Flushed Away: Flux, Fate, and Environmental Impact of Cellulose-Based Wet Wipes within River Systems

Thomas William Allison

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Dedication

For my grandfather, Aubrey, and my nan, Mo – who always supported me in my endeavours. I miss you both dearly.

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Data Accessibility

All data is available upon request (contact allisont2@cardiff.ac.uk or tomallison98@gmail.com).

Data already published are available in the Cardiff University data catalogue (https://research-data.cardiff.ac.uk/authors/Tom_Allison/19088216)

Thesis Abstract

As plastic pollution in aquatic systems becomes a growing concern, “biodegradable” wet wipes made from cellulosic fibres have emerged as popular alternatives. Marketed as eco-friendly and flushable, these products are commonly disposed of in toilets, yet their fate in freshwater environments remains unclear. This thesis investigated the environmental behaviour, degradation, and pollution potential of biodegradable, cellulosic wet wipes using a life cycle-based approach that integrated critical analyses, emissions modelling, and field experiments.

First, this thesis critically evaluated wipe composition and theoretical degradation following flushed toilet disposal. Many wipes, despite green marketing claims, include blends of biological and synthetic fibres, alongside chemical additives that can limit microbial degradation. Their physical fragmentation is common, but molecular degradation is often incomplete, suggesting that most flushed wipes persist in the aquatic environment.

An emissions model was then developed to quantify macro- and microfibre discharges to UK rivers and to assess the risk they pose. Results showed that wastewater treatment plants, sewer overflows, and land-applied sludge are major pathways. While solids are largely removed, microfibres can escape treatment, representing an overlooked pollution source.

Wipe degradation and its environmental drivers were subsequently assessed under both controlled mesocosm and real-world river conditions. Tensile strength loss served as a reliable proxy for degradation, with cotton strip bioassays used as ecological controls. A first brand, composed predominantly of natural cellulose, degraded faster than a second, containing mostly regenerated cellulose. However, both persisted for over five weeks. In rivers, microbial biomass, total dissolved solids, and exposure time were key drivers of degradation, while scanning electron microscopy revealed greater surface wear in natural cellulose fibres.

Despite their biodegradable labels, many wipes do not degrade rapidly in freshwater systems. Finally, the findings were synthesised to propose key recommendations for meeting safe, real-world, environmental standards. These included improved product design and testing protocols, clearer labelling and public education, and better wastewater and waste disposal systems to reduce fibre pollution from “green” consumer products.

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Chapter 1

General Introduction: The Rise of Plastic-Free Wet Wipe Alternatives and Their Fate in River Environments

1 General Introduction: The Rise of Plastic-Free Wet Wipe Alternatives and Their Fate in River Environments

1.1 Background

Plastic pollution is a critical environmental and human health crisis, significantly impacting global aquatic ecosystems (Windsor et al., 2019a; Jâms et al., 2020). Plastics can persist for decades, and their incorrect disposal can pose physical and toxicological threats to aquatic life through ingestion, chemical leaching, bioaccumulation, cytotoxicity, and trophic transfer (Stanton et al., 2019; Windsor et al., 2019b; D'Souza et al., 2020; Miller et al., 2020), with similar adverse risks for humans (Ullah et al., 2023; Yu and Flury, 2024; Muniz and Rahman, 2025). Freshwater environments are now recognised as critical hotspots for plastic pollution (Windsor et al., 2019a), with substantial evidence demonstrating their role as both accumulation points and conduits transporting plastics to marine environments (Horton et al., 2017; Ryan and Perold, 2022). Microfibres, defined as fibrous particles smaller than 5 mm, are generally considered the dominant form of microplastic pollution in freshwater systems (Henry et al., 2019; Stanton et al., 2019). These microfibres may originate from synthetic textiles shed during clothes washing (Napper and Thompson, 2016); however, increased recognition of fibrous consumer products ending up in rivers – particularly wet wipes – suggests an additional but underestimated key contributor of microfibre pollution (Cabrera and Garcia, 2019; McCoy et al., 2020; Ó Briain et al., 2020).

Wet wipes are single-use, non-woven, moistened fabric materials manufactured for a diverse range of cleaning purposes, including surface cleaning and personal care, and provide a quick and easy solution to daily hygiene issues. The rise in consumer hygiene awareness and convenience, particularly following the COVID-19 pandemic (Shruti et al., 2021), has resulted not only in the increased production and consumption of wet wipe products (Hu et al., 2022), but also their inappropriate disposal via toilet flushing behaviours (Mitchell, 2019). This incorrect disposal has also been exacerbated by the marketing of wipes advertised as ‘flushable’ wipes, which encourage consumers to dispose of wipes into sewer systems (Tipper, 2016).

Plastic wet wipes are typically composed of synthetic polymer fibres such as polyester, polyethylene-terephthalate, polypropylene, and polyethylene; all highly resistant to molecular

degradation, ensuring that wipes maintain strength and durability in water (Webb et al., 2013; Pantoja-Munoz et al., 2018; Orr and Karadagli, 2020). However, plastic fibres shed readily under aquatic conditions, including in wastewaters (Lee et al., 2021). Consequently, flushed wipes regularly cause sewage blockages and damage (Drinkwater and Moy, 2017), and their shed fibres enter aquatic environments directly through sewer overflows, wastewater effluents (Fig. 1.1) and other wastewater misconnections (Ó Briain et al., 2020; Royer et al., 2021).

Fig. 1.1. Examples of wet wipes pollution entering freshwaters and surrounding riparian systems via sewer overflow outlets. Images derived from Besley and Cassidy (2022).

Increasing public awareness and regulatory pressures around plastic wet wipe pollution have spurred significant shifts towards plastic-free alternatives. For instance, recent legislative developments in the UK have accelerated the phase-out of plastic-containing products, including wet wipes (DEFRA, 2024a), driving industries towards more biologically-based

(materials derived from biological origins; hereafter bio-based) and biodegradable alternatives. This shift has also been propelled by new consumer behaviours, reflecting a growing preference for environmentally friendly and sustainable products (Herrmann et al., 2022; Tang, 2023; DEFRA, 2024b). However, the assumption that 'plastic-free' translates directly into 'environmentally safe' remains critically unexamined.

Product certification standards for 'biodegradability' – the degree to which materials degrade by microorganisms into harmless organic matter – and 'flushability' – the degree to which flushed materials disperse safely within sewer systems – vary significantly in criteria and are typically presented as voluntary guidelines rather than legally enforced requirements (Kjeldsen et al., 2018; Pantoja-Munoz et al., 2018; Filiciotto and Rothenberg, 2021). Additionally, these standards often fail to replicate the complex dynamics of wastewater and freshwater environments, relying instead on controlled laboratory settings (Harrison et al., 2018; Liao and Chen, 2021). Moreover, biodegradability tests rarely account for chemical additives that can leach into aquatic environments, potentially causing toxic effects (Horton et al., 2017; Harrison et al., 2018; Yu and Flury, 2024). This raises substantial doubts about the adequacy of current testing protocols in accurately predicting real-world degradation and overall environmental impacts.

Until recently, plastic microfibres were predominantly regarded as the primary pollutant in aquatic systems. However, emerging evidence suggests that bio-based cellulosic fibres – including natural (e.g. cotton, wood pulp-derived), and chemically regenerated forms (e.g., viscose, rayon, lyocell) are actually more abundant (Lusher et al., 2013; Woodall et al., 2014; Remy et al., 2015; Henry et al., 2019; Suaria et al., 2020), including in freshwaters (Miller et al., 2017; Dris et al., 2018). White fibres from textiles, including wet wipes, have been increasingly identified in wastewaters and aquatic ecosystems, including cellulosic fibres (Ó Briain et al., 2020; Tserendorj et al., 2024; Bach et al., 2025). Growing evidence further indicates that cellulosic wet wipes can persist under wastewater conditions and in coastal environments (Joksimovic et al., 2020; Pedersen et al., 2022; Kargar and Joksimovic, 2024; Metcalf et al., 2024), and may shed more microfibres than plastic equivalents (Kwon et al., 2022). Despite these preliminary findings, substantial knowledge gaps remain regarding the extent, environmental fate, and ecological impacts of these new cellulosic alternatives, particularly in freshwater environments where wet wipes often accumulate.

