudflat	No							
13	Weitzel at al.	2021	Tidal marsh	No							
14	Pinheiro et al.	2022	Saltmarsh	No							
15	Girones et al.	2024	Saltmarsh	No							

3.5.2 Part 1: Initial test protocols for sediment

The majority of tested protocols were successful at recovering fragments and films at acceptable levels, here defined as >70% recovery of a given microplastic type; however, recovery rates of microplastic fibres were typically <50% (Table S3.1). Across all tested protocols, recoveries for PP fragments ranged from 40% to 100%, PE films ranged from 20% to 100%, PA fibres ranged from 0% to 99.5%, and PET fibres ranged from 0% to 120% (Table S3.1). The iterative approach shows how a method may need a lot of adjusting depending on the substrate of interest. Most methods tested in the literature have reported good recovery of microplastics but showed low recoveries when used here for saltmarsh sediments. For example, the oil extraction procedure (OEP), whereby oil and water are mixed with a sample and left to separate into solid, aqueous, and lipid phases, has been successfully used to recover microplastics from complex sediments across multiple studies (e.g., Crichton et al. 2017, Mani et al. 2019, Lechthaler et al. 2020). However, we could not extract fibres consistently using the OEP (Table S3.1; tests #7, 10, 14, 15, 18-22). Many studies that recommend the OEP do not include fibres in their validation tests. Only Lechthaler et al. (2020) used fibres to test recovery, but these were fibre agglomerates and were much larger than fibres typically found in the environment (Lechthaler et al. 2020). Differences in recovery rates might also stem from differences in the organic content of the substrate, the type of oil used, or the equipment set up (Crichton et al. 2017, Mani et al. 2019, Radford et al. 2021). Constant et al. (2021) also found lower recoveries using the OEP, especially for fibres and higher density microplastics (Constant et al. 2021). However, the low recovery of fibres is not exclusive to the oil method. A review of extraction methods by Monteiro & Pinta da Costa (2022) indicated that fibres had lower recovery rates compared to fragments or granulates across multiple studies (Monteiro and Pinto da Costa 2022). We also found this with a density separation using NaBr highlighted in the 'Method comparison for sediments' section below (test #27). Lower recoveries of fibres may be due to their sensitivity to chemical reagents and their unique morphology and flexibility may increase chances of passing through sieves (Lares et al. 2019, Monteiro and Pinto da Costa 2022). Overall, some methods may be appropriate for certain substrates but may not have the same recovery efficiency in others and using diverse

and environmentally representative reference microplastics is imperative for increasing validation of analytical methods.

Regarding impacts on polymers, we found that some steps in the procedure did not affect particle colour for fragments or films but did affect fibres. We tested the effect of different concentrations of NaClO (0.5%, 1%, 2%, 4%, 7%) on the reference microplastics. Previous studies recommended 7.5% and 10% NaClO at 40-50°C because it removed 88% and 92% of hard and soft organic materials, respectively, with minimal effects on polymer fragment weight (Pfeiffer and Fischer 2020). However, we found a concentration of 7% NaClO removed the dye from the PA fibres (Figure S3.2). PA is often the most sensitive to digestion methods (Hurley et al. 2018, Lusher et al. 2020b, Pfeiffer and Fischer 2020, Duan et al. 2020). For our sediments, we found that 1% NaClO was most effective at reducing organic matter while still recovering all microplastics.

The centrifugation step also required some adjustments, as the number of rotations per minute affected recovery rates for fibres. We found that using a high RPM (i.e., 4000 RPM) during centrifugation did not recover fibres at high rates (<50%) but was successful for fragments and films (Table S3.1, test #8). In comparing the efficacy of 500-2000 RPM, we found that between 1750 and 2000 RPM was the most ideal for extracting fibres while still allowing sediment to separate from the solution. A lower RPM is gentler on sample integrity and allows for a slower separation, potentially allowing more time for fibres to separate out from the sediment. Using a lower RPM still had some disadvantages. For instance, the sediment at the bottom of the tubes was more likely to come loose during vacuum filtration and rinsing, resulting in more sediment coated filters.

All methods reduced initial sediment mass by >99%. Past this level of mass reduction, filter clarity and number of filters were used as a proxy to compare treatment efficacy. The oil extraction procedure resulted in the clearest filters but had low and inconsistent recovery rates for all microplastic types. The method with the highest recovery rates (1% NaClO with NaBr centrifuge extraction; #23-26) maintained a layer of sediment on the filters and often resulted in 1-7 filters per sample, depending on original sample mass (10 g vs. 40 g). More filters required

more time to count the microplastics and the layer of sediment would have inhibited micro-FTIR imaging as a feasible alternative. Using a smaller sample mass (10 g) allows for fewer filters for analysis but could limit the representativeness of the sample (Möller et al. 2020). The trade-off is a higher sample mass that would require more analytical effort. Ultimately, there is a balance that must be met between sediment mass reduction and microplastic recovery.

Here, we took protocols from the literature that were commonly used for sediment microplastic analysis and applied them to saltmarsh sediments – a difficult matrix to extract from owing to its high organic content and small grain size. These tests show that complex substrates will need adjustments to microplastic extraction methods depending on access to materials and matrix properties. Differences in equipment, chemical concentrations, sample mass, and procedural order will all affect microplastics recovery rates. There is no method that will work for every sample type or laboratory, but these differences can be controlled if microplastic recovery rates are reported and potentially used as a correction factor.

3.5.3 Part 2: Method comparison for sediments

Average recovery rates (± standard deviation) for all reference microplastics were 84 ± 8% (Protocol #25), 95 ± 7% (Protocol #26) and 49 ± 3% (Protocol #27; Figure 3.3). All methods were successful at recovering >70% of the fragments and films, however protocol #27 recovered <22% of fibres (Figure 3.3). The added centrifuge step in protocol #26 increased the recovery rates of fibres but not fragments or films. While this added step increased recovery rates, it required seven filters (compared with three filters for protocol #25) and increased overall processing time by an hour. There were two replicates in protocol #25 and #26 where recovery of PP fragments was higher than the number of added fragments. While this could be from contamination, such fragments were not observed in the procedural blank; visual observations and FTIR analysis suggest that the spiked PP plastics broke apart during sample processing. Moreover, the size distributions between the added and recovered PP fragments were similar, with a slight skew toward the lower size range in the recovered fragments (Figure 3.4). Radford et al. (2021) also observed increased fragmentation of spiked microplastics in a $ZnCl_2$ separation (Radford et al. 2021). Using a mass-based approach to quantify microplastics could help account

for potential fragmentation during laboratory processing. However, many studies report environmental microplastic concentrations as a count-based value and researchers may be overestimating some polymers due to fragmentation in the extraction process. Both mass and number-based techniques are important for capturing the impacts of extraction protocols on microplastic recovery, though not every laboratory will be equipped to measure both units and this will be dependent on the research objectives.

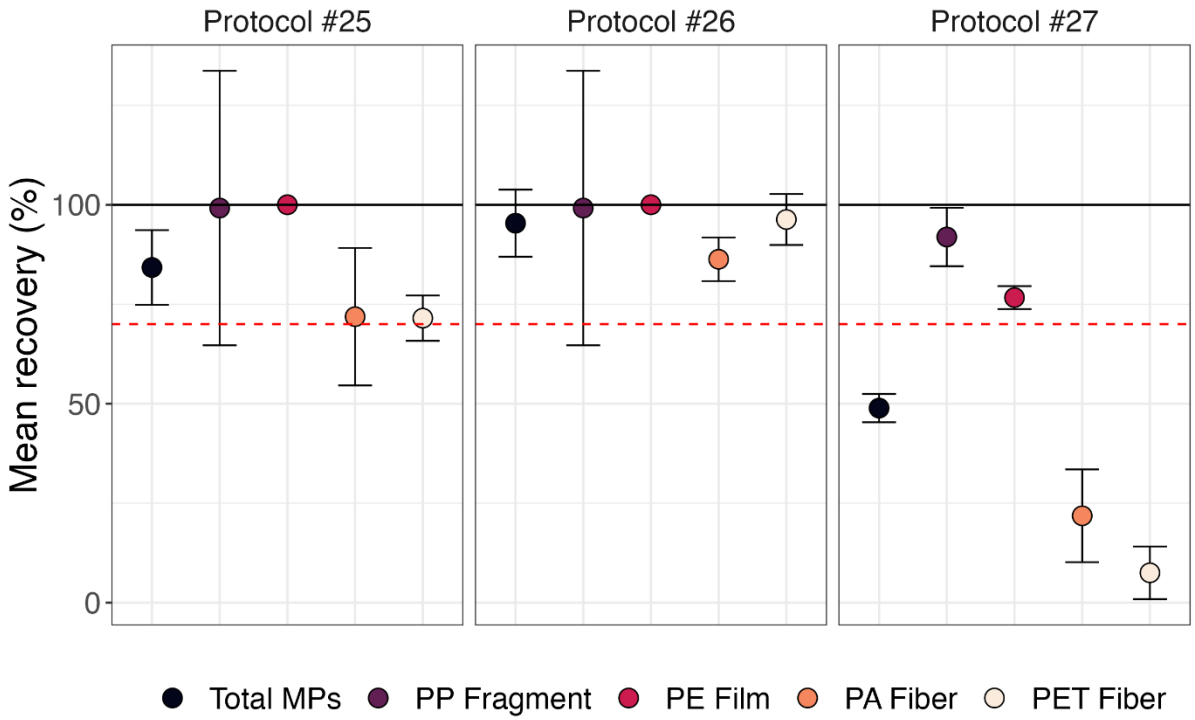

Figure 3.3. Mean ± standard deviation of microplastic recovery rates for three protocols for extracting microplastics from sediments (n = 3; Protocol #25: Centrifuge, Protocol #26: repeated centrifuge step, Protocol #27: SMI unit). Red dashed line marks 70% recovery rate. Black solid line marks 100% recovery rate. Black circles = total microplastic recovery, purple circles = polypropylene (PP) fragments, pink circles = polyethylene (PE) film, orange circles = polyamide (PA) fibres, white circles = polyethylene terephthalate (PET) fibres.

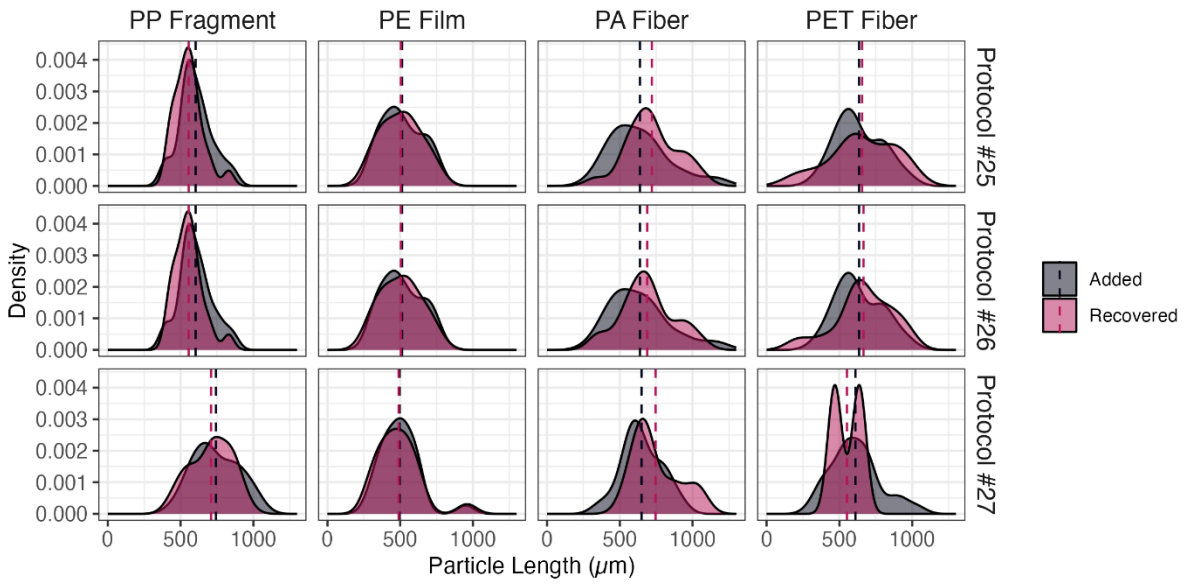

Figure 3.4. Density plot showing the size distributions of microplastics added (grey) and recovered (pink) for tests #25-27. Dashed lines represent the mean.

In Coppock et al. (2017), the SMI unit worked well for recovering fragments and filaments from sand and silty sediments. However, these sediments were collected from sites without vegetation and likely had a lower organic matter content compared to the sediments used here. In contrast, only one method (protocol #26) in our study showed recovery of fibres >80% with the saltmarsh sediments. These comparisons show that there are limitations to any method, and the composition of a sample substrate alters the effectiveness of a method. We therefore cannot rely on previous studies recovery rates when applying a method to a new matrix. However, many methods are useable and can be applied in different contexts.

3.5.4 Part 3: Method comparison for leaf surfaces

The total microplastic recovery rate for leaf surfaces was best for Method 1 at 99 ± 8%. Method 2 had a total recovery of 88 ± 5% and Method 3 had a total recovery of 124 ± 7% (Figure 3.5). Method 1 and Method 2 were both effective at isolating and identifying spiked microplastics. However, Method 1 had more consistent recovery rates across microplastic types, a shorter processing time, and there was less opportunity for transfer loss due to fewer

processing steps compared to Method 2. For these reasons, Method 1 was chosen as the preferred method to analyse microplastics for Chapter 5.

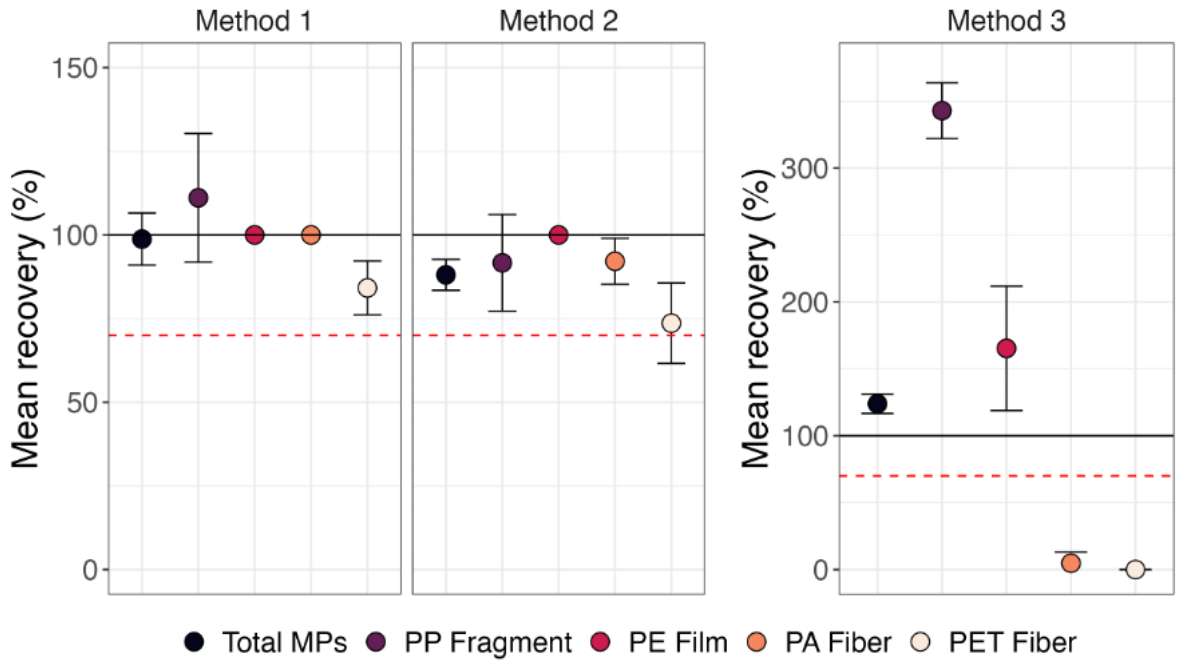

Figure 3.5. The percent mean recovery rate for total microplastics (black dots), polypropylene (PP) fragments (purple dots), polyethylene (PE) films (pink dots), polyamide (PA) fibres (orange dots), and polyethylene terephthalate (PET) fibres across three different methods for extracting microplastics from leaf surfaces. Error bars represent standard deviation, dotted red line marks 70% recovery, and solid black line marks 100% recovery.

Interestingly, Method 3 with FTIR imaging was the least effective method for identifying microplastics in this context. First, fragments and films were overestimated (Figure 3.5). This overestimation could have been from background contamination from the laboratory, from microplastics already present on the leaves that were identified by spectral imaging, or error in the particle building software. FTIR imaging quantifies and records everything on the filter and it is unable to account for microplastic colour. In contrast, using optical microscopy allows for the observer to be selective about particle colour and therefore only quantify the particles of interest. Secondly, the siMPle software system misidentified some of the polyethylene films as

polypropylene (Figure 3.6). This misidentification could be a result of the reference library used or because of the threshold settings. Using a threshold of 0.65 allows for a compromise between allowing some variation in the spectra due to environmental weathering and having a reasonable confidence in the spectral match (Johnson et al. 2020). Moreover, additives and dyes present in the polyethylene films could have interfered with the spectra and affected their identification (Cowger et al. 2020). Lastly, fibres were rarely identified with this technique. Figure 3.6 shows one polyamide fibre was correctly identified but many of the other fibres were not identified at all, even though they were visually present on the filter. The structure of fibres means that they are not always in the same plane of focus during FTIR spectral imaging and therefore may not be analysed consistently (Primpke et al. 2019, Botterell et al. 2022). Primpke et al. (2019) proposed placing a BaF_2 window on top of the filter to press all particles into the same focal plane to overcome this issue.

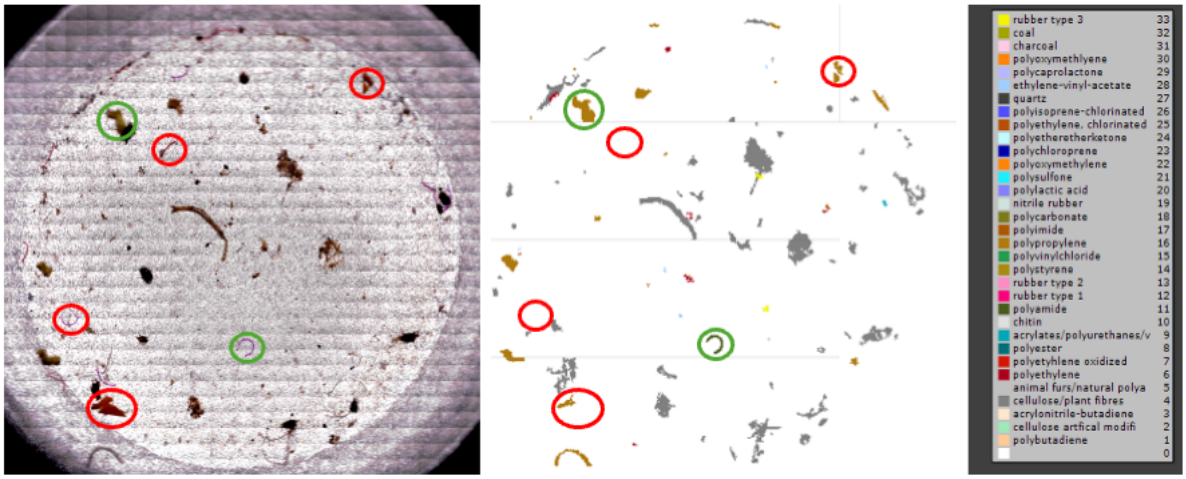

Figure 3.6. Left image shows the visual image survey from the FTIR microscope and the right image shows the image analysis output from siMPle. Red circles highlight a selection of misidentified or not identified particles and green circles highlight a selection of correctly identified particles.

While the FTIR imaging method was unsuccessful to reach the objectives of our research, it could be a worthwhile method for those with hundreds of samples that require a semi-automated approach. Regardless of which method is chosen, it is important to test and report

recovery rate efficiencies prior to analysing samples. Clear reporting will aid in evaluating study results and allow for easier comparisons across studies.

3.5.5 Limitations and further considerations

Moving forward, the challenge will be harmonizing reference materials and the procedure of validation tests. Matrix-matched certified reference materials (CRMs) could be used to validate analytical methods, provide metrological traceability of the results, and generate uncertainty budgets (Ricci et al. 2016, Hildebrandt et al. 2020, Padariya et al. 2022, "ISO/Guide 30:2015(en), Reference materials — Selected terms and definitions" n.d.). This involves creating a matrix resembling the sample matrix of interest (e.g., sediment) containing a known and verified amount of analyte (e.g., microplastics) and is accompanied by a certificate containing the value of the specified property, its associated uncertainty, and a statement of metrological traceability (Venelinov and Sahuquillo 2006, Padariya et al. 2022, Emteborg et al. 2024, "ISO/Guide 30:2015(en), Reference materials — Selected terms and definitions" n.d.). Matrix-matched CRMs have been used for the analysis of organic and inorganic pollutants such as airborne dust, heavy metals, pesticides, and PAHs (Ricci et al. 2016, Padariya et al. 2022, Emteborg et al. 2024). However, there are many difficulties in creating certified reference materials. The creation of CRMs is often dependent on the creation of standardized analytical methods, generating a causality dilemma (Emteborg et al. 2024, Jacob et al. 2024).

Additionally, different environments (marine vs. freshwater; water vs. sediment) and local economic activities (industrial facilities, fishing ports, tourist areas, etc.) may have different compositions of microplastic pollution. Method validation tests for these different systems might require varying combinations of microplastic polymers, shapes, and sizes as reference microplastics to ensure validation tests are representative of what might be expected in a local area. Other considerations for reference microplastics include using aged microplastics and natural particles (e.g., cotton fibers) (Monteiro and Pinto da Costa 2022, De Frond et al. 2022, Thornton Hampton et al. 2023). Finding reference microplastics that are representative of environmental microplastics while still being identifiable from ambient environmental contamination will be difficult, unless matrix-matched CRMs are produced. Alternatively,

reference microplastics could be fluorescently tagged for easy identification. Some studies have used laboratory made sample matrices and controlled the amount of organic matter and soil properties to avoid ambient environmental contamination (Radford et al. 2021). Though not yet standardized, this technique could be used to allow adjustments based on the specific sample composition of interest. This, in combination with the reference materials being developed by many laboratories, could be a path forward for the harmonization of microplastics research (Venelinov and Sahuquillo 2006, Dehaut et al. 2023, Martínez-Francés et al. 2023, Jacob et al. 2024).

3.5.6 Conclusion

Analytical methods to extract microplastics will always differ across laboratories based on availability of funding and resources, the research question, type of substrate, and experimental design. A one-size-fits-all approach would limit access to microplastics research and hinder scientific creativity. Standardized protocols with measurement standards should be reserved for accredited laboratories focused on monitoring. Where the research question investigates beyond monitoring, it may be more valuable to apply harmonized recovery rate tests with verified and diverse reference materials. Results can then be compared via extraction efficiencies with known uncertainties rather than applying a single protocol across one matrix with varying properties. Ultimately, the microplastics research field can only advance when recovery rate tests become a requirement and reference materials are harmonized to facilitate the comparability and reliability of field data. Going forward, microplastic studies would benefit from:

1. Harmonized recovery rate tests wherein the same substrate and processing conditions are used as the ones being investigated in the main study.
2. Representative reference microplastics that contain the scope of microplastic shapes, sizes, densities, and weathering present in the environment.
3. Clear, detailed and consistent reporting of method validation tests and microplastic recovery rates to aid comparisons across the field.

Chapter Three: Supplementary Information

Table S3.1. Table of different methods tested with method details, mass reduction rates, and microplastic recovery rates.

Test #	1	2	3	4	5
Test Name	**$ZnCl_2$-SMI**	**NaBr-SMI**	**Oil-Ctrfg4000**	**(Oil-Ctrfg4000 +) 30% H_2O_2**	**(Oil-Ctrfg4000 +) Fenton's**
Sample details					
Sample mass	50 g	50 g	40 g	40 g	40 g
# of replicates	n = 3	n = 3	n = 3	n = 3	n = 3
Plastics added					
Order of steps				Sediment separation -> organic digestion	Sediment separation -> organic digestion
Organic digestion					
Organic digestion solution				30% H_2O_2	Fenton's reagent (iron sulfate heptahydrate + 30% H2O2)
Organic digestion conditions				50C, 120 RPM. Overnight.	50C
Sediment separation					
Sediment separation solution	$ZnCl_2$ (1.5 g/cm3)	NaBr (1.5 g/cm3)	Rapeseed oil	Rapeseed oil	Rapeseed oil
Sediment separation unit	SMI	SMI	Centrifuge (4000 RPM)	Centrifuge (4000 RPM)	Centrifuge (4000 RPM)
Mass reduction					
% mass reduced	90% ± 1%	93% ± 0.6%	99% ± 0.3%	61% ± 9.1 % *	79% ± 8.4% *
Recovery rates					
% recovered (all plastics)					
% PP fragment					
% PE film					
% PA fibre					
% PET fibre					
Extra details					
# filters per sample	11 to 12	10	4	1 to 2	1
Estimated processing time	24 h	24 h	1 h	24 h	3 h
Notes				* Mass reduction is based on difference from the weight *post* oil separation and th from original 40 g sample). Mass reduction based on original sam	
Method description	$ZnCl_2$ (1.5 g/cm3) in SMI unit. Overnight separation. 50 g sample, left overnight.	Used 1.5 g/cm3 NaBr with SMI to test mass reduction before and after. 50 g per sample, left overnight.	Used oil and ultrapure water with centrifuge (4000 RPM, 5 minutes, 5 brake) to test mass reduction before and after. 40 g per sample.	After oil separation (per test #3), digested in 30% H_2O_2 overnight (50C, 120 RPM).	After oil separation (per test #3), digested with iron sulfate heptahydrate + 30% H_2O_2.

Test #	6	7	8	9	10
Test Name	**(Oil-Ctrfg4000 +) 10% NaClO**	**Oil-Ctrfg4000 + 10% NaClO**	**NaBr-Ctrfg4000 + 7% NaClO**	**7% NaClO + oil-SMI**	**7% NaClO + oil-SMI**
Sample details					
Sample mass	40 g	40 g	40 g	40 g	40 g
# of replicates	n = 3	n = 3	n = 1	n = 1	n = 1
Plastics added		PP fragments, PE films, PA & PET fibres	PP fragments, PE films, PA & PET fibres		PP fragments, PE films, PA & PET fibres
Order of steps	Sediment separation -> organic digestion	Sediment separation -> organic digestion	Sediment separation -> organic digestion	organic digestion -> sediment separation	organic digestion -> sediment separation
Organic digestion					
Organic digestion solution	10% NaClO	10% NaClO	7% NaClO	7% NaClO	7% NaClO
Organic digestion conditions	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.
Sediment separation					
Sediment separation solution	Rapeseed oil	Rapeseed oil	NaBr (1.5 g/cm3)	Rapeseed oil	Rapeseed oil
Sediment separation unit	Centrifuge (4000 RPM)	Centrifuge (4000 RPM)	Centrifuge (4000 RPM)	SMI	SMI
Mass reduction					
% mass reduced	94% ± 2.9% *			99.90%	98%
Recovery rates					
% recovered (all plastics)		**21% ± 6.5%**	**54%**		**20%**
% PP fragment		50.8% ± 9.2%	75%		40%
% PE film		34.7% ± 23.2%	100%		20%
% PA fibre		0%	10%		0%
% PET fibre		0%	38%		20%
Extra details					
# filters per sample	1	1 to 2	2	1	1
Estimated processing time	24 h	24 h	24 h	48 h	48 h
Notes	ıe weight post organic digestion (i.e., not ple weight is 99% for all.				
Method description	After oil separation (per test #3), digested in 10% NaClO overnight (50C, 120 RPM).	Used 40 g sediment, oil and centrifuge method (4000 RPM, 5 brake, 5 min). Shook tubes by hand for 5 min, then vortexed 1 min. Then digested overnight with 10% NaClO (50C, 120 RPM). Vacuum filtered 20 µm.	Used 40 g sediment, NaBr (1.5 g/cm3) in centrifuge at 4000 RPM, 5 min, 5 brake. 7% NaClO for overnight digest (50C, 120RPM). Vacuum filtered 20 µm.	40 g samples added to conical flask with 7% NaClO. Left overnight, 50C, 120 RPM. Sieved 20 µm. Rinsed into SMI unit with ultrapure water and oil. Stirred 5 min. Left 24 hrs. *sieved 20 µm*, then vacuum filtered 20 µm.	40 g sample, added to conical flask with 7% NaClO (50C, 120 RPM). Sieved 20 µm, rinsed into SMI with ultrapure water and oil. Mixed 5 min. left 24 hours. Sieved 20 µm, then filtered 20 µm.

Test #	11	12	13	14	15
Test Name	**7% NaClO + NaBr-SMI**	**Oil-Ctfg2000 + 10% KOH**	**NaBr-Ctfg2000 + 10% KOH**	**7% NaClO + Oil-Ctfg2000**	**7% NaClO + Oil-Ctfg2000**
Sample details					
Sample mass	40 g	10 g	10 g	10 g	5 g
# of replicates	n = 2	n = 1	n = 1	n = 1	n = 1
Plastics added	PP fragments, PE films, PA & PET fibres	PA & PET fibres	PA & PET fibres	PA & PET fibres	PA & PET fibres
Order of steps	organic digestion -> sediment separation	sediment separation -> organic digestion	sediment separation -> organic digestion	organic digestion -> sediment separation	organic digestion -> sediment separation
Organic digestion					
Organic digestion solution	7% NaClO	10% KOH + 0.1% Tween	10% KOH + 0.1% Tween	7% NaClO	7% NaClO
Organic digestion conditions	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.
Sediment separation					
Sediment separation solution	NaBr (1.5 g/cm3)	Rapeseed oil	NaBr (1.5 g/cm3)	Rapeseed oil	Rapeseed oil
Sediment separation unit	SMI	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)
Mass reduction					
% mass reduced	99.90%				
Recovery rates					
% recovered (all plastics)	**45% ± 0%**			**91%**	**45%**
% PP fragment	80% ± 20%				
% PE film	80% ± 0%				
% PA fibre	15% ± 15%			82%	29%
% PET fibre	5% ± 5%			100%	50%
Extra details					
# filters per sample	1	1	3	1	1
Estimated processing time	48 h	24 h	24 h	24 h	24 h
Notes	**Unopened, expired bleach was used here, so concentration of active chlorine was likely lower.**				
		Too much material on filters to count			Spiked with microplastics AFTER dige
Method description	40 g sample, added to conical flask with 7% NaClO (50C, 120 RPM). Sieved 20 µm, rinsed into beaker then into SMI with NaBr. Mixed 5 min. left 24 hours. Sieved 20 µm, then filtered 20 µm.	10 g sample, added to 50 mL falcon tube. Rinsed with ultrapure water and added 2 mL of oil. Shook for 5 min, alternating with hand and vortex. Centrifuged at 2000 RPM, 5 min , 5 brake. Filtered 10 µm. Digested overnight with 10% KOH + 0.1% tween (50C, 120RPM). Filtered 10 µm.	10 g sample, added to 50 mL falcon tube. Rinsed with and added NaBr. Shook for 5 min, alternating with hand and vortex. Centrifuged at 2000 RPM, 5 min , 5 brake. Filtered 10 µm. Digested overnight with 10% KOH + 0.1% tween (50C, 120RPM). Filtered 10 µm.	10 g sample. Digested with 7% NaClO overnight BEFORE adding the spike plastic. Added MPs, then sieved 20 µm. Rinsed with ultrapure water then transferred to 50 mL falcon tube with 2 mL oil. Shook for 5 min, alternating for 1 min with hand and vortex. Centrifuged 2000 RPM, 5 brake, 5 min. Filtered 10 µm.	5 g sample. Digested with 7% NaClO overnight BEFORE adding the spike plastic. Added MPs, then sieved 20 µm. Rinsed with ultrapure water then transferred to 50 mL falcon tube with 2 mL oil. Shook for 5 min, alternating for 1 min with hand and vortex. Centrifuged 2000 RPM, 5 brake, 5 min. Filtered 10 µm.

Test #	16	17	18	19	20
Test Name	**7% NaClO + NaBr-Ctrfg2000**	**7% NaClO + NaBr-Ctrfg2000**	**7% NaClO + Oil-Ctrfg2000**	**20 % KOH + Oil-Ctrfg2000**	**7% NaClO + Oil-Ctrfg1000**
Sample details					
Sample mass	10 g	5 g	10 g	10 g	10 g
# of replicates	n = 1	n = 1	n = 1	n = 1	n = 1
Plastics added	PA & PET fibres	PA & PET fibres	PP fragments, PE films, PA & PET fibres	PP fragments, PE films, PA & PET fibres	PP fragments, PE films, PA & PET fibres
Order of steps	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation
Organic digestion					
Organic digestion solution	7% NaClO	7% NaClO	7% NaClO	20% KOH	7% NaClO
Organic digestion conditions	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.
Sediment separation					
Sediment separation solution	NaBr (1.5 g/cm3)	NaBr (1.5 g/cm3)	Rapeseed oil	Rapeseed oil	Rapeseed oil
Sediment separation unit	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)	Centrifuge (2000 RPM)	Centrifuge x2 (1000, 1750 RPM)
Mass reduction					
% mass reduced					
Recovery rates					
% recovered (all plastics)	**69%**	**110%**	**75%**	**31%**	**32%**
% PP fragment			100%	100%	80%
% PE film			90%	60%	80%
% PA fibre	75%	90%	60%	9%	0%
% PET fibre	65%	120%	50%	14%	20%
Extra details					
# filters per sample	2	2	1	2	2
Estimated processing time	24 h	24 h	24 h	24 h	24 h
Notes	tion step before sediment separation.				
Method description	10 g sample. Digested with 7% NaClO overnight BEFORE adding the spike plastic. Added MPs, then sieved 20 µm. Rinsed with NaBr then transferred to 50 mL falcon tube. Shook for 5 min, alternating for 1 min with hand and vortex. Centrifuged 2000 RPM, 5 brake, 5 min. Filtered 10 µm.	5 g sample. Digested with 7% NaClO overnight BEFORE adding the spike plastic. Added MPs, then sieved 20 µm. Rinsed with NaBr then transferred to 50 mL falcon tube. Shook for 5 min, alternating for 1 min with hand and vortex. Centrifuged 2000 RPM, 5 brake, 5 min. Filtered 10 µm.	10 g sample. Digested overnight with 7% NaClO (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water into 50 mL falcon tube and added 1.5 mL oil. Shook 5 min, alternating with hand and vortex. Centrifuged at 2000 RPM, 5 brake, 5 min. Vacuum filtered 10 µm.	10 g sample. Digested overnight with 20% KOH (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water into 50 mL falcon tube and added 1.5 mL oil. Shook 5 min, alternating with hand and vortex. Centrifuged at 2000 RPM, 5 brake, 5 min. Vacuum filtered 10 µm.	10g sample. Digested with 7% NaClO overnight (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water, then into 50 mL falcon tube and added 1.5 ml oil. Shook 5 min by hand and vortex. Centrifuged at 1000 RPM (5 min, 5 brake). Vacuum filtered onto 20 µm. Sediment resuspended so I added more ultrapure water and oil, shook and centrifuged again at 1750 RPM. Filtered onto a new 20 µm filter.

Test #	21	22	23	24	25
Test Name	**1% NaClO + Oil-Ctrfg1000**	**0.5% NaClO + Oil-Ctrfg1000**	**1% NaClO + NaBr-Ctrfg1250**	**1% NaClO + NaBr-Ctrfg1250 + NaBr-Ctrfg1750**	**1% NaClO + NaBr-Ctrfg1750**
Sample details					
Sample mass	10 g	10 g	10 g	10 g	40 g
# of replicates	n = 2	n = 1	n = 2	n = 2	n = 3
Plastics added	PA & PET fibres	PA & PET fibres	PA & PET fibres	PA & PET fibres	PP fragments, PE films, PA & PET fibres
Order of steps	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation	organic digestion -> sediment separation
Organic digestion					
Organic digestion solution	1% NaClO	0.5% NaClO	1% NaClO	1% NaClO	1% NaClO
Organic digestion conditions	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.
Sediment separation					
Sediment separation solution	Rapeseed oil	Rapeseed oil	NaBr (1.5 g/cm3)	NaBr (1.5 g/cm3)	NaBr (1.5 g/cm3)
Sediment separation unit	Centrifuge (1000 RPM)	Centrifuge (1000 RPM)	Centrifuge (1250 RPM)	Centrifuge x2 (1250, 1750 RPM)	Centrifuge (1750 RPM)
Mass reduction					
% mass reduced					99.7% ± 0.15%
Recovery rates					
% recovered (all plastics)	**41% ± 16%**	**8%**	**75.5% ± 4.5**	**104.5% ± 9.5%**	**84.2% ± 7.7%**
% PP fragment					99.2% ± 28.2%
% PE film					100.0% ± 0%
% PA fibre	36.4% ± 6.4%	0%	85.5% ± 5.5%	99.5% ± 9.5%	71.9% ± 14.1%
% PET fibre	45.7% ± 25.7%	14%	65% ± 15%	110% ± 10%	71.5% ± 4.7%
Extra details					
# filters per sample	1 or 2	1	1 to 2	2 to 3	4
Estimated processing time	24 h	24 h	24 h	24 h	24 h
Notes			The same method, differing sample size (10 g vs 40 g)		
					no mass reduction from rep A
Method description	10g sample. Digested with 1% NaClO overnight (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water into 50 mL falcon tube, added 1.5 ml oil. Shook 5 min by hand and vortex. Centrifuged at 1000 RPM (5 min, 5 brake). Vacuum filtered onto a 20 µm filter.	10g sample. Digested with 0.5% NaClO overnight (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water into 50 mL falcon tube, added 1.5 ml oil. Shook 5 min by hand and vortex. Centrifuged at 1000 RPM (5 min, 5 brake). Vacuum filtered onto a 20 µm filter.	10g sample. Digested with 1% NaClO overnight (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water, then with NaBr (1.5 g/ml) into 50 mL falcon tube. Shook 5 min by hand and vortex. Centrifuged at 1250 RPM (5 min, 5 brake). Sediment looked solid. Vacuum filtered onto a 10 µm filter.	Added more NaBr to the tubes from #23 (1% NaClO + Nabr-ctrg1250). Centrifuged at 1750 RPM (5 min, 5 brake). Vacuum filtered onto 20 µm mesh.	40g sample. Digested with 1% NaClO overnight (50C, 120RPM). Sieved 20 µm. Rinsed with ultrapure water, then with NaBr (1.5 g/ml) into 50 mL falcon tube. Shook 5 min by hand and vortex. Centrifuged at 1750 RPM (5 min, 5 brake). Vacuum filtered onto a 20 µm filter.

Test #	26	27
Test Name	**1% NaClO + NaBr-Ctrfg1750 + NaBr-Ctrfg1750**	**1% NaClO + NaBr-SMI**
Sample details		
Sample mass	40 g	40 g
# of replicates	n = 3	n = 3
Plastics added	PP fragments, PE films, PA & PET fibres	PP fragments, PE films, PA & PET fibres
Order of steps	organic digestion -> sediment separation	organic digestion -> sediment separation
Organic digestion		
Organic digestion solution	1% NaClO	1% NaClO
Organic digestion conditions	50C, 120 RPM. Overnight.	50C, 120 RPM. Overnight.
Sediment separation		
Sediment separation solution	NaBr (1.5 g/cm3)	NaBr (1.5 g/cm3)
Sediment separation unit	Centrifuge x2 (1750, 1750 RPM)	SMI
Mass reduction		
% mass reduced	99.35% ± 0.15%	99.8% ± 0.12%
Recovery rates		
% recovered (all plastics)	**95.3% ± 6.9%**	**48.9% ± 2.9%**
% PP fragment	99.2% ± 28.2%	91.9% ± 6.0%
% PE film	100.0% ± 0%	76.7% ± 2.4%
% PA fibre	86.3% ± 4.5%	21.9% ± 9.5%
% PET fibre	96.3% ± 5.2%	7.5% ± 5.4%
Extra details		
# filters per sample	7	1
Estimated processing time	24 h	48 h
Notes		
Method description	Added more NaBr to the tubes from #25 (1% NaClO + Nabr-ctrg1750). Centrifuged at 1750 RPM (5 min, 5 brake). Vacuum filtered onto 20 µm mesh.	40 g sample. Digested with 1% NaClO (50C, 120 RPM). Sieved 20 µm. Rinsed with ultrapure water, then NaBr (1.5 g/ml) into beaker then into SMI with NaBr. Mixed 5 min, left 24 hours. Vacuum filtered 20 µm.

Table S3.2. Number of added and recovered microplastics for each compared method for isolating microplastics from sediments.

Method tested	**Replicate**	**Microplastic type**	**Number added**	**Number recovered**	**Number recovered with repeat step (Protocol #26)**
Protocol #25 & 26 (centrifuge)	A	*PP fragment*	5	3	3
		PE film	5	5	5
		PA fiber	10	7	8
		PET fiber	9	7	9
		Total microplastics	**29**	**22**	**25**
	B	*PP fragment*	8	10	10
		PE film	8	8	8
		PA fiber	10	9	9
		PET fiber	10	7	10
		Total microplastics	**36**	**34**	**37**
	C	*PP fragment*	8	9	9
		PE film	8	8	8
		PA fiber	9	5	8
		PET fiber	9	6	8
		Total microplastics	**34**	**27**	**33**
Protocol #27 (SMI)	A	*PP fragment*	7	6	
		PE film	8	6	

		PA fiber	9	3	
		PET fiber	9	0	
		Total microplastics	**33**	**15**	
	B	*PP fragment*	10	9	
		PE film	8	6	
		PA fiber	9	2	
		PET fiber	10	1	
		Total microplastics	**37**	**18**	
	C	*PP fragment*	10	10	
		PE film	10	8	
		PA fiber	10	1	
		PET fiber	8	1	
		Total microplastics	**38**	**20**	

Table S3.3. Number of added and recovered microplastics for each tested method for isolating and characterising microplastics from leaf surfaces.