A life cycle assessment (LCA) – a holistic cradle-to-grave analysis – provides a comprehensive method for addressing these knowledge gaps (Miranda et al., 2020; Zhang et al., 2021b; Zhao and You, 2022). By evaluating the entire lifespan of cellulosic wet wipes, from manufacturing processes, through consumer usage and disposal practices, to their ultimate environmental fate, it becomes possible to identify the critical stages contributing most significantly to ecological risk and where targeted interventions are needed most. This thesis adopts an LCA-style approach to systematically investigate these different stages, offering an essential evaluation of whether cellulosic wet wipes genuinely represent a safer environmental alternative to traditional plastic-based products.

1.2 Thesis rationale and importance

Despite the increasing shift towards plastic-free alternatives, including cellulosic wet wipes, there remains a critical lack of understanding about their true environmental behaviour and impacts, particularly in freshwater ecosystems where such products frequently accumulate. Existing assumptions that bio-based materials are inherently safer have not been systematically tested under real-world conditions. Moreover, current biodegradability standards often fail to reflect the complex environmental dynamics that influence material persistence and ecological risk. Addressing these knowledge gaps is vital not only for assessing the sustainability claims of emerging plastic-alternative products but also for guiding responsible production, informed consumer choices, and evidence-based regulatory policies. By combining a life cycle perspective with emissions modelling and experimental degradation studies, this research provides a novel, holistic evaluation of cellulosic wet wipes as an emerging freshwater pollutant. Its findings offer timely insights into the environmental trade-offs associated with plastic-free alternatives, contributing urgently needed data to support a genuinely sustainable transition away from traditional plastics. Findings from Chapter 2 have already informed decision-making in the Welsh Government during the formulation of the Environmental Protection (Single-use Plastic Products) (Wales) Act 2023, highlighting the critical policy relevance and applied value of this work.

1.3 Aims of the thesis

The primary aim of this thesis is to critically evaluate the full life cycle of commercially available cellulosic wet wipes – a widely used and increasingly marketed example of plastic-free alternative products – to understand their environmental fate and assess their ecological risks in freshwater systems. To achieve this, the thesis comprises six chapters, each structured as a publishable research paper employing critical analyses, emission-based mathematical modelling, laboratory tests, and both mesocosm and in-situ field experiments. The specific research objectives are to:

1. Critically analyse existing knowledge on cellulose polymers, their commercial use in wet wipes, and their environmental fate and impacts in freshwater systems.
2. Quantify baseline macro (> 5 mm) - and micro (< 5 mm) -sized emissions of cellulosic wet wipe pollution entering freshwater environments via flushed disposal routes.
3. Assess the degradation dynamics of cellulosic wet wipes in experimental mesocosm and in-situ freshwater conditions, while identifying key drivers for breakdown.
4. Evaluate the ecological risks posed by flushed cellulosic wet wipes within freshwater ecosystems.
5. Identify socio-technical and regulatory interventions necessary for cellulosic wet wipes to achieve environmentally safe standards.

1.4 Thesis overview

Each chapter directly addresses the research objectives outlined above:

Chapter 2 provides a critical analysis of current knowledge on cellulosic wet wipes. It combines fundamental theory and life cycle assessment approaches to evaluate cellulose polymers and their usage in wet wipes from manufacturing to environmental fate. This establishes a foundation for assessing their potential risks in rivers and identifies key knowledge gaps for future research, which this thesis begins to address.

Chapter 3 quantifies the scale of wet wipe pollution in rivers. Using an emissions-based mathematical model with secondary data sources for wet wipe disposal and microfibre release,

this chapter estimates macro- and microfibre emissions of plastic and cellulosic wet wipes entering freshwater systems via wastewater pathways. The model is applied to sub-catchments of two distinct river catchments, illustrating the broader environmental implications under best- and worst-case emission scenarios.

Having established baseline pollution estimates, **Chapter 4** investigates wet wipe degradation under controlled freshwater conditions. Specifically, this chapter tests commercially labelled "biodegradable" cellulosic wipes within field river mesocosms, isolating key environmental and biological factors driving degradation and generating hypotheses on these interactions.

Building on insights from the mesocosm studies, **Chapter 5** tests Chapter 4's hypotheses through field experiments in urban river environments – typical accumulation sites for flushed wet wipes. This real-world assessment quantifies actual degradation rates under dynamic environmental conditions, examining the influence of microbial activity and physical river characteristics.

Collectively, these chapters provide a comprehensive assessment of cellulosic wet wipes as an emerging freshwater pollutant. **Chapter 6** synthesises findings across the thesis to evaluate the ecological risks posed by these materials within their broader life cycle. It outlines implications for regulatory policy, consumer behaviours, and manufacturing practices, identifying targeted socio-technical interventions to mitigate environmental impacts and enhance the sustainability of biodegradable wet wipe products and broader plastic-free alternative products.

Chapter 2

Do Flushed Biodegradable Wet Wipes Really Degrade?

A version of this chapter is available online as:

Allison, T., Ward, B.D., Harbottle, M., Durance, I. 2023. Do flushed biodegradable wet wipes really degrade? Science of the Total Environment 894, 164912. http://dx.doi.org/10.1016/j.scitotenv.2023.164912

In this chapter, the concept and methodology were designed by Thomas Allison in collaboration with Benjamin Ward, Michael Harbottle, and Isabelle Durance. Thomas Allison drafted and edited the manuscript following review and comments from all authors.

Abstract

Consumer wet wipes sold as biodegradable and flushable have tripled in market size in the last decade (>$3 billion in 2022), spurred by concerns over their potential harmful impact. Whilst predominantly composed of cellulosic fibres such as cotton, rayon, or wood pulp, these have been found to persist in sewers and in the environment in near equal abundance to their 'synthetic' counterparts. This raises questions about whether flushed biodegradable wet wipes really degrade.

Working from fundamental principles of cellulosic polymers, the physicochemical composition, environmental interactions, and degradation processes throughout the entire life cycle of cellulosic wet wipe fibres – from production to environmental fate – were explored to understand their likely degradation behaviour in wastewater and freshwater systems.

The results highlight that >50% of biodegradable and flushable wipes are commonly manufactured with both naturally derived, biodegradable cellulosic fibres and synthetic, low-degradable fibres, and that they contain various property-enhancing chemical additives - such as strengthening and antimicrobial agents - that can limit degradation. Whilst cellulose fibres in wet wipes are highly prone to physical fragmentation, their molecular degradation is difficult within the environment. This is due to the physicochemical manufacturing properties of wet wipes and the usually inadequate ambient conditions for its breakdown, creating persistent and possibly biologically harmful microfibres.

It is concluded that currently, most flushed biodegradable wet wipes do not really degrade, and that more empirical investigations are needed on their in-situ degradation behaviour and the environmental and manufacturing processes that may influence this breakdown. In doing so, full life cycle approaches to wet wipes should be adopted, considering their manufacturing properties, consumer disposal behaviour, and environmental implications.

2 Do Flushed Biodegradable Wet Wipes Really Degrade?

2.1 Introduction

Multi-purpose, single-use wet wipes have surged in consumer demand worldwide, namely since the recent pandemic. Their inappropriate domestic disposal presents a significant route of contamination to aquatic ecosystems worldwide (Shruti et al., 2021; Zhang et al., 2021b) and can be easily spotted along rivers (McCoy et al., 2020). As an example, in the UK, their subsequent occurrence on beaches has increased by 94% in 2016, with a 400% increase over the last decade (Pantoja-Munoz et al., 2018), whilst >23,000 wet wipes were recovered from a single area of the Thames foreshore (UK), with an average density of 201 wet wipes/m^2 (Thames21, 2019).

Domestic wet wipes are mainly composed of non-degradable synthetic polymers such as polyethylene terephthalate (PET) and polypropylene (PP) (Pantoja-Munoz et al., 2018; Ó Briain et al., 2020). Over time, these wipes will fragment into smaller but still molecularly intact microfibres in the environment. At this point, they can be ingested by aquatic biota (Hu et al., 2022), with potential physical and ecotoxicological risks to their health (Stone et al., 2020). Ingested microplastic particles have also been found to travel through aquatic food webs (Windsor et al., 2019b; D'Souza et al., 2020), which may, ultimately, transmit these health risks to humans (Zhang et al., 2020; Jiang et al., 2021; Liao and Chen, 2021).