Method tested	**Replicate**	**Microplastic type**	**Number added**	**Number recovered**
Method 1	A	*PP fragment*	9	12
		PE film	5	5
		PA fibre	5	5
		PET fibre	8	7
		Total microplastics	27	29
	B	*PP fragment*	5	5
		PE film	6	6
		PA fibre	7	7
		PET fibre	8	6
		Total microplastics	26	24
	C	*PP fragment*	5	5
		PE film	8	8
		PA fibre	6	6
		PET fibre	10	9
		Total microplastics	29	28
Method 2	A	*PP fragment*	8	6

		PE film	6	6
		PA fibre	5	5
		PET fibre	6	4
		Total microplastics	25	21
	B	*PP fragment*	6	6
		PE film	6	6
		PA fibre	9	8
		PET fibre	8	7
		Total microplastics	29	27
	C	*PP fragment*	9	9
		PE film	5	5
		PA fibre	7	7
		PET fibre	9	6
		Total microplastics	31	27
Method 3	A	*PP fragment*	6	22
		PE film	6	11
		PA fibre	7	1
		PET fibre	9	0
		Total microplastics	28	34

	B	*PP fragment*	9	30
		PE film	8	9
		PA fibre	10	0
		PET fibre	6	0
		Total microplastics	33	39
	C	*PP fragment*	7	23
		PE film	5	10
		PA fibre	5	0
		PET fibre	8	0
		Total microplastics	25	33

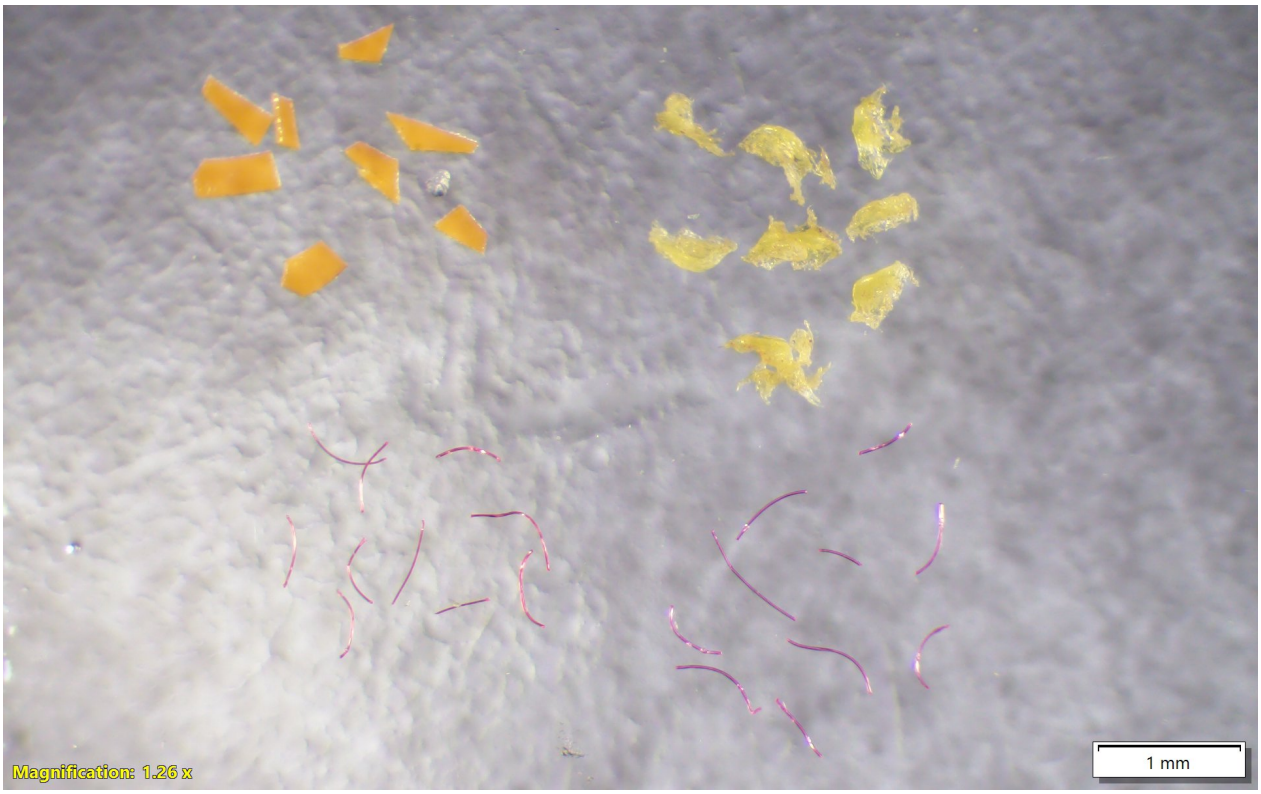

Figure S3.1. Example of reference microplastics. Top left: polyethylene films; top right: polypropylene fragments; bottom left: polyester fibers; bottom right: polyamide fibers.

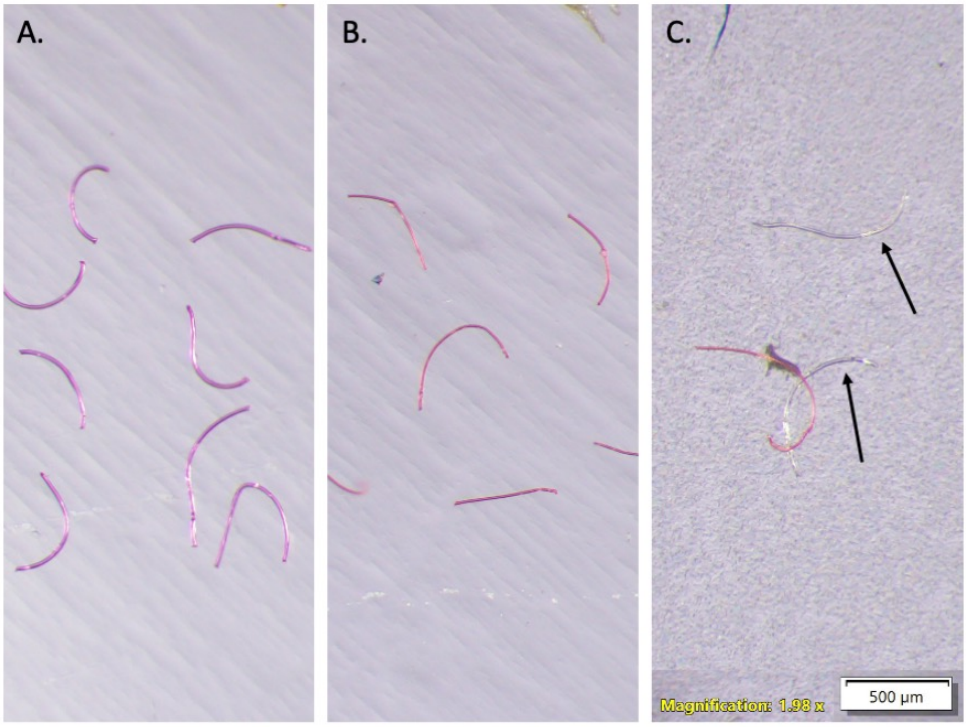

Figure S3.2. Image of (A) purple polyamide fibres and (B) red polyester fibres before and (C) after a 7% NaClO overnight digestion (50°C incubation). Black arrows highlight the polyamide fibres post-digestion.

Chapter Four

Exploring Vegetation Complexity as a Driver of Microplastic Accumulation in a North Norfolk, UK Saltmarsh

This chapter is being prepared for publication.

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HM conceptualized and carried out the experimental design, investigation and data collection, formal analysis, and writing. MC, TT, and PKL contributed to conceptualization, experimental design, and review and editing.

Microplastics are a pervasive global contaminant, posing risk to wildlife and humans. Coastal vegetated wetlands, such as mangrove forests, seagrasses, and saltmarshes, are thought to act as traps for both macro- and microplastic pollution. While microplastics occurrence in coastal vegetated sediments is well documented, there is conflicting evidence on whether the presence of vegetation enhances microplastics trapping relative to unvegetated sites. There are many potential underlying drivers of microplastic entrapment that have yet to be considered. We investigated how vegetation structure and diversity as well as microplastic type relates to microplastic accumulation in a UK saltmarsh (Blakeney National Nature Reserve). To quantify microplastic content and characteristics across increasing levels of vegetation complexity, surficial sediment (5 cm depth) was collected in September 2022 across four levels of species diversity – no vegetation, monospecific grass (*Spartina anglica*), monospecific branched (*Atriplex portucaloides*), and diverse (>3 species). Sediments were processed in the laboratory through oxidative digestion with NaClO followed by a density separation with NaBr. Mean microplastic content (± standard deviation) ranged from 363 ± 67 microplastics kg^{-1} in grassy sediments to 643 ± 358 microplastics kg^{-1} in unvegetated sediments. Branched and diverse vegetated sediments contained 480 ± 152 microplastics kg^{-1} and 379 ± 170 microplastics kg^{-1}, respectively. Microplastic content was significantly higher in unvegetated sediment compared to monospecific grass sediment, but with high variability. Within vegetated sediment, there were no significant differences in microplastic content across levels of vegetation structure and diversity.

Microplastic characteristics remained the same across all unvegetated and vegetated sediments, with small (< 300 μm) fibres and fragments being the most common. Ultimately, microplastic trapping in saltmarsh systems is governed by more than just the presence or absence of vegetation and there are likely many interacting variables influencing microplastic fate.

4.1 Introduction

Microplastics (insoluble plastic particles 0.001 mm - 5 mm in size) are prevalent in coastal ecosystems, evidenced in all environmental compartments including air, water, sediments, fauna and flora (Browne et al. 2010, Cole et al. 2011, Dris et al. 2016, Hoellein and Rochman 2021, Commission Regulation (EU) 2023, Li et al. 2025). Microplastics can be ingested by a wide array of marine organisms, with abiotic and biotic interactions impacting upon biogeochemical processes (Lusher 2015, Seeley et al. 2020, Lee et al. 2025). Coastal environments are home to diverse habitats, ranging from beaches and rocky shores to saltmarshes and mangroves (Harris et al. 2021). The threat of microplastics to coastal vegetated habitats (e.g., saltmarshes, seagrass, mangroves) are of particular concern because these habitats: host a multitude of species of plants, fish, birds, and invertebrates; act as physical barriers between the land and sea, ameliorating storm surges and extreme weather events; promote climate resilience through coastline protection and carbon storage; and filter out contaminants such as heavy metals and organic pollutants (Lee et al. 2006, Barbier et al. 2011, Spalding et al. 2014, Seitz et al. 2014, Hagger et al. 2022). It is therefore feasible that these systems also act as “filters” for microplastic pollution (Biltcliff-Ward et al. 2022, McIlwraith et al. 2024).

Microplastics, stemming from terrestrial and marine sources, enter coastal wetlands via rivers and tidal currents (Biltcliff-Ward et al. 2022, Ouyang et al. 2022). Laboratory and field studies indicate microplastics may adhere to the vegetation or settle in sediment (e.g., Goss et al. 2018, Huang et al. 2020, Jones et al. 2020, de los Santos et al. 2021, Pinheiro et al. 2022, McIlwraith et al. 2024) possibly because of modifications by vegetation on hydrodynamic flow, often reducing velocities and turbulence across the wetland (Möller and Spencer 2002, Leonard and Croft 2006, Möller et al. 2014). As such, saltmarshes, seagrasses, and mangroves have been postulated as potential sinks for microplastic pollution (e.g., Huang et al. 2020, Martin et al. 2020, Kreitsberg et al. 2021, Trusler et al. 2025). A meta-analysis by Hernan et al. (2024) found almost 40% of studies recorded significantly higher microplastics accumulation in coastal vegetated sites compared to unvegetated ones. However, the remaining 60% found no difference or the opposite pattern (Hernán et al. 2024). The dynamic nature of coastal zones makes predicting microplastic transport and deposition challenging, with factors including salinity, wind, currents,

tidal cycle, population density, topography, seasonality, biota, and biotic processes all potentially governing microplastic distribution and accumulation (de Deckere et al. 2001, Tolhurst et al. 2002, Wu et al. 2020, Lloret et al. 2021, Ogbuagu et al. 2022, Zhang et al. 2024a).

Saltmarshes are the least studied coastal vegetative system regarding microplastics transport and fate, with most observations recorded for mangroves (Ouyang et al. 2022, Hernán et al. 2024). Studies focused upon saltmarshes have often found contradictory microplastic accumulation patterns; while some report increased levels of microplastics at marsh edges (Yao et al. 2019, Helcoski et al. 2020), others report more microplastics in the marsh interior (Pinheiro et al. 2022, Trusler et al. 2025). There are also variations on the spatial deposition of different microplastic types; for example, Helcoski et al. (2020) found that fibres were the most abundant microplastic type in an urban tidal wetland, particularly in the bareflat region, whereas Yao et al. (2019) observed fragments as the most abundant type in the bareflat and edges of a saltmarsh. Saltmarshes are uniquely characterized by distinct vegetation zonation, with each zone consisting of a few dominant plant species (Pennings et al. 2005, Moffett et al. 2010). These species differ in shape, flexibility, and dimensions which in turn affects hydrodynamic flow and wave attenuation (Tempest et al. 2015). The role of vegetation and differing vegetation types on microplastic trapping is poorly elucidated. In an experimental flume study McIlwraith et al. (2024) observed vegetation complexity had no influence on microplastic deposition, however the influence of vegetation cover and type on microplastic trapping in complex natural habitats remains untested.

Here, we use field observations to test the hypothesis that vegetation complexity, determined by structural differences and species diversity, will influence underlying sediment microplastic content and composition. We predict that (i) areas with greater plant species diversity will have higher microplastic content than those with low (or no) species diversity; (ii) more structurally complex vegetation will have higher microplastic content than less structurally complex vegetation; and (iii) different microplastic types will have different depositional patterns. By exploring the spatial patterns of microplastics in relation to saltmarsh vegetation we

can further understand the mechanisms behind microplastics trapping and sequestration in coastal wetlands.

4.2 Methods

4.2.1 Study site

Samples were collected from Blakeney National Nature Reserve (North Norfolk coast, England; 52°57'53.7"N 1°01'23.4"E) on the 14th of September 2022 during a low spring tide (Figure 4.1). This site consists of a 6.4 km spit of shingle and sand dunes as well as saltmarshes, tidal mudflats, and reclaimed farmland. It is an important area for wildlife (e.g., sea bird breeding, Common and Grey seals, etc.) and has earned a designation as a Site of Special Scientific Interest (SSSI), Special Protection Area (SPA), and is located within an Area of Outstanding Natural Beauty (AONB) and is a designated Ramsar site. The site is a popular destination for visitors to enjoy recreational activities such as boating, hiking, fishing, and birdwatching. The entire area of the Blakeney National Nature reserve is 11 km^2. The sampling area was located 80 m away from the public footpath (grid reference: TG031451; Figure 4.1). Possible pollution sources in this site include agricultural runoff, development, tourists and visitors, recreational fishing and boating, and sewage discharge (Campos et al. 2018).

4.2.2 Sampling design

Sediment samples were collected from four types of vegetation cover: bare sites, monospecific grass (*Spartina anglica*), monospecific branched (*Atriplex portucaloides*), and diverse (>3 species). A random stratified approach was used with five 0.25 m^2 quadrats for each vegetation type (Figure 4.1; n = 5). Bare sites consisted of pools along a similar tidal elevation as the diverse and monospecific branched sample areas, while monospecific grass areas were located at a lower elevation towards the seaward edge of the marsh (Figure 4.1; Figure S4.1). The bare tidal pools ranged in size from 3.4 – 28.6 m^2. The diverse vegetation areas contained upwards of three species, including *Tripolium pannonicum, Atriplex portucaloides, Pucinellia maritima, Limonium vulgare, Salicornia spp.,* and *Spartina anglica*.

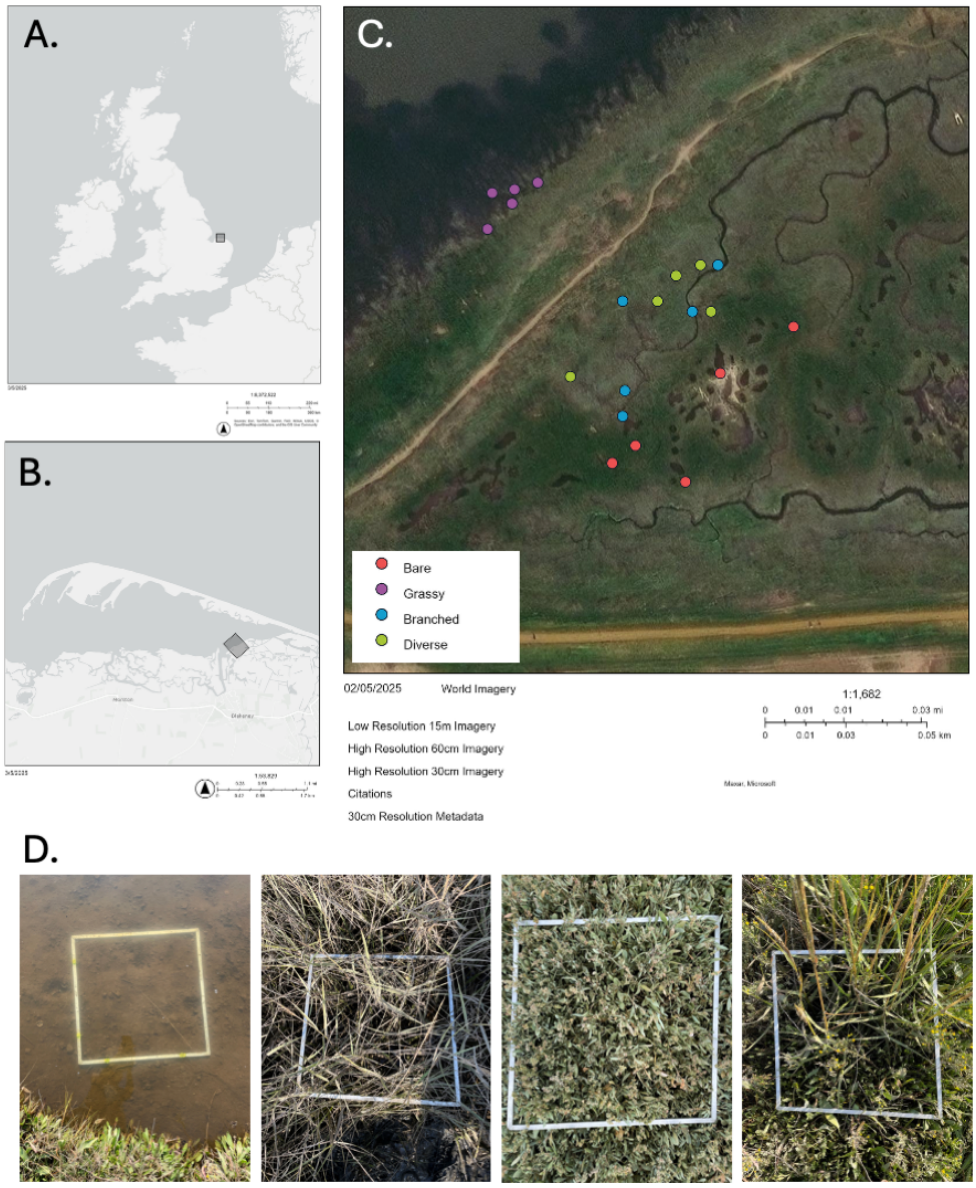

Figure 4.1. (A) Location of sampling was on the North Norfolk Coast of England. (B) Location of sampling within the Blakeney National Nature Reserve. (C) Areal image of sampled areas. Coloured dots represent types of sampled sediment (Red = bare; Purple = monospecific grass; Blue = monospecific branched; Green = diverse). (D) Photo examples of each type of vegetation cover. From left to right: bare sediment, monospecific grass, monospecific branched, diverse.

4.2.3 Sample collection

For all quadrats with vegetation, we recorded species presence, percent vegetation cover, and average canopy height. For bare sites, length and width of the pools and water depth were recorded. Five cores (5 cm depth x 5.4 cm diameter) from within each quadrat were collected with a stainless-steel corer and placed in an aluminium tray, then covered with aluminium foil. The five cores within each quadrat were combined into one sample to capture the heterogeneity within the area. Corers were marked at 5 cm, so only the top five cm of sediment was collected, which is the recommended sampling depth by the Marine Strategy Framework Directive (Hanke et al. 2013) and other researchers in the field (Brander et al. 2020). Field blanks (N=3) were collected by placing empty aluminium trays next to a sample collection point and leaving them exposed for the duration of one sample collection; blanks were then covered with foil and treated as all other samples. Upon return to the laboratory, samples were frozen at -20˚C until later analysis.

4.2.4 Laboratory analysis

Sediment was prepared by freeze-drying in a ModulyoD freeze dryer (Thermo Electron Corporation) for a minimum of two days. Sediment cores were spread on to new aluminium trays to increase surface area for lyophilization and covered with foil to prevent contamination. Once dried, sediment was stored in sealed glass jars at room temperature. Sediment grain size was measured using a particle size analyser (Beckman Coulter LS230) and organic matter content was determined using a loss-on-ignition protocol (Smeaton et al. 2022). We calculated total organic carbon based on a conversion provided by Smeaton et al. (2022) for Great Britain saltmarshes.

For microplastics analysis, dried sediment was sieved with a 5 mm stainless-steel sieve on an Endecotts Octagon 200 Sieve Shaker for 5 minutes to remove any large debris. Sediment was then homogenized by stirring with a stainless-steel spoon and a 30-40 g subsample was collected. Each subsample was then sieved over a 1 mm sieve and the < 1 mm size fraction were used for microplastics analysis. The 1-5 mm size fraction was stored in glass jars for future analysis but were not analysed in this study. Samples were further split into 10 g subsamples to ease

processing. Each 10 g subsample was placed in a 250 mL conical flask and digested overnight with 50 mL of 1% NaClO in an orbital incubator (50°C, 120 RPM; Sanyo) to remove organic matter. Each digested 10 g subsample was sieved onto a 20 µm stainless-steel sieve (Fisherbrand, ISO 3310/1), triple rinsed with ultrapure water and rinsed once with NaBr (1.5 $g \cdot cm^{-3}$) to prepare for density separation. Subsamples were rinsed with NaBr into a 50 mL falcon tube and topped up with NaBr to the 50 mL mark. The samples were mixed for 5 minutes by shaking falcon tubes manually and with a vortex. Samples were centrifuged at 1750 RPM for 5 minutes with 5 brake speed (Eppendorf Centrifuge 5810 R; 17.3 cm radius). The supernatant was vacuum filtered onto 20 µm nylon mesh and triple rinsed with ultrapure water. Final samples consisted of 3-4 filters depending on the number of 10 g subsamples that were necessary. Filters were stored in Petri dishes at room temperature.

Putative microplastics were visually sorted under an Olympus SZX16 microscope and were picked out of the sample based on colour and texture (Lusher et al. 2020a). Each particle was measured and imaged with CellSens software (version 2.1) and then placed onto a glass slide wrapped in aluminium foil to provide a reflective background for spectroscopy. 15% of all particles were randomly selected for micro-FTIR spectroscopy to confirm synthetic origin (n = 112; N = 702). This reaches the recommended minimum number of particles needed to accurately represent the proportion of plastic, anthropogenic, and natural particles in an environmental matrix (De Frond et al. 2023). Random selection of particles was carried out by using a random number generator to select 4 out of 25 slides to analyse in their entirety. Chemical identification was accomplished with a Perkin Elmer Spotlight 400 in reflectance mode and wavelength range of 4000-700 cm^{-1}. Number of scans ranged from 10 to 16 and resolution ranged from 4 cm^{-1} to 8 cm^{-1}, adjusted depending on quality of the spectra. Spectra were matched against in-house, published (Primpke et al. 2018), and commercially available databases from SpectrumIR software (Perkin Elmer 2017, version 10.6.0.893). Library search scores > 70% were accepted. If a match was between 50-70% the spectra was collected again in another spot and with different settings up to three times. If all three scans resulted in the same match, it was accepted. If not, or if a match was < 50% the particle was considered ‘Unknown’. Material types were sorted into five categories of “Plastic”, “Anthropogenic synthetic”, “Anthropogenic

Cellulosic", "Cellulosic", and "Unknown". Semi-synthetic matches, such as rayon or viscose, were classed as 'cellulosic' because the spectra are difficult to differentiate from natural cellulosic spectra. Following Cole et al. (2023), cellulosic particles with a colourful dye, such as blue or red, were classed as 'anthropogenic cellulosic', while particles without a noticeable dye were classed as 'cellulosic'. The number of microplastics per kg dry sediment was corrected based on confirmed plastic from FTIR results. Characteristic properties of particles (e.g., shape and size) were not corrected for confirmed plastic and are referred to as 'anthropogenic particles' hereafter.

4.2.5 QA/QC

Samples were processed in an ultraclean laboratory and under a laminar flow hood and cotton laboratory coats were worn. All surfaces were wiped with 70% ethanol before and after handling a sample and equipment was washed with Decon 90 and triple rinsed with ultrapure water. Field blanks (N = 3) were processed as regular samples. The number of particles in blanks was 20, 22 and 24 particles. We blank corrected the results by taking the average, rounded to the nearest whole number, of each colour and category in blanks and subtracted these from each sample. This resulted in the subtraction of 1 black fibre, 10 blue fibres, 1 brown fibre, 8 clear fibres, 1 green fibre, 1 grey fibre, 1 pink fibre, 3 blue fragments, and 1 green fragment. 60% of particles were classified as anthropogenic cellulosic, cellulosic, or unknown. The remainder were plastic or anthropogenic synthetic. The most common plastic particles in blanks were ethylene vinyl acetate fragments.

We also included eight laboratory air blanks, consisting of a glass fibre filter left in an open Petri dish, to evaluate airborne contamination during laboratory processing. Four were collected during sample processing in the laminar flow hood and four were collected during microscopy. Each air blank was left exposed throughout the duration of one sample. Particles in air blanks ranged from 0 to 4 particles and all were identified as cellulosic with micro-FTIR. Method validation tests were conducted to ascertain microplastic recovery. We added four types of microplastics (polypropylene fragments, polyethylene films, polyamide fibres, and polyester fibres) to dried saltmarsh sediment and processed it according to the procedure above (n = 3).

The average recovery rate for all added microplastics was 83 ± 8%. Fibres had the lowest recovery with 72 ± 5% for PET fibres and 72 ± 14% for PA fibres. Fragments and films had recovery rates at 95 ± 27% and 100 ± 0%, respectively (McIlwraith et al. 2025).

4.2.6 Data analysis

Data was expressed as microplastics per kg dry sediment by dividing the number of counted microplastics (across all filters per sample) by the number of grams of subsampled sediment (30-40 g dry weight) and multiplying by 1000. Data was assessed for normality and homogeneity of variances with a Shapiro-Wilks test and Bartlett's test, respectively. Assumptions were not met, so we used data transformation or generalized linear models (GLM) and Akaike information criterion (AIC) to determine the best fitting models. We used a gamma GLM with log link function to compare the number of microplastics per kg dry sediment among sediments with different vegetation cover and to compare anthropogenic particle sizes among sediments. Anthropogenic particle sizes were further split into three size classes (small (<300 µm), medium (300-1000 µm), large (>1000 µm)) and comparisons were made within each size classes among sediments with different vegetation cover. Small particles were tested with a one-way ANOVA and log-transformed data. Medium and large particles were compared using a negative binomial GLM with a square root link function. We also compared the relative abundance of microplastic shapes in each sediment type using PERMANOVA with 999 permutations and a Bray-Curtis distance matrix.

4.3 Results

4.3.1 Site characteristics.

Sediment properties across all types of vegetation cover were similar (Table 4.1). Across the sampled area, sediment was largely comprised of silts, ranging from 57% in a diverse area to 73% in a grass area. Grass areas had the highest proportion of silt and clay, followed by branched, bare, then diverse (Table 4.1). Grain size distributions were also similar. Mean grain size ranged from 12.9 ± 1.5 µm in grass areas to 22.8 ± 5.9 µm in bare areas. However, sediments varied in mode grain size, with diverse vegetated sediment at 104.0 ± 4.2 µm followed by branched at 87.9

± 28.1 µm, bare at 49.5 ± 33.2 µm, and grass at 29.8 ± 9.3 µm (Table 4.1; Figure 4.2). Mean water content of sediment across all sampled areas was 55 ± 6% and ranged from 44% in a branched area to 69% in a diverse area. Average organic matter and organic carbon was 11 ± 3% and 5 ± 1%, respectively. A diverse area had the highest organic matter (18%) and organic carbon (8%), and a grass area recorded the lowest organic matter (7%) and organic carbon (4%). Vegetation canopy cover varied in the grassy (75% to 90%) and diverse areas (85% to 100%) but branched was consistently 100% covered. Average canopy height was highest in a grass area at 58 cm and lowest in a diverse area at 14 cm.

Table 4.1. Mean ± standard deviation of sediment and vegetation properties for each sampled area.

	Bare	**Grass**	**Branched**	**Diverse**
Sediment properties				
Total organic matter (%)	10.3 ± 1.2	7.3 ± 0.5	11.5 ± 1.5	13.2 ± 2.9
Total organic carbon (%)	5.3 ± 0.4	4.2 ± 0.2	5.8 ± 0.6	6.4 ± 1.1
Mean grain size (μm)	22.8 ± 5.9	12.9 ± 1.5	18.2 ± 2.8	21.9 ± 1.6
Mode grain size (μm)	49.5 ± 33.2	29.8 ± 9.3	87.9 ± 28.1	104.0 ± 4.2
Median grain size (μm)	27.4 ± 7.4	15.2 ± 2.0	22.4 ± 3.9	27.7 ± 2.3
% Sand (63.01 – 2000 μm)	23.7 ± 7.9	10.2 ± 3.2	22.2 ± 5.7	28.8 ±2.5
% Silt (4.01 – 63 μm)	66.4 ± 5.8	71.4 ± 1.7	61.7 ± 4.3	58.8 ± 1.8
% Clay (0.01 – 4 μm)	9.9 ± 3.0	18.4 ± 1.6	15.2 ± 1.6	12.4 ± 1.0
Water content (%)	56.6 ± 6.3	51.6 ± 1.3	51.0 ± 4.2	59.3 ± 6.5
Vegetation properties				
Canopy cover (%)	NA	83.8 ± 7.5	100.0 ± 0.0	95.0 ± 7.1
Number of vegetation species	NA	1.0	1.0	4.6 ± 1.1
Canopy height average (cm)	NA	48 ± 9.1	22.3 ± 3.1	25 ± 9.8

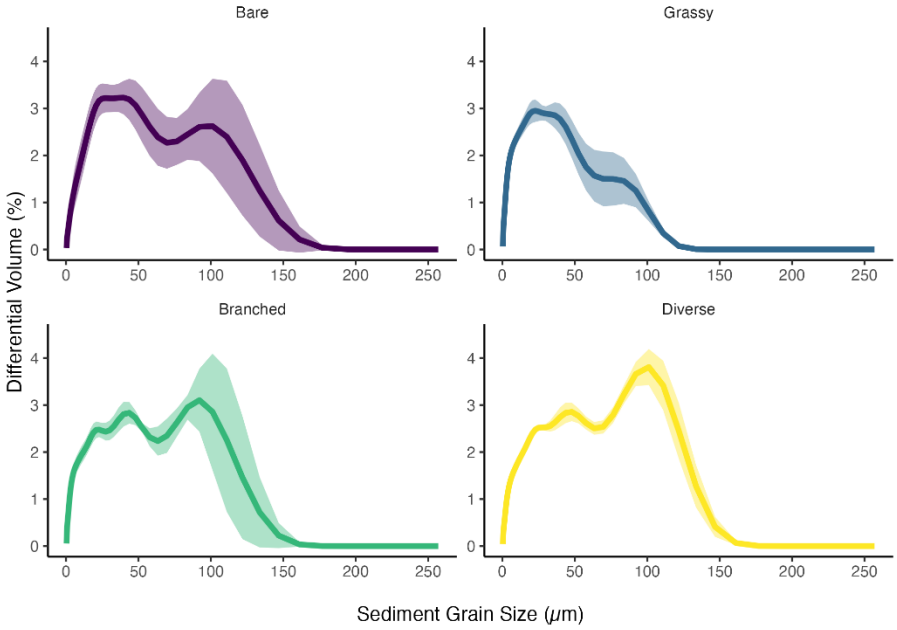

Figure 4.2. Sediment grain size distributions for each type of vegetation cover. Solid line indicates the mean percent differential volume. Shaded areas represent the standard deviation.

4.3.2 Microplastic content was higher in unvegetated areas.

Mean microplastic content (± standard deviation) in sediment across all areas was 466 ± 228 per kg dry sediment. Unvegetated sediment had a higher microplastic content (643 ± 358 microplastics per kg) than vegetated sediment (407 ± 138 microplastics per kg; Figure 4.3). The lowest mean content was 363 ± 67 per kg for grass areas. Branched and diverse areas had mean microplastic content of 480 ± 152 per kg and 379 ± 170 per kg, respectively.

Microplastic content was significantly lower in grass areas compared to bare areas (p = 0.038, pseudo r^2 = 0.27) and diverse areas were borderline significant (p = 0.054) compared to bare areas (Table S4.1; Figure 4.3). There were no significant differences among any other groups.

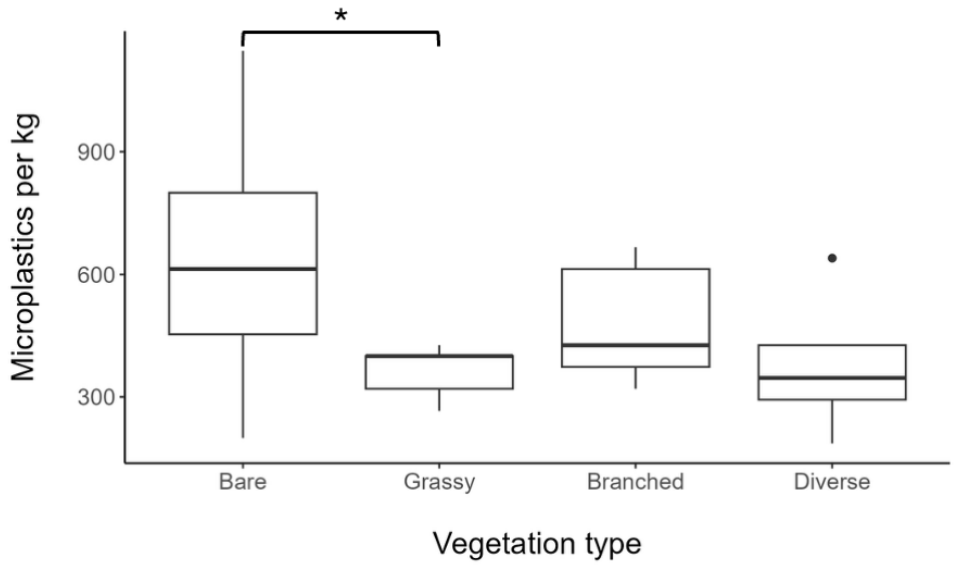

Figure 4.3. Microplastic content in sediments with different types of vegetation cover in microplastics per kg dry sediment. Asterisk and bracket indicate significant differences between groups (gamma GLM with log link function; p = 0.038). Boxes represent the interquartile range (IQR; 25th -75th percentile), horizontal line indicates the median, and whiskers extend to the maximum and minimum values within 1.5x the IQR. Individual points beyond the whiskers are outliers.

4.3.3 Microplastic characteristics were consistent across sediments.

Mean (± standard deviation) anthropogenic particle length was 588 ± 840 µm and median length was 226 µm across all areas (Table S4.2). Particles sizes ranged from 24 to 5484 µm in length and 7 to 1157 µm in width. There was no effect of vegetation type on the size of microplastics recorded (Table S4.3; Figure 4.4). However, when split into size classes of small (<300 µm), medium (300 – 1000 µm), and large (>1000 µm), bare areas had significantly more 'medium' sized microplastics than grass (p = 0.02; pseudo r^2 = 0.32) or diverse areas (p = 0.01; Table S4.5; Figure 4.5). There were no differences in small and large particles among sediment types (Table S4.4, S4.6).

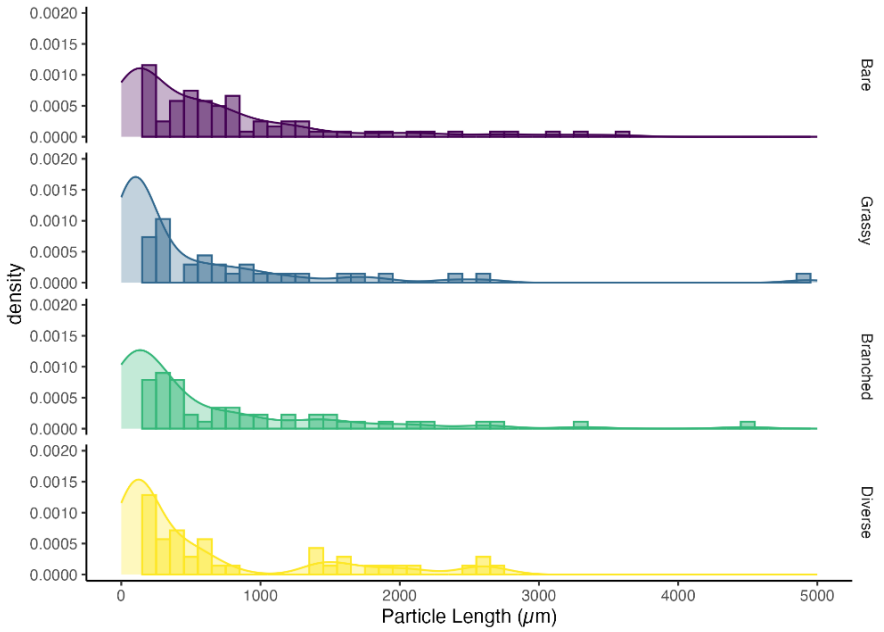

Figure 4.4. Density plot and histogram of anthropogenic particle lengths in sediments of different vegetation cover.

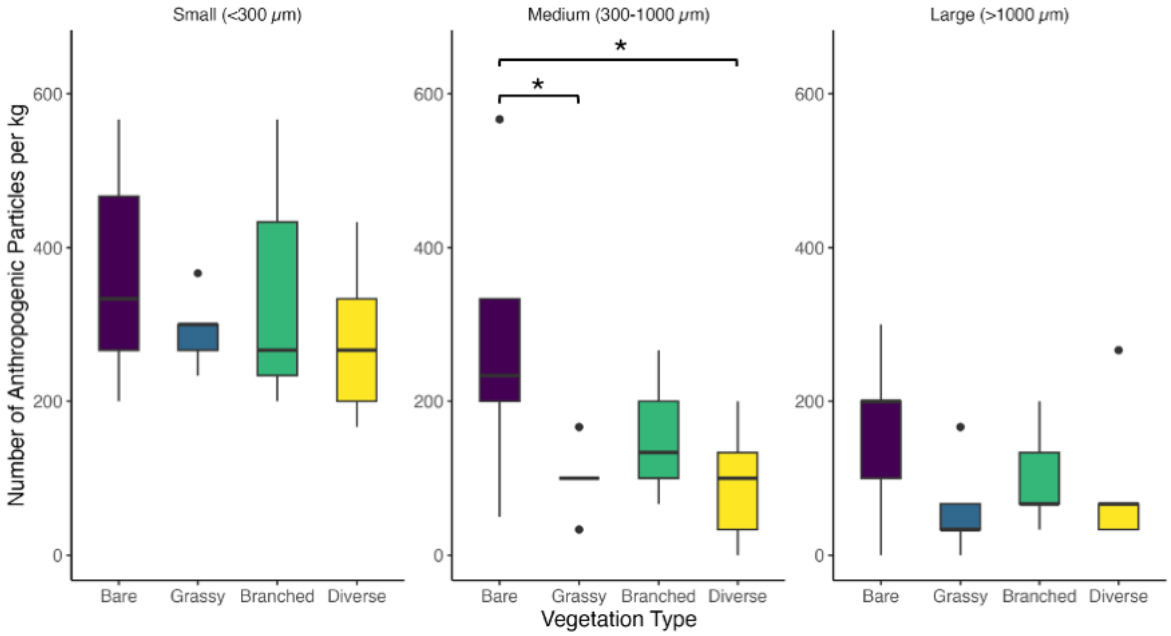

Figure 4.5. Number of anthropogenic particles per kg dry sediment, split into three size classes, across sediments of different vegetation cover.

Across all areas, anthropogenic particles were largely composed of fibres (46.3%) and fragments (44.9%), followed by films (6.5%), spheres (1.7%), and foams (0.6%). Microplastic shape composition within sediments of each vegetation type were similar and there was no significant difference in microplastic composition across areas (Figure 4.6; PERMANOVA, pseudo-$F = 0.8$, df = 3, 16, $p = 0.7$).

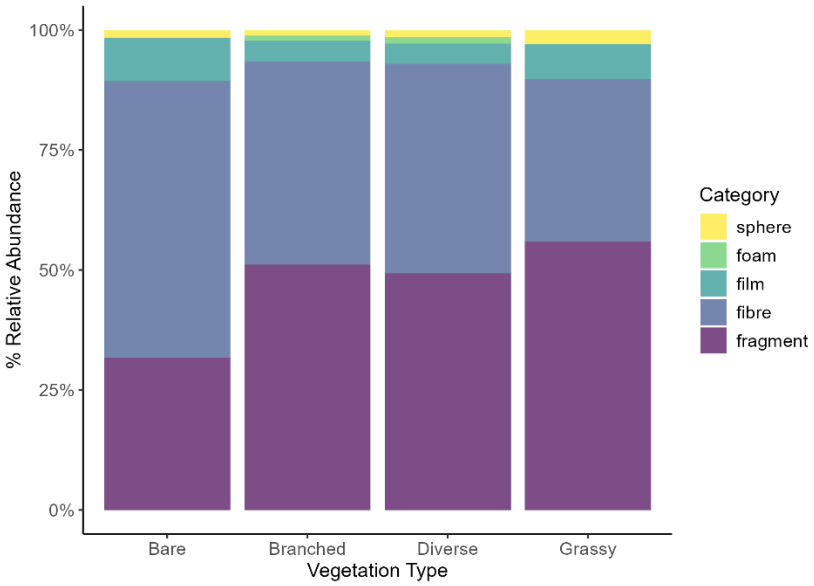

Figure 4.6. Percent relative abundance of microplastic categories in sediments of different vegetation cover.

Of the particles subsampled for micro-FTIR, 80.4% were plastic, 8.0% were anthropogenic synthetic, 5.4% were anthropogenic cellulosic, 3.6% were unknown, and 2.7% were cellulosic (Figure 4.7). The most common plastic polymer was polyester (45.6%) followed by acrylic (18.9%) and alkyds (12.2%). Other identified polymers include polypropylene (7.8%), polyethylene (3.3%), epoxy resins (3.3%), copolymers (3.3%) and other (5.6%). 'Other' included one polybutylene adipate-co-terephthalate particle, one polyisobutene, one polyvinyl alcohol, and two polyamide particles. Based on material ID, shape, texture, and colour, 28.6% of particles were identified as paint particles.

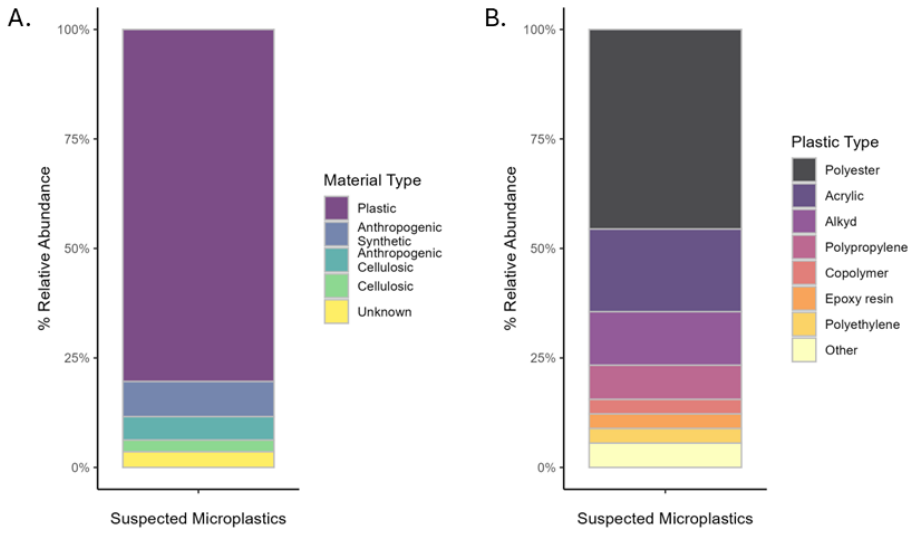

Figure 4.7. (A) Percent relative abundance of anthropogenic particle material types identified by micro-FTIR. (B) Percent relative abundance of microplastic polymer types identified by micro-FTIR.

4.4 Discussion

In this study, we show that microplastics content differed across saltmarsh sediments with varying vegetation structure and diversity. Bare areas had a higher microplastic content and larger variability than any of the vegetated areas. However, there was no difference in microplastic content among vegetated areas with differing structure or diversity. Microplastic shapes and sizes were similar across all sampled areas.

4.4.1 Microplastic content in saltmarsh sediments

Microplastics were present in all areas across the saltmarsh, including in unvegetated areas, ranging from 187 to 1147 microplastics per kg dry sediment, which is lower compared to other saltmarshes in the UK, such as those in the River Thames, which had microplastic contents of 1520 to 2234 per kg dry sediment, but this is a significantly more populated and polluted estuary (Trusler et al. 2025). The microplastics content recorded in this study are still within the range of what others have found in saltmarsh surface sediments across the globe, from 23 ± 18

microplastics per kg dry sediment in the Ria Formosa Lagoon, Portugal to 2500 microplastics per kg dry sediment in the Lima estuary, Peru (Cozzolino et al. 2020, Almeida et al. 2023).

4.4.2 Spatial variability of microplastics

Contrary to our hypothesis, sediments without vegetation had higher microplastic content than vegetated sediments. One reason for this difference could be due to the spatial variability across the site. Topographical changes affect hydrodynamic flow, either causing water to flow faster or slower due to changes in water depth (Christiansen et al. 2000, Torres and Styles 2007) which can affect microplastic resuspension and transport. Moreover, elevation affects the water submergence time and prolonged periods of submergence can decrease microplastic settlement (Wu et al. 2020). Here, bare, branched, and diverse vegetated sediment were located within the mid to high marsh zone and at high elevation, while the areas with monospecific grass vegetation were at the seaward edge of the marsh and low elevation (Figure 4.1; Figure S4.1). The lower microplastic content at the seaward marsh edge and lower elevation compared to the interior of the marsh and higher elevation is in line with other saltmarshes around the globe (Pinheiro et al. 2022, Girones et al. 2024, Trusler et al. 2025). In mangrove sediments, Xu et al. (2023a) also observed lower microplastic content at the lowest elevational gradient compared to the highest elevation. However, others have recorded the opposite trend, with higher levels of microplastics at low elevation marsh edges (Yao et al. 2019, Helcoski et al. 2020). While elevation may be one possible explanatory factor, the conflicting patterns of microplastic accumulation across coastal vegetated habitats highlights how the diversity in site characteristics as well as local hydrodynamics can affect microplastic fate.