Incorrect wet wipe disposal also has significant social and economic impacts. Wet wipe pollution has increasingly gained media attention, often visualised by their presence in "fatbergs" – congealed masses of wet wipes and other wastewater substances that persist within the sewer network (Alda-Vidal et al., 2020). Roughly 50 % of blockages within UK sewage systems are caused by wipes and similar hygiene products (Pantoja-Munoz et al., 2018; Mitchell, 2019). These blockages can promote the surface growth of various microbial communities, thus creating the need for additional biological treatment processes within sewers (Lee et al., 2021). Furthermore, some of these microorganisms can produce toxic or corrosive by-product chemicals that cause damage to the surrounding wastewater infrastructure (Durukan and Karadagli, 2019). The operational and financial costs for wastewater companies can be high (Mitchell, 2019; Ó Briain et al., 2020), reaching annual damages up to $15 million

in Australia, and £23 million in London (UK) alone (Fam et al., 2017). Combined sewage overflows (CSOs), release points for sewage and rainwater run-off from roads and urban areas during heavy rainfall (Scurlock, 2022), can act as additional pathways for wipes into freshwater ecosystems so that downstream, wipes are becoming a frequent source of aesthetic pollution (Horton et al., 2017; Besley and Cassidy, 2022).

Alternative wet wipes, marketed as biodegradable and flushable have been developed to address these issues (Zhang, 2010). For a wipe to be considered as biodegradable, it must be produced from natural polymers, such as cellulose, in order to decompose into harmless biomass (IWSFG, 2020). Alongside biodegradability, cellulose offers the advantage of favourable manufacturing properties (i.e. lightweight, high chemical adhesion, biocompatibility, and natural abundance) and low cost (Ibrahim et al., 2018; Yun et al., 2020; Jiang et al., 2021; Polman et al., 2021). Cellulose has, therefore, become a key polymer for commercially available biodegradable wet wipes.

However, there is limited knowledge on the abundance and degradation behaviour of these alternative wet wipes and their fibres, particularly once they enter the environment (Liao and Chen, 2021). There is growing evidence that many products labelled as biodegradable, such as wet wipes, do not, or do not fully, degrade under natural environmental conditions (Napper and Thompson, 2016; Manfra et al., 2021). This may be because many wipes branded as biodegradable still tend to include plastic (Ó Briain et al., 2020). However, there is evidence that cellulose-based fibres remain undegraded within wastewater effluents and freshwaters (Dris et al., 2018; Lares et al., 2018; Ó Briain et al., 2020; Zambrano et al., 2020b), marine environments (Adams et al., 2021) and municipal sewage sludges (Habib et al., 1998). This environmental persistence may also derive from the presence of additives – chemical substances commonly added to polymers during the manufacturing process to improve product functionality, degradation resistance, and performance (Lambert and Wagner, 2017; Hahladakis et al., 2018), but this has not been thoroughly analysed to date. Whilst outside the scope of this chapter, understanding the extent of the environmental risk posed by cellulosic wet wipes and their fibres also requires both their in-situ identification and quantification – analyses that are only just developing for plastics.

This chapter explores the current knowledge on wet wipes, and questions whether biodegradable wet wipes, and particularly cellulose-based forms, are environmentally better alternatives to traditionally synthetic wipes. Working from fundamental understandings, wet wipes are introduced within the context of a life cycle assessment, and their different manufacturing processes, physicochemical properties, consumer disposal, and fate are explored to evaluate their potential environmental risks. Then, the extent of likely biodegradable wet wipe breakdown within the aquatic environment is highlighted, focusing on wastewater and freshwaters as their disposal routes. In doing so, the environmental degradation mechanisms, the likely influencing environmental variables, and the interplay between these that may influence breakdown are explored. Finally, a discussion was presented on whether flushed biodegradable wet wipes truly degrade, alongside recommendations for future research.

2.2 Wet wipes in their life cycle

Wet wipes are versatile nonwoven textiles that serve a variety of purposes, from disinfection to personal hygiene. They are composed of different polymer fibres and chemical additives (Russell, 2007; Durukan and Karadagli, 2019), that require distinct manufacturing processes and present unique disposal challenges. The breakdown of wet wipes in the environment is largely determined by the choice of manufacturing process. To evaluate the environmental impact of cellulose-based wet wipes, a life-cycle approach is adopted that examines the effects of raw materials and typical manufacturing processes, as well as the physicochemical properties and additives that influence their degradability and fate. Additionally, the influence of different disposal methods on the breakdown behaviours of wet wipes is discussed.

2.2.1 Life cycle assessments of wet wipes

Life cycle assessments (LCA) can be valuable tools for evaluating the environmental impact of wet wipes throughout their entire life cycle, including manufacturing, usage, disposal, and fate (Fig. 2.1). A recent LCA of synthetic and bio-based wet wipes in China found that whilst the overall environmental impact of wet wipe manufacturing is relatively low (0.02 – 7.17 % of the total manufacturing emissions produced across all stages) (Zhang et al., 2021b), the production of wood pulp fibres used in their production can lead to significant eutrophication

due to chemical fertilizer and pesticide application. Besides this however, there are no LCAs on biodegradable wet wipes.

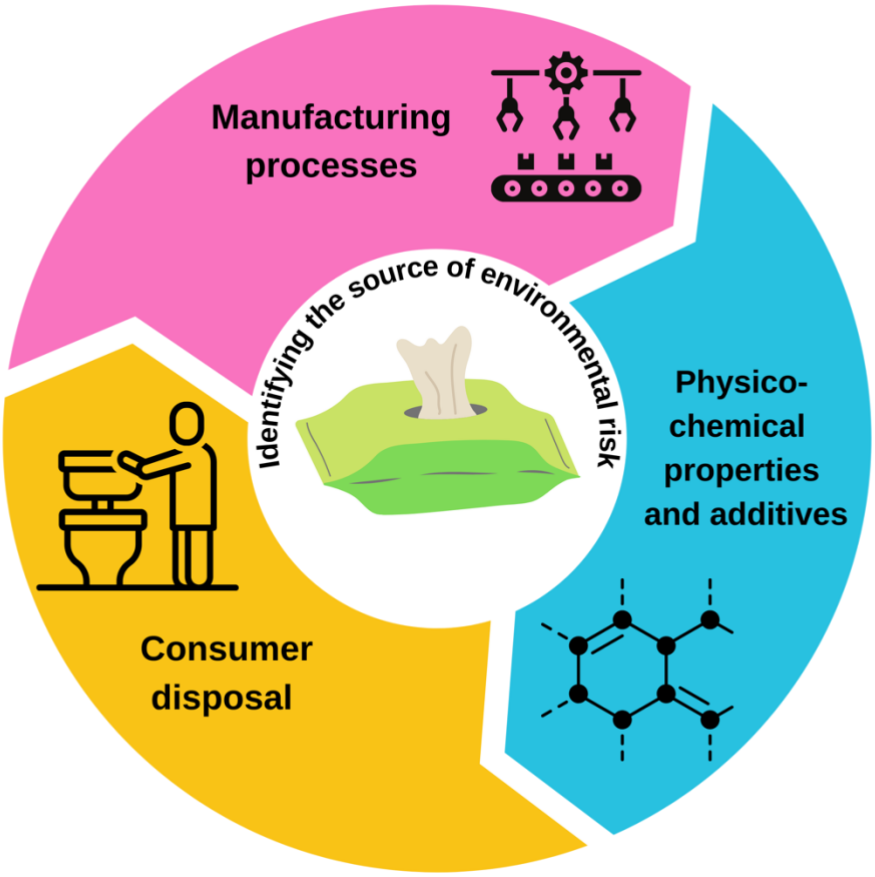

Fig. 2.1. Stages of the cellulosic wet wipe life cycle to investigate as sources of environmental risk.

2.2.2 Raw materials and manufacturing process

Wet wipes can be made from a variety of polymer fibres including synthetic petrochemicals (e.g. PET, PP and polyethylene [PE]), natural sources such as cellulose (e.g. cotton and wood pulp) or chemically regenerated cellulose (e.g. viscose and lyocell) (Dris et al., 2018; Pantoja-Munoz et al., 2018; Orr and Karadagli, 2020).

Synthetic plastic fibres are petroleum-based polymers with added chemical additives that resist degradation but can leach harmful byproducts and pollutants into aquatic environments (Webb et al., 2013; Miller et al., 2017; Stone et al., 2020). Most wet wipes currently available on the market contain at least one type of synthetic fibre (Ó Briain et al., 2020; Lee et al., 2021), as their ability to maintain shape and increase tensile strength when wet makes them more durable during consumer usage (Pantoja-Munoz et al., 2018; Lee et al., 2021).