Microplastic accumulation may also be related to sediment composition (Enders et al. 2019, Radford et al. 2024, Greenshields et al. 2025). Radford et al. (2024) found a negative correlation between microplastics and percent clay content in seagrass sediments. While we did not find a correlation between percent clay content and microplastic content, there was a higher proportion of clay in the grassy sediments than in the bare sediments. There is some evidence to suggest that coarser grained sediments are better able to retain microplastics because there is more space for downward migration (Mancini et al. 2023, Radford et al. 2024, Greenshields et al.

2025). Alternatively, many have suggested that because microplastics are similar in size and density to small particulate sediment, such as clay, they will have similar transport behaviour (Browne et al. 2010, Harris 2020). Indeed, studies comparing across larger spatial scales have found a positive trend between clay content and microplastics in sediment (Enders et al. 2019, Marques Mendes et al. 2021). Microplastic transport and settling has proved difficult to predict because there are many additional factors (e.g., biofouling, salinity) that can influence microplastic transport that are not included in standard sediment transport formulae (Mendrik et al. 2023). This further highlights the complex set of interacting factors controlling microplastics distribution.

Alternatively, the higher levels of microplastics found in the bare areas could be due to the quiescent water within tidal pools, which would allow more time for microplastics to settle out. Saltmarsh pools are not always strongly flushed after tidal flooding and there is limited organic matter trapping and export within them (Spivak et al. 2017). Notably, the variance across bare areas was much larger than the variance observed in any of the vegetated sites (Figure 4.3). This could reflect the temporal variability and fluctuations that are experienced within the saltmarsh and the varying size and depth of the tidal pools that retain microplastic. It is possible that microplastics in vegetated areas are more consistent because there is a reduction in resuspension, erosion, and wave disturbance compared to unvegetated areas which might be more susceptible to fluctuations in microplastic content (Xu et al. 2023a). Moreover, sediment stability (and by association, microplastic stability) differs temporally, for instance when sediment is exposed or submerged (Tolhurst et al. 2006a). There could therefore be variations in microplastic content depending on if sampling occurs during low tide, when only pools are submerged, compared to when the whole marsh is flooded.

There was also no difference in microplastic content across increasing levels of vegetation complexity or diversity. This is consistent with an experimental flume study that found no difference in sediment microplastic concentration between grassy and branched structures (McIlwraith et al. 2024). This suggests that there are factors other than the vegetation cover and complexity that are driving microplastic accumulation dynamics in coastal wetland systems.

However, structural differences measured by inter- and intraspecific variation within other biogenic canopies, such as macroalgae and mussel beds have been shown to influence microplastic accumulation (Nicastro et al. 2022, Cozzolino et al. 2022). This has also been observed when comparing across different biogenic canopy types. Field flume experiments revealed hard corals trapped more microplastics than seagrass or macroalgae (de Smit et al. 2021). There could be many additional factors that may explain these differences. For instance, canopy density and spatial arrangement can influence microplastic accumulation (Lim et al. 2020, de los Santos et al. 2021). Here, percent canopy cover was similar across vegetated areas (Table 4.1) and may not have demonstrated sufficient variation to cause a pattern in microplastics distribution.

4.4.3 Microplastic characteristics

The sizes of anthropogenic particles were similar across sediment of different vegetation cover (Figure 4.4). Most particles were less than 300 µm, which is consistent with others who have found increasing environmental microplastic concentrations with decreasing particle sizes (Lindeque et al. 2020). Smaller particle sizes are more likely to be ingested by or adhere to benthic organisms and pose a higher risk because of an increased surface area to volume ratio and increased likelihood to translocate into the circulatory system (Browne et al. 2008, Wright et al. 2013, Bucci et al. 2020, Woodhouse et al. 2025).

Fibres and fragments were equally dominant across all sediments. Other studies in saltmarsh sediments found fibres as the most abundant shape, followed by fragments (Helcoski et al. 2020, Cozzolino et al. 2020, Trusler et al. 2025). The higher abundance of fragments observed here could be due to the prolific boating activity in the area. Many fragments were acrylic, alkyd, or epoxy resin which are used in boat coatings and paints. Paint particles are only recently being recognized as a type of microplastic (Muller-Karanassos et al. 2019, Turner 2021, Diana et al. 2025). They are often overlooked due to inconsistent identification techniques, and their high density makes them difficult to extract from sediments with current methods (Diana et al. 2025). However, modelling suggests that paints are a considerable source of microplastic to the environment and more effort is needed to recognize and quantify paint particles as a form

of microplastic pollution (Paruta et al. 2022, Zhu et al. 2024, Diana et al. 2025). Many of the fibres identified were polyester and acrylic. These polymers are commonly used in clothing and textiles (Carr 2017). These fibres can enter the environment via wastewater effluent or shedding during use and make up a substantial proportion of global microplastic pollution (De Falco et al. 2020, Athey and Erdle 2022).

4.4.4 Limitations and further considerations

Here, we sampled sediment once in the late summer/early autumn season due to timing constraints, but sampling at other times of the year (e.g., spring, summer) could have revealed alternative patterns. Microplastic depositional patterns in surficial sediment could change depending on the time of year. Storm surges and rainfall can impact microplastic levels by re-releasing particles or flushing upstream microplastics into downstream systems (Wang et al. 2019, Cheung and Not 2023). Low-tide rainfall has been observed to increase sediment erosion in saltmarshes (Torres et al. 2004, Tolhurst et al. 2006b), which could also increase microplastic release. Moreover, the seasonal cycles of vegetation could impact microplastic content in sediments. There is evidence that microplastics can adhere to the vegetation itself (Gutow et al. 2016, Jones et al. 2020) and once leaves are shed, they may contribute microplastics to the sediment. In this study, we did not sample vegetation because of the protected status of the site, but it is possible that the vegetation acted as a filter and intercepted the microplastics prior to reaching the sediment, whereas all microplastics in unvegetated sites would have deposited (McIlwraith et al. 2024). On smaller temporal scales, Wu et al. (2020) observed differences in microplastic content between vegetated and unvegetated sediments only during neap tidal cycles but not spring tidal cycles. This was only observed in the top 2 cm of sediment, suggesting that some microplastics are not sequestered into deeper sediments and are more easily subjected to tidal influence (Wu et al. 2020). While comparing microplastic content across sediment depths was outside the scope of this study, comparing only the top 2 cm of sediment could have revealed more transitory patterns.

It is difficult to compare microplastic content across studies due to differences in laboratory and sampling techniques (McIlwraith et al. 2025). Our methods had an 83% recovery

efficiency, thus microplastic content is likely higher than what we have reported. However, many studies do not report recovery efficiency at all (Way et al. 2022, McIlwraith et al. 2025). It is also important to consider units used to express microplastic abundance. We express microplastic content as number per kg dry sediment. This is the most common and recommended unit used and allows comparison across other studies in microplastics research (Brander et al. 2020). However, in muddy environments, expressing units on a per volume vs. per mass basis can change the patterns in and significance of results (Tolhurst et al. 2005). This is because soft sediments are composed of many different components that co-vary with one another (e.g., cohesive and non-cohesive particles, water, gas, biota, and other matter). Patterns can then be enhanced or masked when standardized by mass because the results will largely be determined by the amount of sand (the heaviest component). Thus, once a biogeochemical variable is standardized by mass, it will not be independent from other biogeochemical properties standardized by mass, which will influence the interpretation of data potentially leading to incorrect inference (Flemming and Delafontaine 2000, Tolhurst et al. 2005).

4.4.5 Conclusions

The spatial distribution of microplastics in sediment differed across the saltmarsh but not in the way we expected. The differences observed across the site were more likely driven by other factors, such as elevational differences, rather than the complexity of vegetation structure or diversity. However, the high variability of microplastic content in unvegetated sediments compared to vegetated sediments is indicative of reduced resuspension of particles, likely from wave dampening by vegetation (Xu et al. 2023b). Ultimately, microplastic fate in saltmarshes is not governed by the simple presence or absence of vegetation but likely a multitude of interacting variables.

Chapter 4: Supplementary Information

Table S4.1. Results of generalized linear model comparing number of microplastics per kg dry sediment across sediments with different vegetation cover using a gamma distribution and log link function.

	Estimate	Standard Error	t-value	p-value
Intercept	-0.4421	0.1797	-2.460	0.0256 *
Bare - Grass	-0.5721	0.2541	-2.251	0.0388 *
Bare - Branched	-0.2918	0.2541	-1.148	0.2677
Bare - Diverse	-0.5290	0.2541	-2.082	0.0538 .

AIC = -5.9314; null deviance = 3.9658 on 19 degrees of freedom; residual deviance = 2.8939 on 16 degrees of freedom.

Table S4.2. Mean ± standard deviation, median, minimum and maximum lengths of anthropogenic particle found in sediments of different vegetation cover.

	Bare	*Grass*	*Branched*	*Diverse*
Mean ± sd (µm)	680 ± 940	475 ± 788	565 ± 799	564 ± 746
Median (µm)	384	134	242	183
Minimum (µm)	35	39	24	36
Maximum (µm)	5484	4930	4484	2732

Table S4.3. Results of a generalized linear model comparing the length of anthropogenic particles among sediments with different types of vegetation cover using a gamma distribution and log link function.

	Estimate	Standard Error	t-value	p-value
Intercept	6.5221	0.1295	50.346	<2e-16***
Bare - Grass	-0.3582	0.2171	-1.650	0.0999 .
Bare - Branched	-0. 1857	0.1999	-0.929	0.3536
Bare - Diverse	-0.1865	0.2151	-0.867	0.3867

AIC = -5152.4; null deviance = 565.63 on 349 degrees of freedom; residual deviance = 559.77 on 346 degrees of freedom

Table S4.4. Comparison of the number of small (<300 µm) anthropogenic particles per kg dry sediment across sediments with different vegetation cover using an ANOVA and log transformed data.

	df	Sum Sq	Mean sq	F-value	p-value
Vegetation type	3	0.1843	0.06144	0.452	0.719
Residuals	16	2.1729	0.13581		

Table S4.5. Comparison of the number of medium (300 – 1000 µm) anthropogenic particles per kg dry sediment across sediments with different vegetation cover using a negative binomial GLM and square root link function.

	Estimate	Standard Error	t-value	p-value
Intercept	-2.5826	0.3789	-6.816	4.15e-06 ***
Bare - Grass	-1.1626	0.4720	-2.463	0.0255 *
Bare - Branched	-0.7499	0.4892	-1.533	0.1448
Bare - Diverse	-1.2214	0.4698	-2.600	0.0193 *

AIC = 104.88; null deviance = 29.45 on 19 degrees of freedom; residual deviance = 20.13 on 16 degrees of freedom.

Table S4.6. Comparison of the number of large (> 1000 µm) anthropogenic particles per kg dry sediment across sediments with different vegetation cover using a negative binomial GLM and square root link function.

	Estimate	Standard Error	t-value	p-value
Intercept	-3.2550	0.4087	-7.965	5.87e-07 ***
Bare - Grass	-0.8806	0.5059	-1.741	0.101
Bare - Branched	-0.4902	0.5301	-0.925	0.369
Bare - Diverse	-0.5489	0.5261	-1.043	0.312

AIC = 97.43; null deviance = 24.22 on 19 degrees of freedom; residual deviance = 20.80 on 16 degrees of freedom.

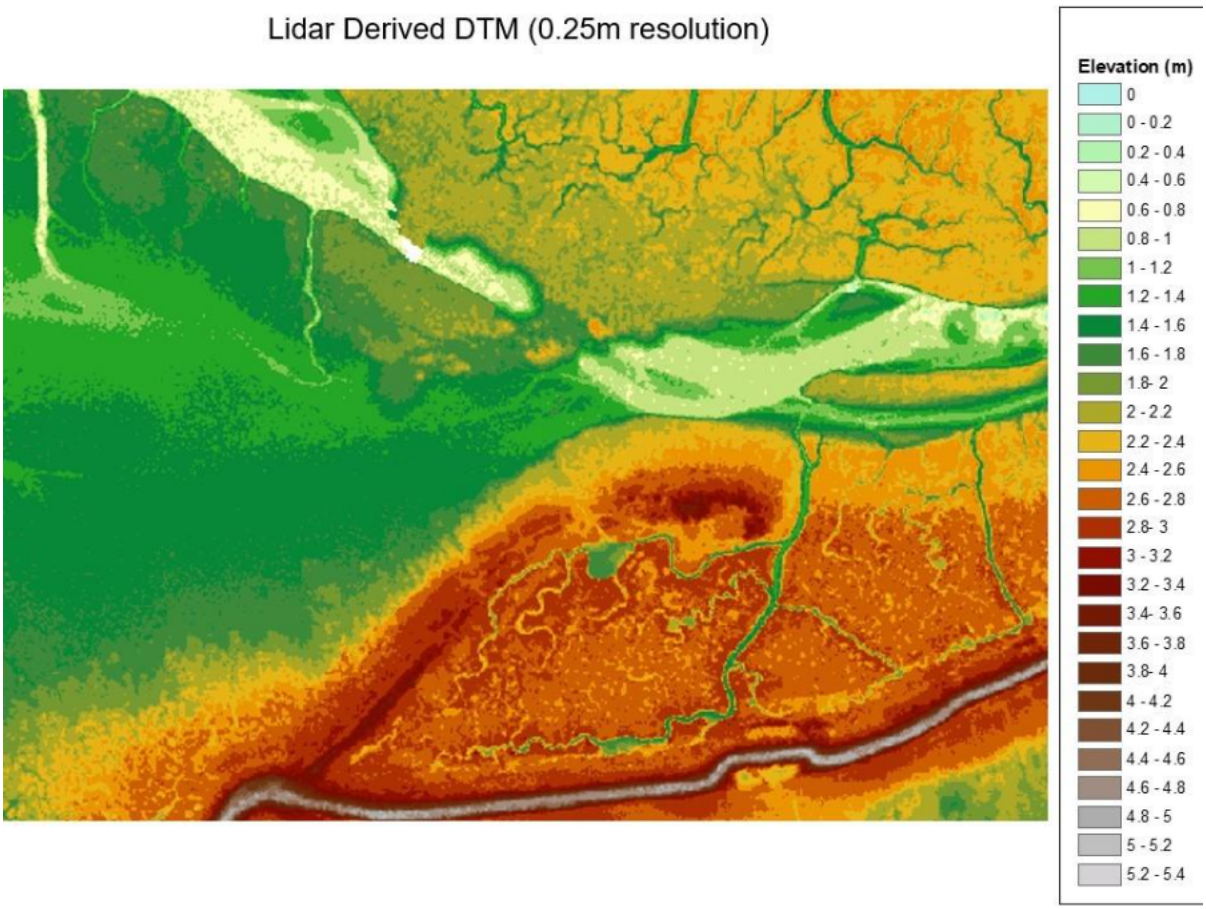

Figure S4.1. Digital Terrain Model (DTM) of the sampled area. Courtesy of Raymond Torres.

Chapter Five

Saltmarsh Sink: The Role of Saltmarsh Vegetation in Microplastic Flux in the Tamar Estuary, UK

This chapter is being prepared for publication.

McIlwraith, H.K., Lindeque, P.K., Tolhurst, T.J., and Cole, M.,

HM conceptualized and carried out the experimental design, investigation and data collection, formal analysis, and writing. MC, TT, and PKL contributed to conceptualization, experimental design, and review and editing.

Coastal vegetated habitats are known to accumulate plastic debris stemming from land and sea. Entrapment of microplastics occurs within sediment and on the vegetation. However, little is known about the transport of microplastics after vegetation senescence. Through a field investigation we explored whether microplastics are exported from a saltmarsh by adhering to detritus. Microplastics and other anthropogenic particles were quantified and characterised across three leaf stages (alive, dead, outflowing detritus) of sea purslane (*Atriplex portulacoides)* and from an ambient control (outflowing tree detritus) from a saltmarsh in the Tamar estuary, UK in October 2024. Live and dead leaves were collected from six quadrats within the saltmarsh while outflowing and ambient control leaves were collected via six net samples off a bridge. Microplastics on leaves were isolated in the laboratory by washing on an orbital shaker with NaClO and Tween80, vacuum filtered and characterised by micro-FTIR. Anthropogenic particles were present on all leaf stages and concentrations were highest on dead, detached leaves within the saltmarsh (0.74 ± 0.33 particles cm^{-2}). Concentrations for live leaves, outflowing detritus, and the ambient control were 0.30 ± 0.40, 0.33 ± 0.27, and 0.19 ± 0.08 particles cm^{-2}, respectively. The number of anthropogenic particles per leaf was related to leaf surface area, with larger leaves carrying more particles. Moreover, the presence of anthropogenic particles, including microplastics, on outflowing saltmarsh detritus indicates that microplastics can be exported out of saltmarshes. With even a small proportion of export, we estimate that saltmarsh vegetation could transport between 22 million to 4.4 billion anthropogenic particles into the estuary with

every tide. This value will vary significantly because of the complex nature of these systems, and the dynamic and transient features of coastal vegetated ecosystems should be accounted for when considering them as a microplastic sink. Still, recognizing the importance of above-ground vegetation as a compartment for microplastics can further our understanding of microplastic risk in the environment relevant to environmental loads and organism exposure.

5.1 Introduction

The prevalence of plastics in saltmarsh, seagrass, and mangrove habitats is generating concern for ecosystem health and management (Rangel-Buitrago et al. 2024). Coastal vegetated ecosystems contribute to many ecosystem services that are relied on by society, such as storm protection, biological productivity, and water quality maintenance (Barbier et al. 2011, Spalding et al. 2014, Hagger et al. 2022). Plastics generated from land and marine-based activities enter these habitats and pose a threat to their functioning (Rangel-Buitrago et al. 2024). For example, macroplastic debris can cause negative effects to marine life via entanglement and ingestion (Barboza et al. 2018); and when plastic breaks down into small plastic pieces called microplastics (insoluble plastic 0.001 – 5 mm in size), it becomes bioavailable to a host of organisms, with the potential to impact on organism health and ecological function (Browne et al. 2011, Lusher 2015, Seeley et al. 2020, Coppock et al. 2021, Pinheiro et al. 2022, Thompson et al. 2024, Lee et al. 2025).

There is increasing evidence that coastal vegetated habitats can act as reservoirs for microplastic pollution, with larger concentrations observed in vegetated sediments compared to unvegetated sediments (Huang et al. 2020, 2023, Jones et al. 2020, Zhao et al. 2022, Pinheiro et al. 2022). Microplastic deposition in these systems is postulated to be driven by the physical influence of biogenic canopies on turbulence and water flow, a property that increases sediment deposition (Leonard and Croft 2006, Spalding et al. 2014, Waldschläger et al. 2022). However, microplastics may also be trapped by epibenthic organisms such as filter feeding bivalves, and by adherence to the vegetation itself (Goss et al. 2018, Jones et al. 2020, Lim et al. 2020, Nicastro et al. 2022). Adherence of microplastics on vegetation has been observed on macroalgae, seagrasses and mangrove leaves (e.g., Gutow et al. 2016, Goss et al. 2018, Jones et al. 2020, Feng et al. 2020, Sfriso et al. 2021, Mateos-Cárdenas et al. 2021, Li et al. 2022a). Adherence to vegetation is not unique to marine species, with microplastics observed on terrestrial and freshwater plants as well (e.g., Liu et al. 2020, Mateos-Cárdenas et al. 2021, Perera et al. 2024). In an enclosed flume set-up, microplastics were demonstrated to adhere to both simple and branched artificial plants (McIlwraith et al. 2024). Some have suggested that terrestrial and mangrove leaves are an important sink for microplastics and could act as bioindicators of

pollution (Liu et al. 2020, Li et al. 2022a, Sudhakar and Mathew 2025). Only one previous study has investigated a saltmarsh species and found no microplastics adhered (Cozzolino et al., 2020); although, the presence of microplastics on other macrophytes suggests they should also adhere to saltmarsh vegetation. The ability to capture and retain microplastics is likely dependent on leaf morphology (Bi et al. 2020, Sudhakar and Mathew 2025). Microplastic concentrations could also be affected by factors such as geographic location, urbanization, species differences, and methodology (de Smit et al. 2021, Lloret et al. 2021, Hernán et al. 2024, McIlwraith et al. 2025).

The adherence of microplastics to plants poses potential concerns for organism health. Micro- and nanoplastics can adversely impact upon photosynthetic ability, growth, and nutrient cycling in higher plants (Gerstenbacher et al. 2022, Menicagli et al. 2022). There is also evidence that leaves can absorb nanoplastics through stomatal pathways and migrate to vascular tissue or accumulate in trichomes (Li et al. 2025). Epibionts living on the plants, and herbivores and detritivores that rely on living and decaying plant matter will also be exposed to adhered microplastics (Goss et al. 2018, Gutow et al. 2016). Indeed, ingestion of microplastics by common saltmarsh species, such as *Littorina Littorea* and *Corophium volutator*, has been observed, including directly from macrophytes (Gutow et al. 2016, Woodhouse et al. 2025). Adherence to plants thereby provides an additional entry point to marine food webs that poses a risk to the health and ecological functioning of these biotic communities (Gutow et al. 2016, Sussarellu et al. 2016, Paul-Pont et al. 2016, Goss et al. 2018, Coppock et al. 2021).

Saltmarshes are highly productive habitats, with net primary production (NPP) reaching up to 3700 g m^{-2} y^{-1} (Gallagher et al. 1980). However, part of this productivity can be exported and exchanged with adjacent estuarine and coastal ecosystems according to the “outwelling” hypothesis (Odum 1980). This states that coastal ecosystems are interconnected and exchange energy in the form of nutrients, organic matter, and organisms but this is dependent on the locality and physical characteristics of the wetland (Odum 1980). In the case of saltmarshes, detrital export rates are highly variable and can range from <1% to 45% of NPP (Teal 1962, Bouchard and Lefeuvre 2000). The retention or export of detritus is dependent on physical and biological influences such as tidal regime, storm surges, elevational gradients, topography, wind,

decomposition rates, and infauna and microbial communities (Dame 1982, Valiela et al. 1985, Halupa and Howes 1995, Bouchard et al. 1998, Bouchard and Lefeuvre 2000, Caçador et al. 2009). The life cycle of leaves is an important aspect when considering the environmental fate of microplastics that adhere to this transient vegetative matter. Sanchez-Vidal et al. (2021) observed microplastics in seagrass leaf-detritus balls which get transported onto beaches, ultimately bringing microplastics out of the vegetated area. The release of contaminants via detritus from estuarine and coastal systems has been quantified for heavy metals and this export is expected to increase due to sea level rise and increased flooding events (Caçador et al. 2009, Duarte et al. 2017). As such, it is important to consider the transient nature of above-ground vegetation when evaluating microplastic adherence and investigating the role of above-ground vegetation on microplastic fluxes will further our understanding of vegetated wetlands as a microplastics source, sink or temporary repository.

To this end, we tested the hypothesis that microplastics are exported out of vegetated systems via detritus because they adhere to saltmarsh leaves. We used field measurements to characterize and quantify microplastics on saltmarsh leaves at different stages of the leaf life cycle. We predict that saltmarsh leaves will have adhered microplastics, including on leaves that are outflowing from the system; that concentrations will be largest on leaves that are oldest and detached from the plant due to larger surface areas; and that although export is small compared to what is retained in the marsh, a non-negligible amount of microplastics will leave saltmarsh systems via detritus.

5.2 Methods

5.2.1 Study site

The sampling area was a semi-enclosed tidal creek located to the north-west of Plymouth in the Tamar Estuary, United Kingdom (50°25'23"N 4°11'03"W; Figure 5.1). Collection occurred in October 2024 (Temperate autumn) during a spring tide, with a maximum height of 5.51 m, wherein the entire marsh is submerged. The sampling area contains 2600 m^2 of saltmarsh and the wider area is a Marine Conservation Zone, Special Area of Conservation, Site of Special Scientific Interest, and a Special Protection Area. This public recreational area is primarily used

for walking, fishing, and baiting and is bordered by a residential area and a wastewater treatment site. However, it has a history of being subject to fly-tipping, littering, and abandoned vessels (Colwill 2024). In 2016, there was a major pollution incident in Tamerton stream, upstream of the sampling area, caused by a blocked sewerage pipe from commercial wet wipes, resulting in substantial impacts on the local fish population (Environment Agency 2019).

5.2.2 Sample design

Microplastics adhered to leaves were quantified from three leaf stages of one saltmarsh species – sea purslane *Atriplex portulacoides*, and one ambient control group. The first two leaf stages were collected within the saltmarsh and included leaves still attached to the plant (S_{live}) and leaves that had fallen (detached; S_{dead}). The third stage were leaves outflowing from the marsh as floating detritus ($S_{outflowing}$). Tree leaves that had fallen into the water and were also outflowing from the marsh area were included as an ambient control (C_{tree}). Two methods of sampling were used. For S_{live} and S_{dead} leaves, samples were collected at low tide, when the whole marsh was exposed, and used quadrats with a random sampling design. Outflowing detritus was collected during an ebb tide with nets dropped from two footbridges.

Figure 5.1. (A) Location of the sampling area was in the Tamar Estuary, United Kingdom. (B) Sampling was located near a residential area, north-west of Plymouth. (C) Yellow arrows indicate the direction of ebb tide and the location of outside marsh sampling where both $S_{outflowing}$ and C_{tree} leaves were sampled. Orange circles represent the within marsh sampling where both S_{live} and S_{dead} leaves were sampled during low tide.

5.2.3 Sample collection

Within marsh sampling

For leaf samples within the marsh, we used six 0.25 x 0.25 m quadrats placed in a random sampling design (Figure 5.1c). A random sampling approach was selected to capture microplastic concentrations across the whole marsh and therefore compare the overall within marsh levels to

outflowing marsh leaves. Using ArcGIS software, a grid was overlaid on the sampling area, with each square equivalent to a 3 x 3 m area. A random number generator was used to select grid coordinates for sampling. If a sampling point was placed on an area with no vegetation, the random number generator was repeated to get new grid coordinates. After points on the grid were determined, latitude – longitude coordinates were recorded using the location tool in ArcGIS Online. Waypoints were saved to a GPS app (GAIA GPS) and used in the field to place the quadrats.

Within each quadrat (N = 6), three samples of S_{live} leaves were collected by cutting at the petiole with scissors (N = 18). Each sample included five leaves from one branch, for a total of 15 leaves per quadrat. Leaves were cut from nodes across the branches from mid-canopy level to top canopy level and young leaves (less than 1 cm in length) were avoided. Sets of five leaves were placed into covered aluminium foil trays. All of the S_{dead} leaves within the quadrat were collected and placed into one aluminium tray and total number of S_{dead} leaves per quadrat was counted once in the laboratory. Total sample size was N = 18 for S_{live} and N = 6 for S_{dead} leaves. All leaves were handled with stainless-steel tweezers, and all equipment was rinsed with ultrapure water between quadrats. Percent canopy cover, species presence, average and maximum canopy height, and GPS coordinates were also recorded. We also recorded the number of branches within each quadrat and the number of leaves on three branches to estimate the number of S_{live} leaves per quadrat (Table 5.1; Equation 1). Field blanks were collected by placing an empty aluminium tray next to a quadrat throughout the duration of a sample collection (n = 4; two during S_{live} sampling; two during S_{dead} sampling).

Outside marsh sampling

There were two outflows located on the East and West side of the saltmarsh, along the footpath where the sampling area connected with the wider estuary (Figure 5.1c). At the start of an ebb tide, we dropped a net (5 mm pore size; 60 x 50 x 50 cm; polyethylene terephthalate mesh) into each outflow from a footbridge and submerged it to 50 cm depth, with the upper 10 cm of the net entrance above the water surface, for 4 minutes, and repeated this three times at each bridge (N = 6). Debris caught in the net was placed into a stainless-steel mixing bowl (35 cm

x 13 cm). Any saltmarsh leaves that were >90% intact and identifiable were picked out with tweezers and placed into a covered aluminium tray ($S_{outflowing}$; N = 6). Thus, highly degraded leaves were not included. For ambient controls (C_{tree}), 10-16 tree leaves of varying species were collected from the net sample and placed into covered aluminium trays (N = 6). Flow velocity was recorded with a Valeport 801 Electromagnetic Flow Meter for each sample. Three 20 second recordings were taken at the midpoint of the collection time (Table S5.1). Between samples, nets were rinsed off in a bucket containing seawater and tweezers were rinsed with ultrapure water. Sample collection start and end time and sample exposure time during sorting was recorded. Two field blanks were collected by placing an empty aluminium tray next to a sample during the sorting process to capture any atmospheric contamination (one blank per bridge). Upon return to the laboratory, all samples were frozen at -20°C.

5.2.4 Laboratory analysis

Under a laminar flow hood, leaves were photographed with a ruler on aluminium foil for surface area analysis. For S_{dead}, $S_{outflowing}$, and C_{tree} leaves, five of each were selected for microplastic quantification and surface area analysis. Any remaining leaves were also counted, photographed, and species was recorded. Surface area of leaves was determined using the polygon tool in ImageJ software (ImageJ 1.5j8/Java 1.8.0_112), summing the surface area across all five leaves per sample and multiplying by two.

For microplastics analysis, leaves (five per sample) were washed by placing in 720 mL glass jars with a 1% NaClO and 0.5% Tween80 solution and placed in an orbital shaker (Cole-Palmer SI600) for 30 minutes at 180 RPM. Saltmarsh leaves were washed with 100 mL of solution, while tree leaves were washed with 300 mL of solution to ensure they were completely submerged. Leaves were then removed from solution, and the outside of the leaves were rinsed with ultrapure water into the jar. The solution was filtered onto 20 µm nylon mesh filters with vacuum filtration and triple rinsed with ultrapure water. Leaves were placed into glass beakers to oven dry at 50°C for a minimum of two days to measure dry weight.

Putative microplastics were visually sorted under an Olympus SZX16 microscope and were picked out of the sample based on colour and texture (Lusher et al. 2020). Each particle was measured and imaged with CellSens software (version 2.1) and then placed onto a glass slide wrapped in aluminium foil to provide a reflective background for spectroscopy. Black fragments, suspected to be tyre particles, that cannot be characterised using FT-IR, were placed into a 4 mL glass vial for analysis by Py-GC-MS. Micro-FTIR spectroscopy is not sufficient for tyre particle identification, due to the infrared absorbance by the carbon black components of the particles (Rosso et al. 2023).

Of the particles that were not suspected tyre particles (N = 158), 20% were randomly selected for micro-FTIR spectroscopy to confirm synthetic origin (n = 33). This reaches the recommended minimum number of particles needed to accurately represent the proportion of plastic, anthropogenic, and natural particles in an environmental matrix (De Frond et al. 2023). Random selection of particles was carried out by using a random number generator to select one of five glass slides to analyse in its entirety. Chemical identification was accomplished with a Perkin Elmer Spotlight 400 in reflectance mode and wavelength range of 4000-700 cm^{-1}. Number of scans ranged from 8 to 16 and resolution ranged from 4 cm^{-1} to 8 cm^{-1}, adjusted depending on quality of the spectra. Spectra were matched against in-house, published (Primpke et al. 2018), and commercially available databases from SpectrumIR software (Perkin Elmer 2017, version 10.6.0.893). Library search scores > 65% were accepted. If a match was between 50-65% the spectra was collected again in another spot and with different settings up to three times. If all three scans resulted in the same match, it was accepted. If not, or if a match was < 50%, the particle was considered ‘Unknown’. These thresholds were chosen to strike a balance between sample processing time and confidence in spectral matches, and it is within the range used by other studies (60% - 90%; Cowger et al. 2020). Material types were sorted into four categories of “Plastic”, “Anthropogenic Cellulosic”, “Unknown Cellulosic”, and “Unknown”. Semi-synthetic matches, such as rayon or viscose, were classed as ‘cellulosic’ because the spectra are difficult to differentiate from natural cellulosic spectra. Following Cole et al. (2023), cellulosic particles with a colourful dye, such as blue or red, were classed as ‘anthropogenic cellulosic’, while particles without a noticeable dye were classed as ‘unknown cellulosic’ because the origin could not be

determined and could be either anthropogenic or natural. Microplastic concentrations were not corrected for confirmed plastic because 90% were suspected tyre particles and their polymeric origin could not be confirmed. For this reason, particles will be referred to as anthropogenic particles.

5.2.5 QA/QC

Samples were processed in an ultraclean laboratory and under a laminar flow hood and cotton laboratory coats were worn. All surfaces were wiped with 70% ethanol before and after handling a sample and equipment was washed with Decon 90 and triple rinsed with ultrapure water. All blanks (N = 6) were processed as regular samples. The average number of particles in within-marsh and outside marsh blanks was 9 ± 3.7 and 3.5 ± 0.7 particles, respectively. We blank corrected the results according to sampling method by taking the average, rounded to the nearest whole number, of each colour and category in blanks and subtracted these from each sample. For within-marsh samples, this resulted in the subtraction of 1 blue fibre, 4 clear fibres, 1 grey fibre, 1 purple fibre, 1 yellow fibre, 1 blue fragment, 1 clear fragment, 1 green fragment, and 3 black fragments. For outside-marsh samples, this resulted in the subtraction of 3 clear fibres, 1 grey and 1 pink fibre, in addition to all 3 green fibres to account for any potential contamination from the PET net used for sampling. Particles in blanks identified by micro-FTIR were classed as unknown cellulosic, acrylic, or polyester.

Laboratory methods were validated with a spike-recovery experiment (following McIlwraith et al., 2025; Chapter 3). Three sets of five saltmarsh leaves (*Atriplex portulacoides*; n = 3) were spiked with four types of microplastics (polypropylene fragments, polyethylene films, polyamide fibres, and polyester fibres), ranging in size from 244 to 1372 µm in length. Spiked samples were processed as described and recovery of spiked microplastics was recorded to evaluate recovery rates. Average total recovery was 98.8 ± 7.8% (Table S5.2).

5.2.6 Data analysis

Data was expressed in anthropogenic particles per cm^2 leaf surface area by dividing the number of anthropogenic particles by the surface area of the sampled leaves. Data was assessed

for normality and homogeneity of variances with a Shapiro-Wilks test and Bartlett's test, respectively. Assumptions were not met, so a Generalized Linear Mixed Model (GLMM) with a negative binomial distribution and log link function was used to compare anthropogenic particle concentrations across leaf types. Leaf type nested within sampling site was included as a random effect to account for variability at the site level (quadrat and bridge) and the sample type level (S_{live}, S_{dead}, $S_{outflowing}$, C_{tree}). A post-hoc analysis of estimated marginal means was used to compare across all groups. We also used a separate test (negative binomial GLMM with sampling time as a random effect) to see if time influenced anthropogenic particle concentrations during the outside-marsh sampling and there was no significant effect of time on the explained deviance. To assess correlations between site factors and number of microplastics, a Kendall's rank correlation was used. All analyses were conducted in RStudio (2024.12.0).

We also expressed data as anthropogenic particles per m^2 of saltmarsh for both S_{live} and S_{dead} leaves by multiplying the number of anthropogenic particles per leaf by the number of leaves in a quadrat and dividing by the area of the quadrat (Table 5.1; Equation 2). This was then used to calculate the total number of anthropogenic particles on saltmarsh vegetation in the sampling area and on saltmarsh vegetation in the Tamar estuary (Table 5.1; Equation 3). Surface area (m^2) of saltmarshes in the Tamar estuary and of the sampling area was obtained from the Priority Habitats Inventory (England) dataset provided by Natural England (https://naturalengland-defra.opendata.arcgis.com/datasets/Defra::priority-habitats-inventory-england/about).

We also estimated the potential number of anthropogenic particles entering the Tamar estuary from macro-detritus export. We calculated the net change in anthropogenic particle concentrations from S_{live} to $S_{outflowing}$ (Table 5.1; Equation 4) and multiplied that by the number of anthropogenic particles on saltmarsh vegetation in the Tamar estuary and by two possible levels of macro-detritus export (Table 5.1; Equation 5). We used a low estimation of 0.05% (Bouchard & Lefeuvre, 2000) and a high estimation of 10% export of net primary productivity (Bouchard, 1998). Both estimates are relevant to European saltmarshes, with the higher value specifically based on *A. portulacoides* productivity.

Table 5.1. List of equations used. APs = anthropogenic particles. COM = coarse organic matter.

1	Number of S_{live} leaves per quadrat	$= Average\ number\ of\ leaves\ per\ branch\ \times\ Number\ of\ branches$
2	Number of anthropogenic particles per m^2 of saltmarsh	$= \frac{Number\ of\ APs\ per\ leaf\ \times\ Number\ of\ leaves\ per\ quadrat}{Area\ of\ quadrat\ (0.0625\ m^2)}$
3	Number of anthropogenic particles on saltmarsh vegetation (Tamar APs and sampling area APs)	$= Number\ of\ APs\ per\ m^2 \times vegetation\ cover\ (m^2)$
4	Percent change in APs from S_{live} to $S_{outflowing}$	$= \left[\frac{Average\ APs\ per\ cm^2\ (S_{outflowing}) - Average\ APs\ per\ cm^2\ (S_{live})}{Average\ APs\ per\ cm^2\ (S_{live})}\right] \times 100$
5	Number of anthropogenic particles exported via detritus	$= (Tamar\ APs\ \times percent\ change\ in\ APs)\ \times \%\ COM\ export$

5.3 Results

5.3.1 Microplastics adhered to vegetation

S_{dead} leaves had the largest number of anthropogenic particles per leaf area (cm^2). Mean anthropogenic particle concentrations on S_{live}, S_{dead}, $S_{outflowing}$, and C_{tree} leaves were 0.30 ± 0.40, 0.74 ± 0.33, 0.33 ± 0.27, and 0.19 ± 0.08 particles cm^{-2}, respectively (Table S5.3, S5.4). There were significantly more anthropogenic particles per leaf area (cm^2) on S_{dead} leaves than S_{live} leaves (p = 0.001) and C_{tree} leaves (p = 0.02; marginal R^2 = 0.22; conditional R^2 = 0.49; Figure 5.2).

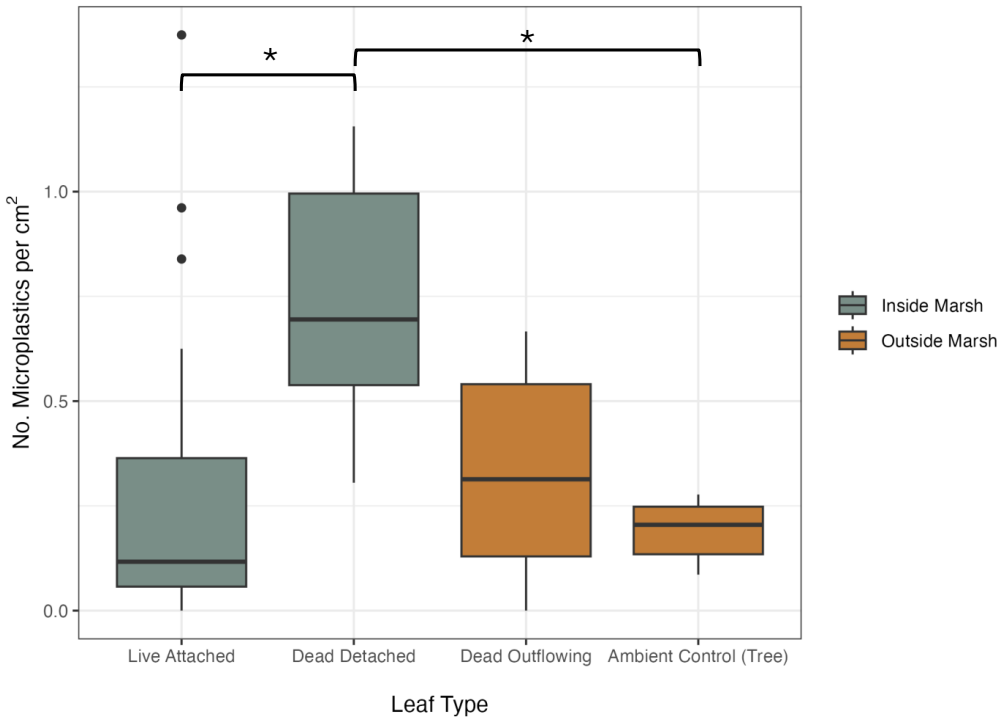

Figure 5.2. Number of anthropogenic particles per cm^2 of leaf for each leaf life stage (S_{live}, S_{dead}, $S_{outflowing}$) and for an ambient control (outflowing tree leaves; C_{tree}). Asterisk and bracket indicate significant differences between groups (negative binomial GLMM with log link function). Boxes represent the interquartile range (IQR; 25^{th} -75^{th} percentile), horizontal line indicates the median, and whiskers extend to the maximum and minimum values within 1.5x the IQR. Individual points beyond the whiskers are outliers.

Percent canopy cover ranged from 50% to 90% (mean: 75 ± 16%). Average canopy height ranged from 13 to 52 cm (mean: 25 ± 14 cm) and maximum canopy height ranged from 27 to 70 cm (mean: 41 ± 16 cm). *Atriplex portulacoides* was the dominant vegetation species and the only species present in any of the quadrats. *Spartina spp.* was also present in the surrounding saltmarsh. Tree species analysed include *Quercus robur* (English Oak), *Fagus sylvatica* (European Beech), *Corylus avellana* (Common Hazel) and *Fraxinus excelsior* (European Ash).

The number of anthropogenic particles per cm^2 did not correlate with percent canopy cover (tau = 0.23, p = 0.15) or with average (tau = -0.25, p = 0.12) or maximum canopy height (tau = -0.16, p = 0.33). However, number of anthropogenic particles per leaf had a significant and

positive correlation with leaf surface area (tau = 0.60, p = 0.000001), this pattern was maintained when only saltmarsh leaves were included (tau = 0.51, p = 0.0001; Figure 5.3).

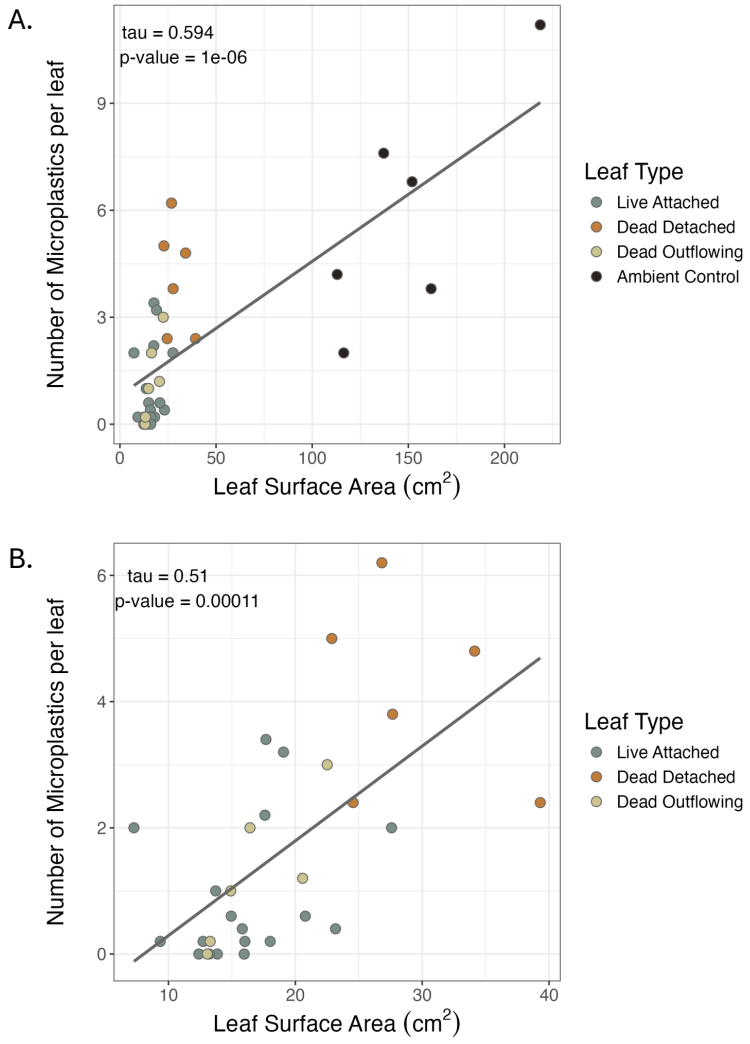

Figure 5.3. Number of microplastics per leaf compared to leaf surface area (A) for all leaf types (B) for only *A. portulacoides* leaves. Kendall's rank correlation is represented in the top left of each graph.