Natural cellulosic plant fibres are biopolymers derived from renewable plant feedstock sources (Shaghaleh et al., 2018; Liu et al., 2021). Among these, cotton is most commonly used for manufacturing wet wipes, accounting for almost 30 % of all 113 million tonnes of polymer fibres produced in 2021 (Carr, 2017; Barrows et al., 2018; Textile Exchange, 2022). Cellulosic fibres make up over 50 % of all raw materials used in wet wipes and are added to increase biodegradability, softness, and water absorbency (Zhang et al., 2018; Durukan and Karadagli, 2019; Orr and Karadagli, 2020; Harter et al., 2021). Regenerated fibres, including viscose, rayon, lyocell, are also derived from cellulose biopolymers, but have been physically and chemically modified during manufacturing (Gago et al., 2018). However, the inconsistent labelling of regenerated fibres as either natural or synthetic in environmental pollution studies makes it challenging to accurately estimate their environmental persistence and impact.

The manufacturing processes involved in producing wet wipes from selected raw materials include web formation, bonding, and finishing processes. Web formation involves arranging fibres of different sources into layers (EDANA, 2022), which can be achieved through dry-laid (i.e. web forming in a dry state), wet-laid (i.e. web forming in a liquid substance) or spun melt methods (i.e. wet forming by heat) (Mitchell, 2019; Atasagun and Bhat, 2020). The fibrous web networks are then mechanically or chemically bonded to increase their strength (Russell, 2007; Pantoja-Munoz et al., 2018; Durukan and Karadagli, 2019; EDANA, 2022). Finally, different chemical additives are added during the finishing process to improve a specific product's functionality (EDANA, 2022). These additives can include antibacterial and antistatic properties, dyeing, flame retardancy, anti-shrink agents, and surface softening (Varadarajan and Venkatachalam, 2016; Dris et al., 2018; Stone et al., 2020; Harter et al., 2021; Jahandideh et al., 2021).

2.2.3 The specific properties of cellulose wet wipes

In this section, particular attention is given to the specific chemical (Section 2.2.3.1) and physical (Section 2.2.3.2) properties of cellulose fibres in wet wipes, as well as applied chemical additives (Section 2.2.3.3) that may affect their degradation and fate.

2.2.3.1 Key chemical properties of cellulose

Cellulose is a bio-renewable polysaccharide that makes up most plant tissue (Yuan and Cheng, 2015; Adams et al., 2021). It consists of β-D glucose repeating units covalently linked by acetal functions. Each monomeric unit of cellulose has three highly reactive hydroxyl groups, giving the polymer its hydrophilic and biodegradable properties (Klemm et al., 2005; Shaghaleh et al., 2018).

Cellulose fibres are structured hierarchically as single chains, microfibrils, and macrofibrils (Fig. 2.2) (Belgacem and Gandini, 2011). Strong intra and intermolecular bonds formed by hydroxyl groups and oxygen atoms create highly ordered crystalline regions in the microfibrils (Yuan and Cheng, 2015; Ghasemi et al., 2017). The degree of crystallinity determines the overall strength, durability and biocompatibility of the polymer (Shaghaleh et al., 2018). For example, cotton has a higher degree of crystallinity (~70 %) than other cellulosic fibres (Wood, 1988), making the polymer more resistant to degradation. In contrast, amorphous regions are loosely structured, susceptible to degradation processes, and tend to include additional biopolymers such as hemicelluloses and lignin (Ghasemi et al., 2017). Therefore, the de-crystallisation process plays an essential role in the extent of degradation in cellulose (Ghasemi et al., 2018).

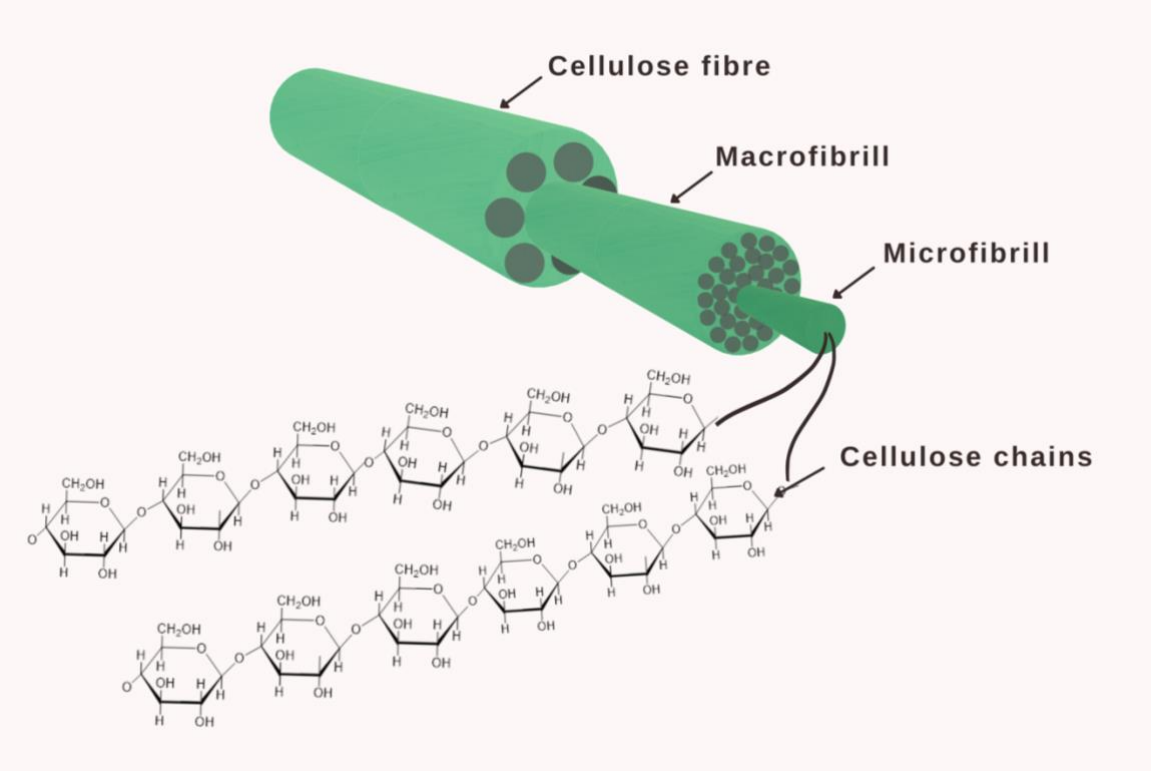

Fig. 2.2. Cross section of the different hierarchical levels of cellulose fibres. Adapted from Belgacem and Gandini (2011).

Cellulose has a strong affinity for itself and hydroxyl-containing materials such as water (Khazraji and Robert, 2013). Cellulose exhibits amphiphilic properties, due to its extensive intra and inter molecular hydrogen bonds and simultaneous hydrophobic molecular interactions, which can significantly impact its solubility in water and most organic solvents (Väisänen et al., 2021b).

The degree of polymerisation (DP), representing the average number of monomer repeat units in a polymer, varies depending on the origin and processing of cellulose (Klemm et al., 2005; Yuan and Cheng, 2015). For instance, filter paper consists of purified cellulose with a DP of 2900 (Malešič et al., 2005), whilst birch and dissolving pulps have DPs of 1800 and 3600, respectively (Sirviö and Lakovaara, 2021). However, the typically high DP of cellulose is a major obstacle to its chemical breakdown (Väisänen et al., 2021b).

Cellulosic fibres from different sources also vary in molecular weight (MW) which can affect the rate and extent of polymer degradation. You et al. (2021) reported the weight-average MWs

(measured in kDa) of various cellulosic fibres, with raw cotton having the highest MW (444 kDa), followed by dissolving wood pulp (208 kDa), Tencel (lyocell) (127 kDa), and viscose (127 kDa).

2.2.3.2 Key physical properties of cellulose

Wet wipe manufacturers need raw materials with modifiable physicochemical properties to meet a variety of consumer and industrial needs. These properties may include high strength, high absorbency for chemical additives, or biodegradability for fast disintegration in the natural environment (Mitchell, 2019). Unmodified cellulose alone has poor adsorption capacity and mechanical properties, but it has a large number of hydroxyl polar groups that can be modified to suit various applications (Habibi, 2014; Jiang et al., 2021).

The morphology of cellulosic fibres is important for their application and biological degradation (Klemm et al., 2005). Cellulosic fibres in wet wipes are typically short in length (0.3-10 mm) (Zhang et al., 2018; Mitchell, 2019). Regenerated fibres like viscose are longer than natural wood pulp fibres (Zhang et al., 2018). The length of fibres affects the breakdown of wipes. For instance, shorter fibres improve physical dispersibility and chemical degradation processes, whilst longer fibres increase wet tensile strength and resistance to breakdown (Tipper, 2016).