5.3.2 Microplastic characteristics

The majority of anthropogenic particles on vegetation were fragments (94.8%), followed by fibres (5.0%), and spheres (0.2%; Figure 5.4). The relative abundance of particle shapes adhered to the different leaf stages and control leaves were similar. Though, S_{live} leaves had a

higher proportion of fibres compared to any of the other groups (Figure 5.4). Out of all particles, 89% were black fragments, suspected to be tyre particles indicated by their colour, shape, and texture.

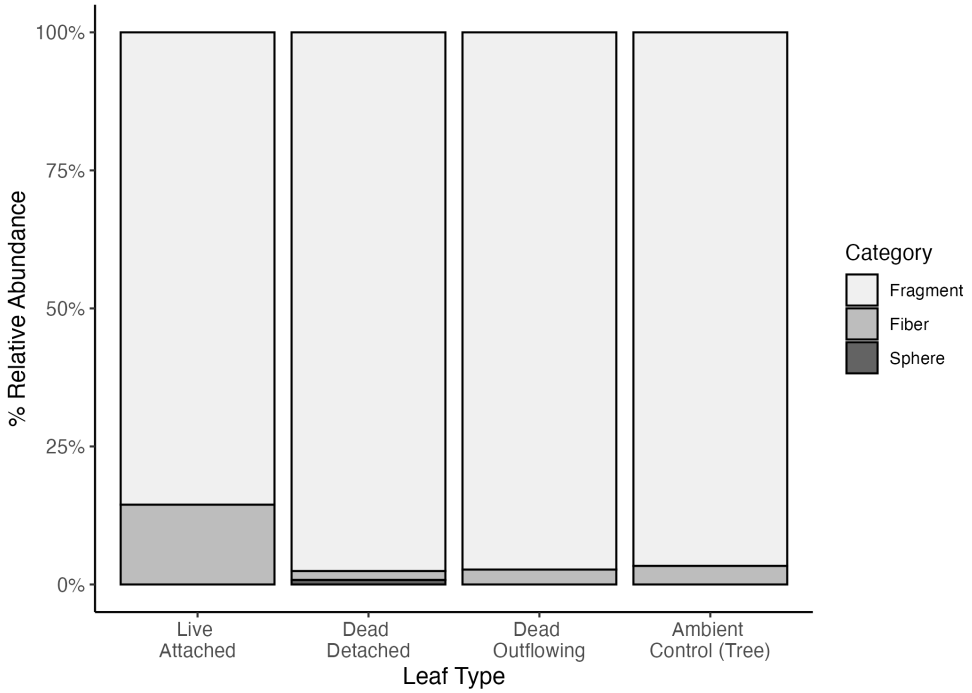

Figure 5.4. Relative abundance of anthropogenic particle shapes on each leaf type.

Median particle size was 94 µm (mean ± s.d.: 172 ± 661 µm; Figure 5.5a). Most particles were less than 300 µm. There was one fibre adhered to a C_{tree} leaf that was greater than 5000 µm (Table S5.5). Particle lengths were largely similar across the four groups (Table S5.5; Figure 5.5b). However, S_{live} and C_{tree} leaves had a higher frequency of longer particles compared to S_{dead} and $S_{outflowing}$ leaves. S_{live} leaves also had fewer particles in the < 125 µm size range compared to the other groups (Figure 5.5b).

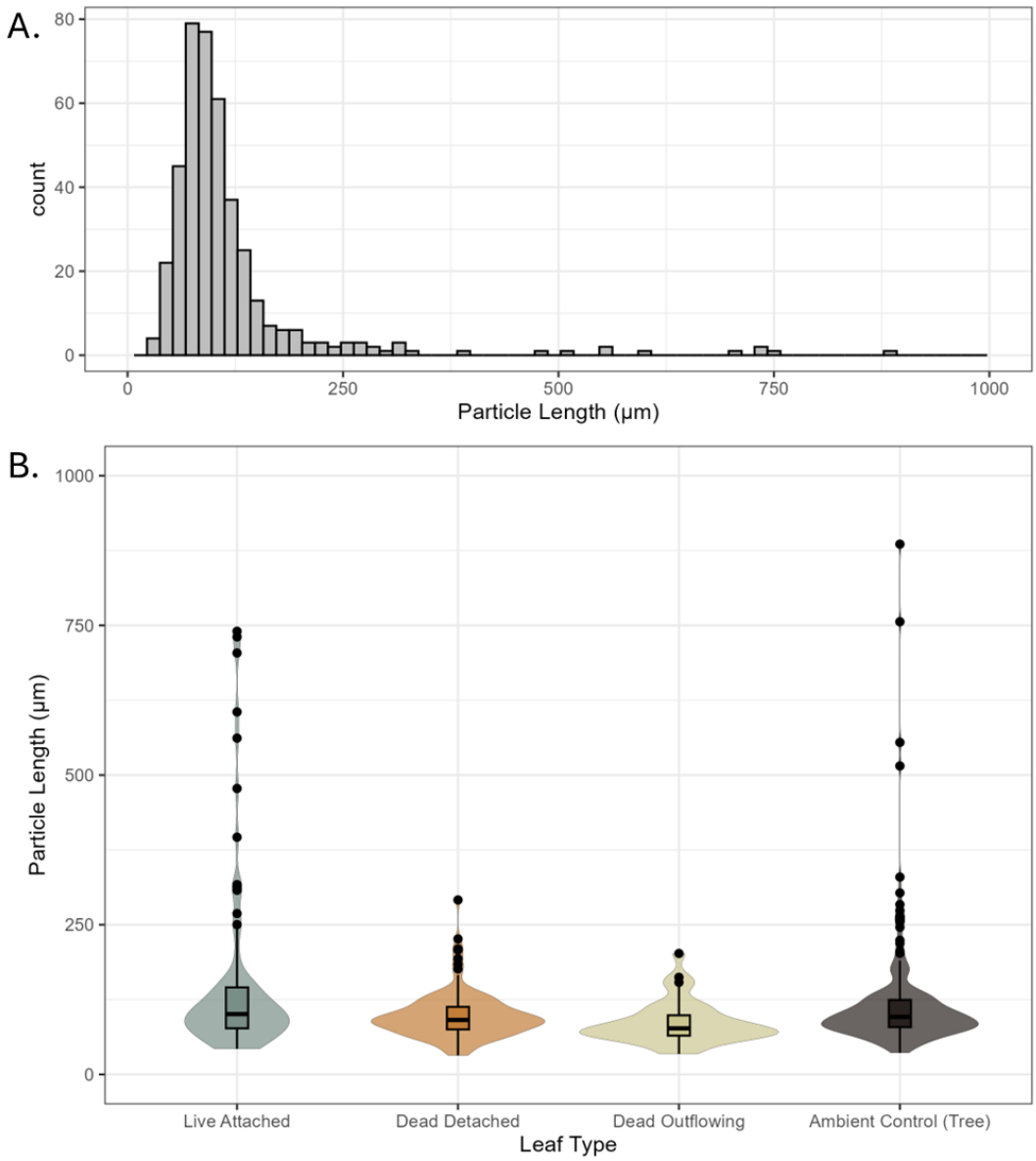

Figure 5.5. (A) Frequency of particle sizes in all samples. (B) Distribution of particle sizes for each leaf type.

Of the particles that were subsampled for micro-FTIR, 43.3% were confirmed plastic, 50.0% were unknown cellulosic, 3.33% were anthropogenic cellulosic, and 3.33% were unknown (Figure 5.6a). Polymers identified included polyester (30.8%), acrylic (23.1%), alkyd (15.4%), polypropylene (7.7%), polyisobutene (7.7%), polyamide (7.7%), and a copolymer (7.7%; Figure 5.6b).

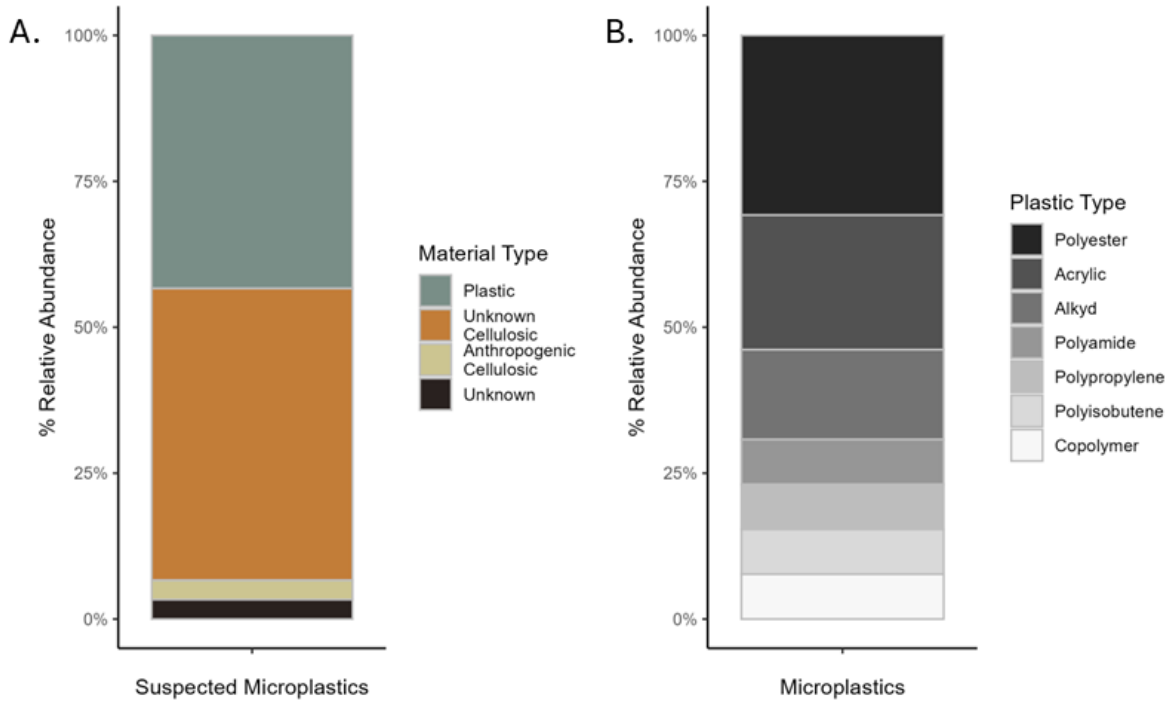

Figure 5.6. (A) Relative abundance of anthropogenic particle material types identified by micro-FTIR. (B) Relative abundance of polymer types identified by micro-FTIR.

5.3.3 Capacity for microplastic export

There were 10.1% more anthropogenic particles per cm^2 on $S_{outflowing}$ leaves compared to S_{live} leaves. The number of anthropogenic particles per m^2 was 13,914.6 ± 14,719.4 on S_{live} leaves and 3,320.3 ± 2,208.4 on S_{dead} leaves. For the sampling area, this equates to 36,177,986 anthropogenic particles adhered to live saltmarsh vegetation at a given time. If we extrapolate this to the saltmarshes of the Tamar estuary, not considering differences in vegetation species or canopy density, there could be 39,884,838,104 anthropogenic particles adhered to the vegetation canopy at a given time. Using two estimates of macro-detritus export (0.05% and 10% of net aerial primary production), there could be between 21,957,313 and 4,391,462,665 anthropogenic particles exported from saltmarshes into the Tamar estuary during tides.

5.4 Discussion

Here, we show that anthropogenic particles, including microplastics, adhered to saltmarsh vegetation and are exported on outflowing detritus, but concentrations are largest on detritus within the marsh. This confirms an additional pathway for microplastics to remain in saltmarsh systems, first adhering to the above-ground canopy then transferring to sediment as the leaves fall and are buried by sediment or faunal bioturbation. The number of anthropogenic particles on leaves increased with leaf surface area and most anthropogenic particles were suspected tyre particles, though fibres were also observed.

5.4.1 Microplastics adherence to saltmarsh vegetation

Anthropogenic particles were present on saltmarsh leaves at different leaf stages and ranged from 0 to 1.37 particles cm^{-2} (Table S5.3). To date, only one other study has investigated microplastic adherence on a saltmarsh species (the cordgrass *Sporobolus maritimus*) and found no presence of microplastics (Cozzolino et al. 2020). In comparison to other marine macrophytes, the concentration of anthropogenic particles on live saltmarsh leaves (0.30 ± 0.40 particles cm^{-2}) is larger than that on seagrass blades but similar to mangrove leaves. A review of microplastics on seagrasses calculated an average of 0.085 ± 0.031 particles cm^{-2} on seagrass blades (Li et al. 2023a). Submerged mangrove leaves from the Beibu Gulf had concentrations of 0.11 – 0.49 particles cm^{-2}, which was larger than non-submerged leaves (0.09-0.24 particles cm^{-2}; Li et al. 2022a). Mangrove leaves in the Gulf of Mannar had even larger concentrations (0.6 to 1.2 particles cm^{-2}; Sudhakar and Mathew, 2025). These differences may be due to variations in leaf morphology, diurnal fluctuations, tidal influence, leaf salt content, and spatial arrangement (Wei et al. 2024, Sudhakar and Mathew 2025).

Contrary to other studies, fragments were more abundant than fibres on saltmarsh leaves. The dominant microplastic shape found on both seagrass blades and mangrove leaves have been fibres (Li et al. 2022a, 2023a, Sudhakar and Mathew 2025). This could indicate differences in pollution sources or methodology, as most of the fragments were suspected tyre particles. Tyre particles are a significant source of plastic pollution but are often underreported due to difficulties with identification (Kole et al. 2017, Wagner et al. 2018, Knight et al. 2020,

Rosso et al. 2023). Most conventional methods for identifying microplastics (e.g., FTIR and Raman spectroscopy) do not work effectively for identifying tyre particles (Knight et al. 2020). Moreover, they have a high density and generally settle out of the water column quickly and are therefore less likely to be transported away from their source (Parker-Jurd et al. 2025). However, small sized particles (<200 µm) can remain in suspension and be flushed further downstream (Ziajahromi et al. 2020, Parker-Jurd et al. 2025). Here, the closest road was located only 15 m away from the sampling site and the closest major road was located < 1 km away. If suspected tyre particles are excluded, fragments are still the most abundant microplastic shape on saltmarsh vegetation but the proportions of fibres to fragments becomes more evenly spread (44% fibres, 54% fragments, 2% spheres). S_{live} leaves had a higher proportion of fibres compared to the other leaf stages (Figure 5.4). With black fragments excluded from S_{live} leaves, 80% of particles are fibres and 20% are fragments, which is more comparable to other marine macrophytes (Datu et al. 2019, Sudhakar and Mathew 2025). This suggests that though fibres may be more abundant on leaves while attached to the plant, they may be more readily released, particularly after the leaf detaches. There are few studies that have tested the strength of adherence of different microplastic types. One experimental study with freshwater duckweed (*Lemna minor*) showed strong adherence from polyethylene fragments compared to spheres but they did not compare this to fibres (Rozman et al. 2022). Further work is needed to understand the variables affecting the strength of microplastic adherence such as microplastic shape, size, surface charge, and leaf surface morphology.

5.4.2 Microplastic Vegetation-Sediment pathway

The higher concentration of anthropogenic particles on S_{dead} leaves compared to S_{live} or $S_{outflowing}$ leaves suggests that saltmarshes are still ultimately a sink for microplastics (Figure 5.2). It confirms, in addition to the route of direct deposition via reduced turbulence and water flow, a second pathway for microplastics to remain in saltmarsh systems. The above-ground vegetation can intercept microplastics in the water column (McIlwraith et al. 2024). As vegetation ages and the leaves increase in surface area, they accumulate more microplastics (Figure 5.3), eventually senescence will result in leaves falling on to the sediment as detritus. Microplastics can then be transferred into the sediment as the leaf is buried or breaks down from microbial decomposition

or shredding by detritivores (Newell et al. 1989). Detritivores that ingest microplastics on macrophytes can also further the transport of microplastics into sediments through egestion in faecal pellets (Gutow et al. 2016, 2019).

5.4.3 Capacity for microplastics export

Despite larger concentrations of anthropogenic particles on leaf detritus within the saltmarsh, there is still a proportion of particles that are exported with outflowing detritus (Figure 5.2). While some of the anthropogenic particles on outflowing detritus could be acquired during transport across the water surface, the high variability on $S_{outflowing}$ compared to C_{tree} leaves suggests that some particles are from the saltmarsh and not just from the ambient seawater (Figure 5.2). Based on the amounts recorded here, there is potential for 22 million to 4.4 billion anthropogenic particles being washed into the Tamar estuary during tides from saltmarsh vegetation. In comparison, microplastic discharge estimates from wastewater treatment plant effluent range from 3.6 million to 460 million per day depending on treatment processes employed (Ziajahromi et al. 2017). Thus, there is potentially a considerable amount of microplastic flux from saltmarsh vegetation with every tide. Macro-detritus export is, however, highly variable. Depending on the functional age of the marsh, organic matter export can vary significantly. Functionally old marshes largely act as sources of organic matter compared to functionally young marshes (Boorman 2003, Chen et al. 2016). Moreover, flood tides may re-introduce detritus back into the marsh (Bouchard et al. 1998, Bouchard and Lefeuvre 2000). There can also be species-specific and seasonal differences. Saltmarshes have high floristic diversity and therefore have a wide range of detritus levels throughout the year (Caçador et al. 2009, Duarte et al. 2017). For instance, *Spartina alterniflora* does not typically shed its leaves and there is very little flux of particulate shoot material (Dame 1982, Newell et al. 1989). While the exact estimate of the number of microplastics exported from saltmarshes is unknown, there is a sizable amount that could be transported via the above-ground vegetation. Yet research on this compartment is limited, with most studies focused on sediments (Hernán et al. 2024). To fully constrain the plastic cycle and locate global reservoirs, investigations that include all parts of an ecosystem are needed.

5.4.4 Limitations and further considerations

This work can be further explored in three ways. First, the site in this study was heavily polluted, with bike and car tyres, clothing, and other discarded plastic observable in the surrounding area (Figure 5.7). This likely contributed to the high loading of microplastics on saltmarsh leaves and it would be interesting to observe how these patterns compare to a less polluted site. Notably, microplastics were also adhered to C_{tree} leaves. The tree leaves acted as a background measurement of microplastics adherence. The leaves sampled were a mix of different species to account for any species-specific variability, however, it would be interesting to compare microplastic loadings across tree species. There could be differences in the level of microplastic adherence due to changes in leaf morphology and surface microstructures, as was observed between species of mangrove and terrestrial tree leaves in the Southeast Coast of India (Sudhakar and Mathew 2025). Lastly, this work could be taken further by conducting a mass balance of a saltmarsh system by quantifying microplastic levels in sediments, water, air, vegetation, and fauna. This data, combined with microplastic inputs and outputs, would more readily increase our understanding of saltmarsh systems as a source or sink of microplastics. Attempts at quantifying a global ocean mass balance of microplastics are occurring (Harris et al. 2023), but mass balance approaches focused on small scale coastal zones have yet to be explored. While the results of the present study showcase saltmarsh vegetation as both an accumulation point and pathway of export for microplastics, it is limited by a single study site and time point, thus the role of saltmarshes in the global plastic cycle requires further investigation.

Figure 5.7. Image of discarded plastic in the sampling area.

5.4.5 Conclusions

Anthropogenic particles, including microplastics, were present on saltmarsh vegetation on live, dead, and outflowing leaves. The above-ground vegetation of saltmarshes can contain a substantial amount of microplastics, a proportion of which is exported via macro-detritus transport. Nevertheless, concentrations were highest for detritus within the marsh, indicating that saltmarshes are primarily a sink for microplastics. However, this pattern may change as microplastic flux in coastal vegetated canopies is dependent on innumerable factors (e.g., tidal regime, species differences, pollution sources, seasonality). Therefore, the dynamic and transient nature of these ecosystems must be considered before coastal vegetated canopies can truly be considered a microplastics sink.

Chapter 5: Supplementary Information

Table S5.1. Average flow velocity for each sample.

Bridge Number	Sample Number	Average Flow Velocity (m/s)
1	1	0.143
	2	0.228
	3	0.317
2	1	0.316
	2	0.389
	3	0.616

Table S5.2. Recovery rates of microplastics from method validation tests.

Spike Plastic	***Mean Recovery ± standard deviation***
Polypropylene fragment	111 ± 19%
Polyethylene film	100 ± 0%
Polyamide fibre	100 ± 0%
Polyester fibre	84 ± 8%
Total	99 ± 8%

Table S5.3. Total number of anthropogenic particles counted per sample after blank subtraction.

Sample type	***Leaf type***	***Site number***	***Replicate***	***No. MPs***	***Per cm²***	***Per leaf***	***Per g dry weight***
Net	Marsh	1	A	0	0	0	0
		1	B	10	0.61	2.0	156.25
		1	C	5	0.34	1.0	69.44
		2	A	6	0.29	1.2	78.60
		2	B	1	0.08	0.2	16.95
		2	C	15	0.71	3.2	148.15
	Tree	1	A	21	0.19	4.2	74.03
		1	B	38	0.28	7.6	90.12
		1	C	34	0.22	6.8	91.32
		2	A	19	0.12	3.8	47.62
		2	B	56	0.26	11.2	109.45
		2	C	10	0.09	2.0	36.45
Quadrat	Dead	1	A	25	1.09	5.0	306.12
		2	A	24	0.70	4.8	157.21
		3	A	31	1.16	6.2	370.52
		4	A	19	0.69	3.8	259.09
		5	A	12	0.49	2.4	171.43
		6	A	12	0.31	2.4	108.11
	Live	1	A	1	0.06	0.2	8.62
		1	B	1	0.06	0.2	11.54

		1	C	10	0.36	2.0	49.75
		2	A	0	0	0	0
		2	B	0	0	0	0
		2	C	0	0	0	0
		3	A	11	0.62	2.2	198.80
		3	B	17	0.96	3.4	94.62
		3	C	16	0.84	3.2	230.77
		4	A	10	1.37	2.0	232.56
		4	B	0	0	0	0
		4	C	5	0.36	1.0	75.38
		5	A	1	0.11	0.2	22.73
		5	B	1	0.08	0.2	18.87
		5	C	2	0.09	0.4	20.83
		6	A	3	0.14	0.6	33.21
		6	B	2	0.13	0.4	29.41
		6	C	3	0.20	0.6	43.69

Table S5.4. Mean number of anthropogenic particles for each group, standardized by different measures.