The internal morphology of cellulosic fibres is also important for understanding the degradation of cellulose-based wet wipes (Abu-Rous et al., 2006). Amorphous regions in natural cellulose are more porous than crystalline regions, making them more susceptible to water and enzymatic hydrolysis (Holtzapple, 2003). Therefore, wipes with greater porous and cracked fibre structures may promote fibre fragmentation in aquatic environments (Enfrin et al., 2020; Duan et al., 2021). However, more porous wipes may also accumulate more small sewer solids during wastewater transport, increasing their density and reducing their porosity, and thus, hindering their transport and degradation (Durukan and Karadagli, 2019).

Few studies have explored the physical properties of wet wipes, especially biodegradable forms, and those that have been done are usually experimental in design and lab-based. Durukan and Karadagli (2019) found that the physical properties of dry and moist-state

flushable and non-flushable wipes were very similar in terms of sheet mass, thickness, volume per unit mass, and moisture content, likely due to similar manufacturing processes.

High wet tensile strength and durability are desirable qualities in synthetic wet wipes (Orr and Karadagli, 2020). However, producing bio-based, biodegradable wipes that balance wet strength, dispersibility and softness is challenging (Sawhney et al., 2012; Zhang et al., 2018; Yun et al., 2020). A study comparing the wet and dry tensile strength of "flushable" nonwoven wipes to toilet paper found that these wipes had almost 23 times greater wet breaking force and retained their strength when wet, unlike toilet paper (Durukan and Karadagli, 2019). The wet strength loss of flushable wipes was only 29 % on average, compared to 91 % for toilet paper.

Petrochemical-derived additives are commonly used to improve the wet strength of cellulose wet wipes (see Section 2.3.3.3), but they can limit degradation processes (Yun et al., 2020). However, blends of cellulosic fibres can provide a balance of wet strength and degradability without the use of chemical additives. An experimental study on biodegradable wet wipes found that increasing the viscose content increased wet strength but decreased degradation, whilst increasing the wood pulp content added softness and degradability (Zhang et al., 2018).

The orientation of individual fibrils within cellulose fibres affects their strength, elasticity, elongation at breakage, and therefore, their degradation behaviour. Fibres with higher microfibril orientation are stronger but have lower elongation at breakage compared to fibres with low microfibril orientation (Klemm et al., 2005). Natural fibres and lyocell seem to have higher orientation than viscose (Abu-Rous et al., 2006). However, viscose and cotton have been found to share poor elasticity properties (Remy et al., 2015), suggesting that they may also have similar degradation behaviour.

High liquid absorbency is critical in the production and application of wet wipes, and bio-based cellulosic fibres have a strong advantage in fulfilling this requirement. Natural and regenerated cellulose fibres have low wet strength and high absorption properties, which makes them more readily available to biodegrade (Hauser, 2015; Mitchell, 2019; Harter et al., 2021). Furthermore, fibres with greater absorption properties tend to have a deep-grooved geometry due to the higher specific surface area this creates (Soukupova et al., 2007; Duan et al., 2021). However, pre-treated cotton fibres tend to be more hydrophobic (Sawhney et al., 2012).

2.2.3.3 Chemical additives

Chemical additives are commonly added to materials during manufacturing to improve their properties and durability (Campanale et al., 2020). These additives are present in plastic products but also cellulosic wet wipes, where they can act as strengthening agents or preservatives to prevent microbial growth (Hahladakis et al., 2018; Alvim et al., 2020; Adams et al., 2021). Examples of such additives include polyacrylic ester and urea-formaldehyde resin, phenoxyethanol, parabens, and benzoates (Obokata and a. I., A., 2007; Yun et al., 2020; Pack et al., 2021). However, these additives can leach out over time since they are weakly bound to the molecular structure of polymers, leading to potential ecotoxicological issues (Horton et al., 2017; Windsor et al., 2019a; Luo et al., 2022).

Moreover, additive contaminants can also adsorb onto cellulose fibres in the environment, resulting in similar issues (Jâms et al., 2020; Adams et al., 2021). It is currently unclear however, how different additives affect microfibre degradation in the environment (Henry et al., 2019; Ebrahimbabaie et al., 2022). Additionally, biodegradability tests for polymers do not consider the impact of any additives, although biodegradable additives are necessary for a polymer to be considered biodegradable (Lambert and Wagner, 2017; Harrison et al., 2018).

2.2.4 Disposal pathways and fate

Wet wipes are commonly disposed of through either domestic toilets (flushable) or domestic waste (non-flushable) pathways (Mitchell, 2019). This categorisation is based on a wipe's ability to be flushed down the toilet, known as “flushability” (INDA and EDANA, 2018; Orr and Karadagli, 2020). Ideally, the disposal pathway of wet wipes should also consider the biodegradability and dispersal of their polymers, and logically, synthetic petrochemical fibres should be disposed of through bin waste pathways and bio-based fibres through toilet pathways. However, the actual process is more complicated, and fibres from wet wipes of different origins and designated disposal pathways are found undegraded in wastewater and river environments (Drinkwater and Moy, 2017; Mitchell, 2019; Ó Briain et al., 2020). This emphasises the need for a better understanding of the differences between flushable and non-flushable wipes (Section 2.2.4.1), and between biodegradable and non-biodegradable polymers (Section 2.2.4.2).

2.2.4.1 Flushable vs non-flushable wipes

Wet wipes and other sanitary products are increasingly marketed worldwide as “flushable” (Atasagun and Bhat, 2020). Packaging instructions claim that these wipes can be disposed of through household toilets and into the sewage system (Orr and Karadagli, 2020; Harter et al., 2021). These wipes are mainly made of biodegradable bio-based fibres that have low tensile strength in their wet state and are typically used as moist toilet tissues and personal hygiene wipes (Zhang et al., 2018). In contrast, non-flushable products are made of synthetic fibres with much higher wet strength (Mitchell, 2019).

Ideally, a wipe should only be considered flushable if it is made entirely of biodegradable bio-based (i.e. cellulosic) fibres without any nondegradable components that could affect sewage infrastructure and freshwater ecosystems (IWSFG, 2020). However, Joksimovic et al. (2020) found that all tested fabrics labelled as flushable, including wet wipes, only partly degraded when assessed against the International Water Services Flushability Group (IWSFG) standards.

The confusion about flushable wipes stems from inconsistent criteria and regulations for assessing flushability (Pantoja-Munoz et al., 2018). Manufacturers and consumers often assume that flushable products move easily through wastewater to treatment plants and degrade during or after transport (Durukan and Karadagli, 2019). However, this depends on their physicochemical properties, as well as infrastructural and mechanical factors in the wastewater system, including wastewater flow velocity and pipe dynamics (Orr and Karadagli, 2020). Both flushable and non-flushable wipes have caused sewage blockages (Drinkwater and Moy, 2017; Mitchell, 2019). To address these misperceptions, several industries have produced “flushability criteria” guidelines (INDA and EDANA, 2018; WaterUK, 2019; Wildlife and Link, C., 2021). Despite these methodological efforts, there are still no universally standardised and legally-binding regulations placed on manufacturers regarding what can be deemed flushable (Drinkwater and Moy, 2017).

2.2.4.2 Biodegradable vs non-biodegradable polymers

Biodegradable polymers are materials that can be broken down by natural microorganisms, whereas non-biodegradable polymers, such as traditional plastics, resist degradation due to

strong apolar bonds (Fig. 2.3) (Sun et al., 2022b). However, the definition of biodegradable polymers can vary, and it is often mistakenly believed that 'bio-based' and 'biodegradable' mean the same thing (Filiciotto and Rothenberg, 2021). It is important to distinguish between bio-based and biodegradable polymers to understand their environmental fate and impact. As pointed out by Lambert and Wagner (2017), bio-based materials come from renewable organic sources, but not all of them biodegrade, including some bio-based plastics. Instead, degradability depends on a polymer's chemical structure rather than its biological source. Sun et al. (2022b) also emphasise that polymer degradation that lowers its molecular mass facilitates the most efficient biomass generation and microbiological assimilation. The breakdown of a biodegradable polymers occurs through chemical reactions in the environment that weaken chemical linkages and polar bonds, breaking molecular chains into smaller compounds that microorganisms can assimilate (Wang et al., 2011; Law and Narayan, 2021).

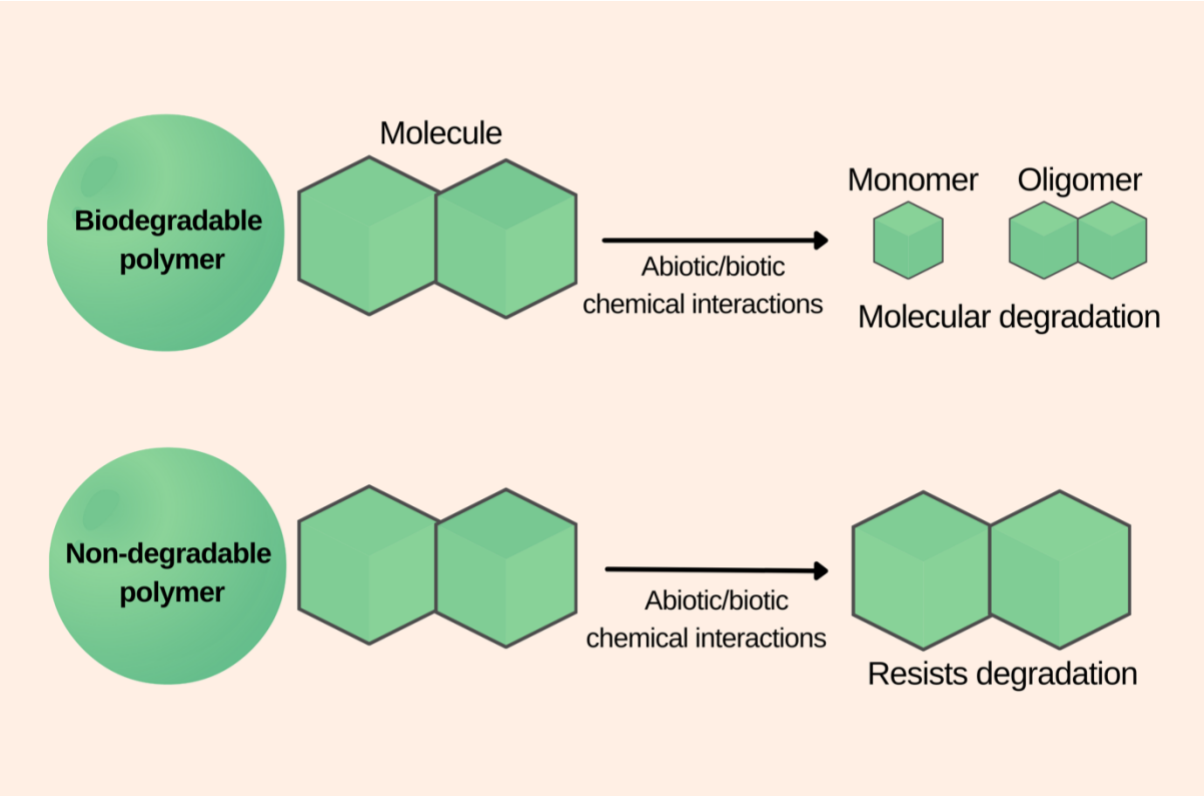

Fig. 2.3. Molecular degradation pathways for biodegradable and non-degradable polymers in response to abiotic/biotic chemical interactions.

Cellulose-based fibres in wet wipes are widely understood to be bio-based and biodegradable materials (Leja and Lewandowicz, 2010; Wei et al., 2021), but the biodegradation process is influenced by their manufacturing processes and chemical properties (Park et al., 2004). Standard biodegradation test protocols require high temperature and moisture settings (Straub et al., 2017; Kjeldsen et al., 2018; GOV.UK, 2021), which are mostly limited to industrial and lab-based facilities, and the conditions required for biodegradation in natural environments vary significantly (Wei et al., 2021). Therefore, biodegradable polymers like cellulose may not efficiently degrade outside laboratory conditions (Lambert and Wagner, 2017) and have been found to accumulate as microparticles or fibres in aquatic environments (Brinsko et al., 2016; Dris et al., 2018; Wei et al., 2021). However, there are no studies to our knowledge that have properly assessed the fate impact of cellulose-based fibres across their entire life cycle.

2.3 Cellulosic wet wipe degradation mechanisms in different environments

Degradation refers to any process (chemical, physical or biological) that breaks down the large molecular chains of a polymer into smaller units with low molecular weight such as carbon dioxide, water, and methane (Guo and Wang, 2019; Ebrahimbabaie et al., 2022). The underlying principle of a biodegradable polymer is that it will completely degrade (Shen et al., 2020; Liao and Chen, 2021). The primary degradation processes in aquatic systems are mechanical fragmentation, photo-degradation, and biodegradation. These often occur together, making it challenging to predict the total life cycle and environmental fate of materials (Chen et al., 2019; Duan et al., 2021; Luo et al., 2022). To better understand the degradation complexity of biodegradable wet wipes that enter the aquatic environment, this section investigates how wet wipes might degrade across the different environments encountered throughout their disposal pathways, from flushing (Section 2.3.1) to wastewater treatment plants (Section 2.3.2) to receiving freshwaters (Section 2.3.3) (Fig. 2.4). Intermediate degradation states are also highlighted, and the potential interactions between identified mechanisms are described (Section 2.3.4).

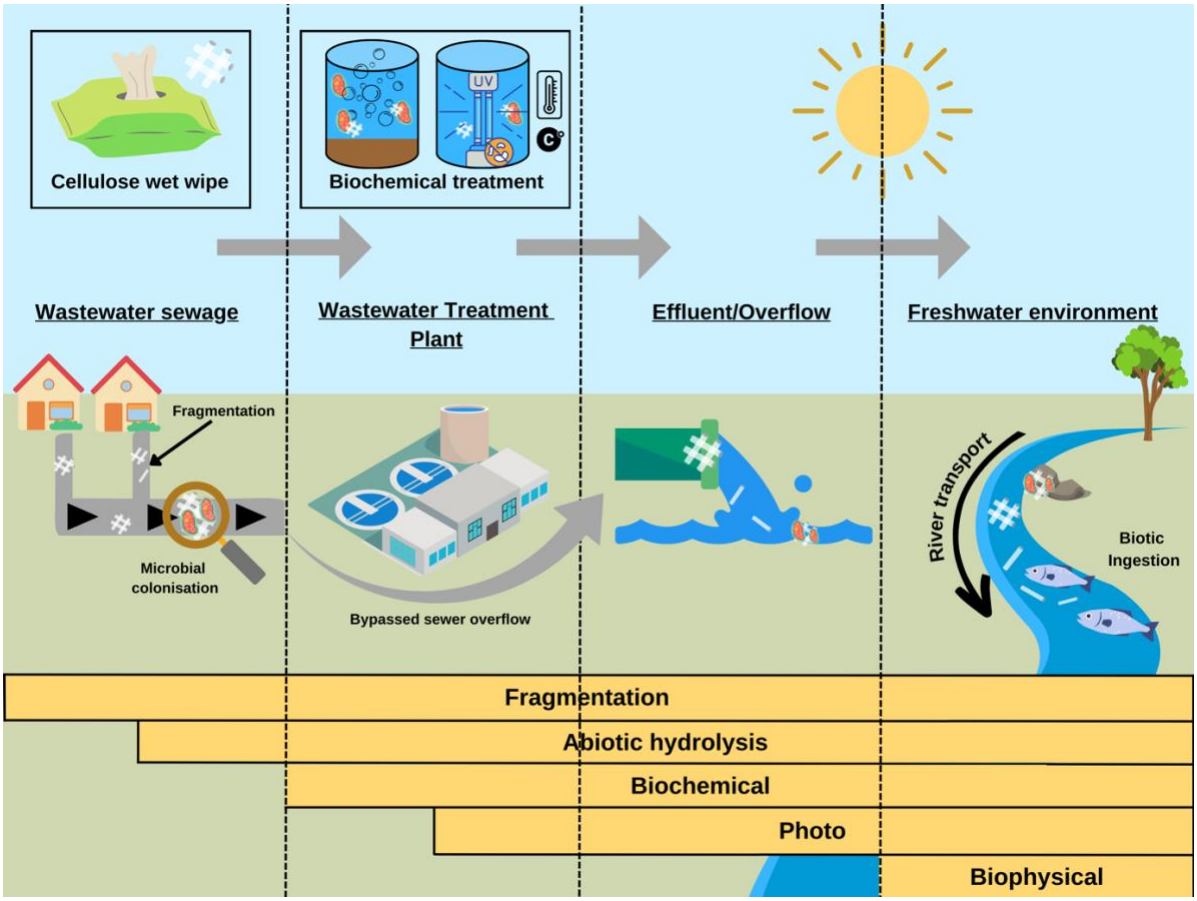

Fig. 2.4. Flushed cellulose wet wipe disposal pathway and expected degradation processes at each stage.