Sample Type	***Mean per cm²***	***Mean per leaf***	***Mean per weight (g)***
Live within marsh	0.30 ± 0.40	0.92 ± 1.13	59.49 ± 78.84
Dead within marsh	0.74 ± 0.33	4.1 ± 1.5	228.75 ± 99.96
Dead outflowing	0.33 ± 0.27	1.23 ± 1.13	76.69 ± 62.78
Dead trees outflowing	0.19 ± 0.08	5.9 ± 3.30	74.83 ± 27.99

Table S5.5. Mean, median, maximum, and minimum particle lengths (µm) on each leaf type.

Sample Type	Mean ± sd particle length	Median particle length	Max length	Min length
Live	220.1 ± 369.8	104.2	2163.7	42.9
Dead	110.3 ± 142.5	91.1	1617.4	31.7
Marsh	124.2 ± 232.0	79.2	1481.2	34.2
Tree	203.6 ± 971.3	96.2	12897.7	36.1
Blank	870.6 ± 722.7	855.3	3057.91	75.0

Chapter Six

Key Findings and Recommendations

Microplastics are a diverse and multifaceted contaminant and there is still much to understand about microplastic pollution. An emerging area of research is microplastic fate and effects in coastal wetland ecosystems due to these ecosystems' societal importance and delivery of key ecosystem services such as habitat provisioning, carbon sequestration, and coastal protection. However, several key knowledge gaps remain. In this thesis, I aimed to better understand the role of coastal vegetated habitats as a reservoir for microplastics within the global plastics cycle. To this end, I used a controlled flume experiment (Chapter 2) and two field investigations (Chapter 4 and 5) to investigate some of the underlying physical and biological drivers of microplastic deposition and transport in vegetated canopies, with a focus on saltmarshes. In Chapter 3, I compared methods for isolating and identifying microplastics from saltmarsh sediments and leaf surfaces to demonstrate the importance of laboratory methods validation in microplastics research.

When I first started the PhD in 2022, there was conflicting information on whether seagrass and saltmarsh habitats trapped more microplastics than unvegetated areas. I hypothesized that these differences were due to the complex nature of these ecosystems, and identified many abiotic and biotic factors potentially influencing microplastics entrapment in coastal vegetated habitats (Figure 6.1). Since then, the number of field observations has increased but the evidence remains inconsistent, with some studies recording higher concentrations of microplastics in vegetated areas compared to unvegetated areas (e.g., Girones et al. 2024, Zhang et al. 2024b, Trusler et al. 2025, Bappy et al. 2025, Paray et al. 2025) and others not recording a difference (e.g., Boshoff et al. 2023, Ledet et al. 2024, Radford et al. 2024). This thesis aims to shed light on what is driving these differences and what variables affect microplastics accumulation in coastal wetland systems.

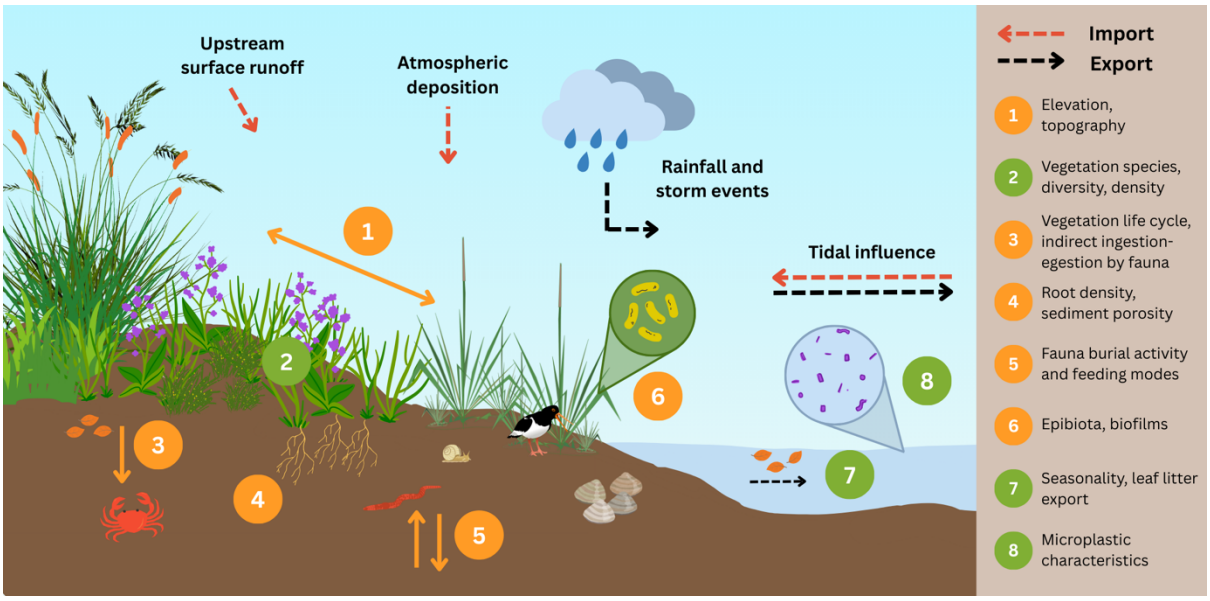

Figure 6.1. Conceptual diagram showcasing the potential influencing factors on microplastic retention and release in saltmarshes. Orange circles represent hypothesized variables, and green circles represent variables explored in this thesis.

6.1 Key findings and limitations of the research

In Chapter 2, I used a controlled flume experiment to investigate a subset of variables influencing microplastic entrapment and deposition: plant presence; plant structural complexity; and microplastic characteristics (Figure 6.1). I found that microplastic shape was the determining factor of microplastic fate in a simulated coastal system and that the presence and structure of the vegetation did not affect the number of deposited microplastics but did affect areas of microplastic accumulation (McIlwraith et al. 2024). Since publication, other experimental studies have investigated additional factors such as plant spatial configuration (Cousins et al. 2025), canopy height (Cousins et al. 2025), canopy density (Kerpen et al. 2024, Cousins et al. 2025) particle settling velocity (Kerpen et al. 2024), sediment composition (Greenshields et al. 2025), and microplastic size (Greenshields et al. 2025). Notably, these controlled studies still have deviating results. For example, Cousins et al. (2025) recorded that microplastics retention increased with decreasing canopy density, whereas Kerpen et al. (2024) found the opposite pattern. Even in controlled systems, there are many different factors to consider (e.g., flow velocity, microplastic type, flume size, artificial vs. live plants) and this makes comparisons across

studies difficult. As such, one of the limitations of using a flume is that results are confined to the conditions of the study and it is nearly impossible to simulate the complexity of real-world conditions. Despite this, Chapter 2 showcases that microplastic shape is an important variable to be considered in microplastic transport and deposition in vegetated systems, as opposed to vegetation structure and presence.

A common constraint in microplastics research is methodology. Different field and laboratory methods are used across studies, and this makes comparisons difficult. Chapter 3 proposes that recovery rate tests with harmonized reference microplastics could be employed to improve method validation and aid study comparisons in microplastics research (McIlwraith et al. 2025). In Chapter 3, I showed that microplastics recovery rates not only differed by method and sample properties (sediment types, leaf surfaces), but also by microplastic type, with fibres often having lower recoveries than other types of microplastics. This work demonstrates that there is no perfect method in microplastics research. There is a toolbox of methods to choose from depending on the sample matrix and accessibility of materials, but we can improve comparability through method validation tests with a range of environmentally representative microplastics. A limiting factor to achieve consistency across the field will be access to reference microplastics. In McIlwraith et al. (2025) I used in-house reference microplastics and this works for creating small quantities of material. However, to carry this idea forward, there needs to be harmonised reference microplastics accessible to all researchers that are created with a standardised protocol, and there are many working towards this goal (Martínez-Francés et al. 2023, Sørensen et al. 2024, Badzoka et al. 2025). This chapter was integral for validating and informing the methods used for Chapters 4 and 5 and provides important considerations for researchers investigating microplastics in complex environments, such as coastal wetlands. This work will ultimately improve our understanding of microplastic fate by harmonising and validating research methods.

To investigate how the patterns observed in Chapter 2 replicate under environmental scenarios, I analysed saltmarsh sediments collected from Blakeney National Nature Reserve (UK) and tested the same variables as Chapter 2 (plant presence and complexity; microplastic type) but with the added dimension of vegetation diversity in Chapter 4. Consistent with Chapter 2,

microplastic content was similar across sediment with different types of vegetation structure and diversity. However, one result diverged from the flume experiment: unvegetated sediments had higher microplastic content than any of the vegetated sediments albeit with high variability. Only two other studies (in mangrove sediments) have found this pattern and also found high variability in unvegetated sediments (Li et al. 2018, Cordova et al. 2021). One reason why I found diverging results from others could be because of the type of unvegetated sediment that I sampled. A meta-analysis by Hernán et al. (2024) found that the number of microplastics in unvegetated areas decreased as distance from vegetated areas increased. Most studies on coastal wetlands use the nearest tidal flat to represent unvegetated sediment; in my study, I chose to sample the tidal pools within the saltmarsh to limit differences in elevation with the other sites. This proximity to vegetation could have increased the microplastic content in bare sediments. While my results suggest that unvegetated intertidal sediments trap more microplastics than vegetated sediments, the mechanism behind this observation requires further exploration. Uncoupling the effects of vegetation from other factors, like hydrodynamic and geographical differences, on microplastic accumulation is a difficult challenge. My work begins to pick apart these factors. Together, Chapter 2 and 4 show that microplastic fate in saltmarshes is determined by more than just the presence or absence of vegetation, or vegetation structural complexity, and is instead likely governed by many interacting factors. Namely, these environments are complex and differences on a local scale will affect whether microplastics are trapped in these systems, and consequently, whether they act as a sink for microplastics.

Another variable that I hypothesized could influence microplastic deposition and retention in vegetated wetlands is through adherence to the aboveground vegetation. As a result, Chapter 5 shows that saltmarsh leaves were a significant reservoir for adhered microplastics in the Tamar Estuary (UK). These results also revealed that saltmarsh leaves could be a vector for transferring microplastics to both the sediment and to the wider estuary. Few studies have looked at microplastic adherence to vegetation, though it has started to become a focus of research, particularly in mangroves and terrestrial plants (Bi et al. 2020, Li et al. 2022a, 2025, Yu et al. 2024). Previous studies have mainly focused on microplastics adhered to leaves attached to the plant. My research adds to this by investigating microplastics adherence at

different leaf stages. From this, I estimated that saltmarsh detritus could export upwards of 22 million anthropogenic particles into the Tamar Estuary with each tide. While it is important to consider temporal and seasonal changes when examining the role of biota in microplastics cycling, this work highlights that saltmarshes are not a one-way system for microplastics. Moreover, by quantifying microplastics in the vegetative compartment, in addition to sediment and water, we can better understand the levels of microplastic contamination in intertidal habitats and consequently, organism exposure. This chapter again highlights the dynamic nature of coastal systems and that microplastic flux out of vegetative systems is possible, thus providing evidence that saltmarshes may act as temporary reservoirs of microplastics rather than a sink.

6.2 Future research

This thesis begins to answer the question of whether saltmarshes act as a sink for microplastics but there are still numerous influential factors on microplastic entrapment that have yet to be explored. As depicted in Figure 6.1, there are multiple pathways of import and export into a saltmarsh, within which, there are abiotic and biotic factors that impact the sequestration and release of microplastics. For instance, rainfall at low tide can mobilize saltmarsh sediments and cause significant erosion (Torres et al. 2004, Tolhurst et al. 2006b), possibly also releasing microplastics. Mobilization of microplastics by rainfall has been observed from terrestrial to coastal environments (Cheung and Not 2023) but not yet from coastal vegetation to the open ocean. Rainfall events could also induce the opposite pattern: coastal vegetation could intercept microplastics from land-based surface runoff during rainfall, accumulate microplastics and inhibit their release to the ocean, which has been observed for terrestrial plants (Han et al. 2022).

Biological differences across systems can include vegetation differences, as explored in this thesis, however, these habitats are also home to diverse benthic communities. Benthic fauna have different functional roles and rework sediment in different ways (e.g. bioturbation), which could affect microplastic cycling into, within, and out of sediment (Hale et al. 2014, Näkki et al. 2019, Coppock et al. 2021). Another underexplored area of research are the extracellular polymeric substances (EPS) secreted by many intertidal organisms such as bacteria, fungi, and algae. Consisting mostly of polysaccharides and proteins, EPS promotes flocculation of

suspended particles and increases sediment stabilisation (Decho 1990, Tolhurst et al. 2002) and could therefore play a role in the retention of microplastics. Indeed, the role of EPS in promoting particle sequestration has been observed for other contaminants, such as heavy metals (Cheng et al. 2022). Ultimately, the sequestration of microplastics into coastal wetland systems is subject to a myriad of interacting parameters and this thesis is a starting point for untangling these effects.

6.3 Are saltmarshes a sink for microplastics?

Collectively, my research shows that saltmarshes are a reservoir for microplastics and are subject to microplastic flux, but plant presence and complexity may not be the main mechanism for microplastics entrapment. Due to the spatial and temporal heterogeneity experienced by coastal systems, patterns of microplastics retention may only be visible at large scales. Three meta-analyses have been published since the start of my PhD and each found greater numbers of microplastics in vegetated sediment than unvegetated sediment, with varying effect sizes (Biltcliff-Ward et al. 2022, Hernán et al. 2024, Qi et al. 2025). Therefore, coastal vegetated habitats might be considered a sink when combined at large spatial scales, but this is not observable at the local level. Determining when and why some coastal vegetated habitats have enhanced microplastic concentrations and others do not will require a deeper understanding of the intricate biological and physical interactions of these systems.

6.4 Wider implications and recommendations

Investigating the transport and accumulation of microplastics in coastal wetlands has important implications for understanding pollution levels relevant to risk management frameworks. My thesis highlights the heterogenous nature of microplastic deposition across spatial scales (vegetated vs. unvegetated sediments) and across compartments (sediment and vegetation), but how this heterogeneity affects organism exposure requires further exploration. For instance, the final fate of microplastics adhered to leaf litter is unknown, and consequently relevant exposure scenarios. Another consideration stemming from this thesis is whether these systems should be considered as a novel ‘clean-up’ mechanism for microplastic pollution. However, the idea that coastal wetlands could contribute to microplastic ‘clean-up’ remains

unsettled due to three reasons: 1) it is unclear what factors enhance microplastic entrapment and therefore, which aspects should be optimized, 2) how microplastics would be removed from the system is unknown, and 3) if not they are removed, the threshold at which these ecosystems can no longer perform other ecosystem functions due to the impacts of microplastics must first be determined. Lastly, contextualising coastal wetlands as a reservoir instead of a sink opens up speculation on how coastal wetlands fit into the global plastics cycle, what microplastic transformation mechanisms and migration pathways occur, and the resulting environmental impacts.

Table 6.1. Recommendations for future research.

1. What is the net flux of microplastics in coastal vegetated systems?
2. What other underlying mechanisms influence microplastics retention and deposition, as outlined in Figure 6.1?
3. What are the ideal parameters for enhancing microplastic entrapment and sequestration?
4. Does coastal vegetation reduce the resuspension of microplastics compared to unvegetated areas? What other factors reduce the resuspension of microplastics (e.g., sediment properties)?
5. How do the levels of microplastics on aboveground vegetation compare to what is contained in sediments?
6. How do microplastic concentrations in water, sediments, and vegetation vary seasonally? Or before, during, and after a storm event?
7. Is the strength of microplastics adherence dependent on microplastic type and vegetation species? What surface properties, of plants or microplastics, affect the relative strength of microplastic adherence?
8. How long do microplastics remain adhered to vegetation? And where does this detritus transport microplastics?
9. Does the presence of adhered microplastics affect epifaunal colonization or grazing on vegetation?

10. At what threshold will microplastics in coastal vegetated habitats impact ecosystem functioning?

11. Can (and should) microplastic entrapment by coastal vegetation be considered a novel ecosystem service?

6.5 Conclusion

To complete the picture of microplastic cycling in coastal vegetated habitats, there are still numerous research questions remaining (Table 6.1 outlines some of these). My research provides evidence that what promotes microplastic entrapment is not clear-cut and the dynamic nature of coastal vegetated systems must be contextualized when considering them as a sink. Still, the presence of microplastics in these ecosystems is evident and a cause for concern regarding ecosystem health and functioning (Rangal-Buitrago et al. 2024). By increasing our knowledge of microplastic transport and fate in previously underexplored habitats, we can better quantify microplastic contamination relevant to organism exposure. Moreover, the global cycling of microplastics through various ecosystems and compartments highlights the transboundary nature of microplastics and, consequently, the need for global coordination to reduce microplastic pollution.

Appendix

Research Dissemination

During my PhD, I have disseminated my research through a variety of formats, including peer-reviewed publications, international and national conference presentations, media, and public outreach.

Peer-reviewed publications resulting from my PhD research:

McIlwraith, H.K., Lindeque, P.K., Tolhurst, T.J. and Cole, M., 2025. Positive controls with representative materials are essential for the advancement of microplastics research. *Microplastics and Nanoplastics*, *5*(1), p.9. 10.1186/s43591-025-00115-y

McIlwraith, H.K., Lindeque, P.K., Miliou, A., Tolhurst, T.J. and Cole, M., 2024. Microplastic shape influences fate in vegetated wetlands. *Environmental Pollution*, *345*, p.123492. 10.1016/j.envpol.2024.123492

Peer reviewed publications from work alongside my PhD:

Jolly, D.J., Allen, E., Olah-Kovacs, B., **McIlwraith, H.,** Warren, R.J., Woodhouse, C., Staines, M., Wright, A.C., Boots, B., Tolhurst, T.J. and Green, D.S., 2025. Eco-friendly or eco-threat? The environmental risks of natural and semi-synthetic fibers. *Environmental Research Communications*, *7*(5), p.052502. 10.1088/2515-7620/add860

Hermabessiere, L., Best, C., Zaidi, S., **McIlwraith, H.K.**, Jeffries, K.M. and Rochman, C.M., 2025. Understanding the contribution of plastic additive in microplastic toxicity from consumer products using fathead minnow (Pimephales promelas). *Environmental Science and Pollution Research*, pp.1-14. 10.1007/s11356-025-36523-z

Hataley, E.K., **McIlwraith, H.K.,** Roy, D. and Rochman, C.M., 2024. Response to: On the need to avoid apple-to-orange comparisons in microplastic research. *Canadian Journal of Fisheries and Aquatic Sciences*, *81*(7), pp.972-977. 10.1139/cjfas-2024-0074

McIlwraith, H.K., Dias, M., Orihel, D.M., Rennie, M.D., Harrison, A.L., Hoffman, M.J., Provencher, J.F. and Rochman, C.M., 2024. A Multicompartment Assessment of Microplastic Contamination in Semi-remote Boreal Lakes. *Environmental toxicology and chemistry*, *43*(5), pp.999-1011. 10.1002/etc.5832

Hataley, E.K., **McIlwraith, H.K.,** Roy, D. and Rochman, C.M., 2023. Towards a management strategy for microplastic pollution in the Laurentian Great Lakes—ecological risk assessment and management (part 2). *Canadian Journal of Fisheries and Aquatic Sciences*, *80*(10), pp.1669-1678. 10.1139/cjfas-2023-0023

McIlwraith, H.K., Hataley, E.K. and Rochman, C.M., 2023. Towards a management strategy for microplastic pollution in the Laurentian Great Lakes—monitoring (part 1). *Canadian Journal of Fisheries and Aquatic Sciences*, *80*(10), pp.1653-1668. 10.1139/cjfas-2023-0022

Conference and workshop presentations

(2025) SETAC Europe 35th Annual Meeting

Exploring Vegetation Complexity as a Driver of Microplastic Accumulation in Coastal Marshes (Oral)

Location: Vienna, Austria

(2025) Revolution Plastics PhD Conference

Exploring Vegetation Complexity as a Driver of Microplastic Accumulation in Coastal Marshes (Oral)

Location: Portsmouth, UK

(2024) Micro 2024: Plastic Pollution from Macro to Nano

What influences microplastic trapping in coastal marshes? Exploring vegetation diversity as a driver of accumulation (Poster)

Microplastic type influences fate in vegetated wetlands (Oral)

Location: Lanzarote, Spain

(2023) SETAC Europe 34th Annual Meeting

A plastic trap? Factors influencing microplastics trapping in coastal vegetated canopies (Poster)

Location: Seville, Spain

(2023) Legacy Plastics Workshop by the Royal Society

Participant

Location: London, UK

(2023) ASLO Aquatic Sciences Meeting

Microplastic type influences fate in vegetated wetlands (Oral).

Location: Palma de Mallorca, Spain

(2022) Micro 2022 Online Atlas Edition: Plastic Pollution from Macro to Nano

A plastic trap? Factors influencing microplastics capture in coastal vegetated habitats (Poster)

Location: Online

(2022) Microplastics Workshop for Early Career Researchers: Best Practices and Expert Insights

A plastic trap? Factors influencing microplastics capture in coastal vegetated habitats (Oral and Poster)

Location: Athens, Greece

Awards

(2025) Awarded £500 grant in aid from Plymouth Marine Science and Education Foundation to present research at SETAC Europe 35th Annual Meeting.

Outreach and Media

(2025). Invited as a guest speaker for Environmental Industries Commission (EIC) – Environmental Laboratory Group Meeting to present on microplastic extraction methods.

(2023 – 2025). Collaborated with the Primary Science Teaching Trust to develop educational lessons and resources about plastic and pollution for primary science students in the UK.

(2024). Various media interviews for radio and print to talk about my research work at the Experimental Lakes Area in Northwestern Ontario, Canada (CBC Thunder Bay Radio, Water Canada, Kenora Miner & News, Toledo Blade, 89.5 The Lake Radio, FOX Detroit).

(2023 – 2024) Co-editor for Scienvy, an environmental science blog for ARIES DTP PhD students to share about their research and life as a PhD student.

(2023). Co-organised the PlyMSEF conference for postgraduate students in Plymouth.

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