2.3.1 Degradation in the wastewater sewage environment

2.3.1.1 Fragmentation

When wet wipes are flushed down the toilet, they enter the sewage system and mix with wastewaters (Fig. 2.4). During sewage transport, two primary degradation mechanisms are expected. The first process is fragmentation, whereby exposure to abrasive and mechanical forces from physical interactions with other (semi)solid particles break a material down into smaller pieces (Duan et al., 2021; Drummond et al., 2022). For wet wipes, physical interactions in sewers will likely result in material surface crack defections that initiate further fragmentation processes throughout the sewer, creating an abundance of increasingly smaller fibres (Enfrin et al., 2020; Drummond et al., 2022).

The overall physical damage caused to wet wipes significantly depends on their tensile strength and elastic modulus (Ghorbani et al., 2013), although their overall mechanical stability will be reduced as a result of these abrasive and mechanical forces (Duan et al., 2021). In the case of cellulose-based wet wipes, physical degradation is expected to be high as their fibres typically have weaker tensile strength and elasticity than their synthetic counterparts (Klemm et al., 2005; Remy et al., 2015). Recent experiments suggest that cellulose-based wipes may shed an average of 548,000 microfibres per gram in simulated wastewater conditions (Kwon et al., 2022).

2.3.1.2 Abiotic hydrolysis

The second degradation mechanism expected for cellulosic wet wipes abiotic hydrolysis (Fig. 2.4), a process where water reacts with the material's surface to break down its molecular chains (Speight, 2017). Hydrolytic degradation is influenced by the presence of hydrophilic monomers and end groups, degree of crystallinity, overall material size, and oxygen-containing functional groups in the polymer backbone (Kale et al., 2007; Farah et al., 2016; Filiciotto and Rothenberg, 2021). In biodegradable plastics, hydrolytic degradation occurs in two stages: 1) random hydrolytic scission of ester bonds in the amorphous regions leading to increased crystallinity, and 2) inward degradation of the amorphous regions towards the polymers' core (Elsawy et al., 2017).

For cellulose, abiotic hydrolytic degradation involves the molecular scission of its glycosidic bonds, which typically requires industrial acidic or alkaline conditions that are not commonly found in wastewaters (Sharma et al., 2013). Hydrolytic degradation in cellulose is also made difficult due to its high degree of insolubility, inflexible molecular chains, and self-aggregation of fibres with higher hemicellulose content into gelatinous substances in water (Ghasemi et al., 2017; Väisänen et al., 2021a; Väisänen et al., 2021b). Cellulose-based wet wipes exacerbate these issues by aggregating in wastewaters and causing blockages (Drinkwater and Moy, 2017).

In principle, for cellulose fibres to degrade hydrolytically, water must diffuse into its structure and affect both the amorphous and crystalline regions, however, the efficiency of this molecular degradation relies on the reduction of its crystalline region and the disentanglement of its molecular chains simultaneously (Cheng et al., 2012; Ghasemi et al., 2017; Ghasemi et al., 2018), which is often not possible by abiotic hydrolysis alone. Therefore, hydrolytic

degradation of cellulose-based wet wipes is likely only effective when other degradation mechanisms such as fragmentation are also occurring (Fig. 2.4).

Hydrolysis causes cellulose fibres to swell in water, especially in fibres with smaller diameters and lower crystallinity (Ghasemi et al., 2018; Väisänen et al., 2021a). It occurs as a result of the high number of hydroxyl groups within the secondary wall that allows water molecules to easily penetrate under osmotic pressure, increasing the overall solvent concentration inside (Peng et al., 2017). Fibre swelling can reduce the material's overall ability to degrade within aquatic environments and may be visually identifiable or detected through initial polymer weight gain (Khazraji and Robert, 2013; Harter et al., 2021). This raises concerns about the pre-treatment processes involved in making biodegradable wet wipes and their solubility for effective hydrolytic degradation within the aquatic environment.

2.3.2 Wastewater treatment plant

Following their sewer transport, cellulosic wet wipes and their fibres enter wastewater treatment plants (WWTPs) and are subject to a range of filtration processes. These include physical filtration processes that remove the larger fractions of wet wipe; however, their smaller fragmented microfibres can pass through into the biochemical filtration stages of treatment. Physical fragmentation and abiotic hydrolysis processes are expected to continue during this wastewater stage due to their sustained physical interactions with wastewater and solid particles. Furthermore, abiotic hydrolysis of cellulose-based wet wipes and their fibres may be enhanced by the introduction of two expected chemical degradation mechanisms during this stage – biodegradation and photodegradation (Fig. 2.4).

2.3.2.1 Biodegradation

Polymers in the environment can be biodegraded through two main pathways: biochemical microbial degradation and biophysical biotic ingestion (Duan et al., 2021; Ebrahimbabaie et al., 2022). As cellulosic wet wipe fibres are fundamentally organic and biodegradable, biochemical degradation is expected to play a key role in their breakdown in both wastewater systems and freshwater environments downstream.

Biochemical degradation is a complex process that occurs in multiple stages, some of which overlap with biophysical processes (Luo et al., 2022). In aquatic systems (Fig. 2.5), microorganisms first colonise the polymer surface, attracted by physical properties such as porosity, density, surface tension, and size (Windsor et al., 2019a; Zambrano et al., 2020b; Luo et al., 2022), as well as environmental factors including pH nutrient availability (Roohi et al., 2017). As they colonise, the microorganisms secrete extracellular polymer substances, which form a sticky biofilm on the polymer surface to aid further microbial growth (Roohi et al., 2017; Duan et al., 2021; Ngyuen et al., 2021). This is followed by a simultaneous biophysical and biochemical process in which hydrolase enzymes catalyse the hydrolysis of the molecular chains, leading to progressive dissimilation of the polymer and surface fragmentation (Bano et al., 2017; Liao and Chen, 2021; Ebrahimbabaie et al., 2022). This process generates low molecular weight oligomers and monomers, which are released into the environment due to chemical degradation and can be used as biomass energy sources for microbes through further bio-assimilation, mineralisation, and metabolite production (Bano et al., 2017; Duan et al., 2021; Liao and Chen, 2021; Ebrahimbabaie et al., 2022; Sun et al., 2022b).

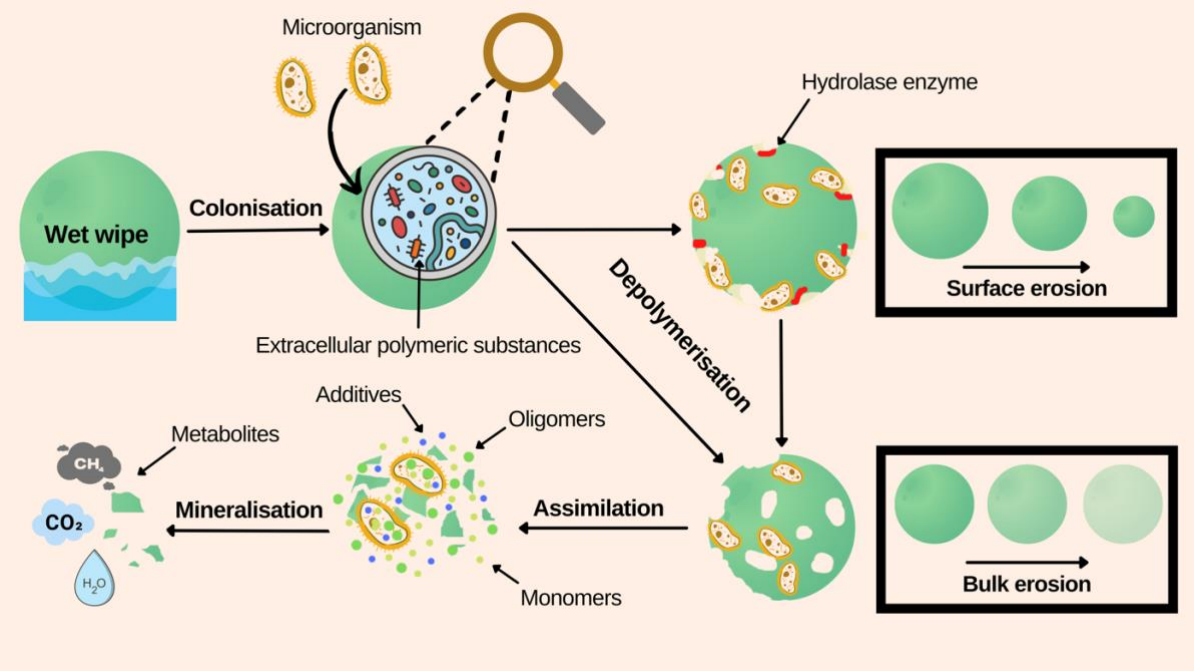

Fig. 2.5. Microbial degradation pathway for biodegradable materials in aquatic environments. Adapted from Duan et al. (2021).

Significant morphological changes occur during biochemical degradation, including changes in overall MW, tensile strength, surface area, adhesion, and hydrophilicity (Luo et al., 2022). Enzymes play a vital role in these changes, leading to loss of mechanical stability and embrittlement (Yuan et al., 2020), as well as the breakdown of polymer additives, which can be released into the environment or promote further biological assimilation (Duan et al., 2021; Luo et al., 2022). However, microbial hydrolytic processes within the natural environment are influenced by various polymer properties, including stereochemistry (Shen et al., 2020; Liao and Chen, 2021), crystallinity (Emadian et al., 2017; Wei et al., 2021), MW (Wei et al., 2021), size and surface area (Duan et al., 2021), as well as environmental conditions and other degradation mechanisms such as light availability, water quality, nutrients, microorganism communities and mechanical fragmentation forces (Zambrano et al., 2020b).

Limited research exists on the biochemical degradation of cellulosic wet wipes and their fibres in WWTPs. However, studies on the biodegradability of various cellulose-based materials in different environments provide valuable insights into their fate. For instance, rayon fibres had the highest rate of biodegradation due to their low crystallinity, degree of orientation, and high moisture regain (Park et al., 2004). Moreover, microbial biodegradation (>70 %) of cotton and rayon fibres was observed in WWTP activated sludge and freshwater, whilst poorer biodegradation occurred in seawater conditions and for cotton/polyester blends in general (Zambrano et al., 2020b). However, the extent of biodegradation depends on the types of enzymes and microorganism communities present (Nagamine et al., 2022). Experiments in rivers and lakes with cellulosic cotton strips also show how their biodegradation varies with temperature, nutrient concentrations, sediment load, and acid-base status (Tiegs et al., 2013; Colas et al., 2019; Carballeira et al., 2020).

It is expected that biodegradation processes in cellulosic wet wipes are most effective during their passage through WWTPs, where a wide range of aerobic and anaerobic microbial communities are present and where environmental conditions are controlled to facilitate molecular breakdown. Additionally, the degradation may occur earlier in the wastewater system when cellulosic wet wipes contribute to sewer blockages and are colonised by microbial communities, leading to subsequent biological breakdown (Drummond et al., 2022).

2.3.2.2 Photodegradation

Photodegradation is a significant cause of plastic breakdown (Zhang et al., 2021a), as well as cellulose in lab-based (Malešič et al., 2005), and quite possibly in aquatic environmental conditions (Fig. 2.4). This process is predominantly caused by ultraviolet (UV) energy from sunlight, which reacts with the photo-sensitive components of polymers (Luo et al., 2021). Photodegradation can cause molecular chain scission, surface oxidation processes, and the loss of mechanical properties, ultimately leading to MW loss into smaller harmless compounds (Rummel et al., 2017; Shao et al., 2018; Liao and Chen, 2021; Luo et al., 2022). UV filtration technology utilising photo-catalytic reactions is often used to treat and remove pollutants from wastewater (Ebrahimbabaie et al., 2022).

UV radiation with a wavelength >340 nm provides enough energy to degrade cellulose with the formation of hydroxyl radicals and oxidation (Malešič et al., 2005). Previous studies on cellulose photodegradation have also found hydroxyl radical breakdown products to form in cotton cellulose at wavelengths between 330 and 360 nm (Phillips et al., 1966; Hon, 1976).

Oxidation plays a critical role in cellulose molecular degradation (Shao et al., 2018). Free radicals and reactive oxygen species form when UV energy photons are absorbed by photo-sensitive chromophores in the macromolecules, causing molecules to enter excited energy states (Ebrahimbabaie et al., 2022). These excited states are then transferred to the nearest C–C and C–H covalent bonds through intramolecular energy transfer processes, overcoming their bond enthalpies and initiating molecular breakages (Duan et al., 2021; Filiciotto and Rothenberg, 2021). Reactive oxygen species have also been found to continue molecular degradation by migrating into the deeper layers of polymers (Ebrahimbabaie et al., 2022).

Manufactured cellulosic fibres have been found to contain a small number of chromophoric compounds, which are believed to derive from thermal, acidic, or basic condensation products of saccharides created during cellulose degradation (Rosenau et al., 2004). These same chromophores often show discolouration and surface damage in response to photodegradation in the aquatic environment (Cai et al., 2018; Ebrahimbabaie et al., 2022). Photodegradation processes in microparticles and fibres are highly influenced by their crystallinity, with higher crystallinity causing light to scatter and reflect (Duan et al., 2021), reducing light penetration distance. As a result, most photodegradation processes occur around the surface layers of

crystalline structures instead of the bulk (Luo et al., 2022). UV irradiation and oxidation changes the surface hydrophobicity and adsorption capacity of microparticles (Lin et al., 2020), and these morphological changes are likely increased by additional polymer degrading processes and environmental conditions (Duan et al., 2021). Although there is no study on the subject, it is expected that cellulosic wet wipes could also be impacted by these processes.

Thermal degradation treatment is sometimes used to improve the performance of anaerobic digestion of pollutants and sewer sludge dewatering processes (Hii et al., 2014). This process involves the absorption of energy (i.e. heat) to overcome the bond dissociation energy of C–C and C–H bonds, leading to chain scission, crosslinking, oxygenated free-radical formation, and a loss of molecular weight (Zhang et al., 2021a; Luo et al., 2022). Unlike photodegradation, thermal degradation affects the entire polymer rather than just its surface layers (Lambert, 2013). However, thermal degradation is often expensive and not feasible in wastewater treatment plants due to its high operating costs, and it typically requires temperatures exceeding 300 °C for natural cellulosic fibres (Jandura et al., 2000; Yang et al., 2007). This makes it an unlikely mechanism for cellulose wet wipes both in industrial and natural environments.

2.3.3 Freshwater environment

Two disposal scenarios exist for wastewater into freshwater environments (Fig. 2.4). Raw wastewater either enters WWTPs, undergoes a range of filtration treatments, and is then pumped out into the aquatic environment, or this pathway is bypassed, and wet wipes enter the aquatic environment through sewer spills – for example from combined sewer overflows. Spills are the predominant source of wet wipes directly into the freshwater environment (Besley and Cassidy, 2022). Given the evidence so far on freshwater pollution from cellulose fibres (Dris et al., 2018; Stanton et al., 2019; McCoy et al., 2020; Ó Briain et al., 2020), it is likely cellulosic wet wipe fibres might enter from both raw sewage spills and from treated sewage effluents.

Within freshwater environments, all previously highlighted degradation mechanisms for cellulosic wet wipes (Fig. 2.4) are expected to continue, particularly, physical fragmentation processes, due to the abrasive and mechanical forces caused by riverbed sediment, solid particles, and hyporheic processes (Duan et al., 2021; Drummond et al., 2022). Fragmentation rates for fibres can vary depending on their size, density, and transport within the aquatic

system. Larger and denser fibres are likely to sink, become buried within river sediments, and thus fragment more slowly (Nizzetto et al., 2016), although this may depend on the benthic and hyporheic exchange rates within freshwater sources (Eerkes-Medrano et al., 2015; Drummond et al., 2022). No studies were found on the impacts of physical degradation on cellulosic wet wipes within freshwater ecosystems. Nevertheless, there have been reports of wipes composed of cellulose and synthetic fibre blends found in downstream sewage effluent and freshwater that have likely been fragmented through crack defects and shear stress forces of mechanical mixing from WWTPs (McCoy et al., 2020; Ó Briain et al., 2020; Lee et al., 2021). During this freshwater transport stage, cellulosic wet wipes and fibres are likely to also be degraded through biophysical interactions with aquatic life.

Biophysical degradation results from the physical interaction and consequent ingestion and digestion of debris by biota within the natural environment. This is a well-evidenced process in freshwater for microfibres of natural, regenerated and synthetic origin (Remy et al., 2015; McGoran et al., 2017; Miller et al., 2017; Dris et al., 2018; Mateos-Cárdenas et al., 2020; Stone et al., 2020; Mateos-Cárdenas et al., 2021). Overall, it is seen as an ecologically negative process, due to the possible toxic and physically harmful nature of manufactured particles and their chemical additives (Ó Briain et al., 2020; Luo et al., 2022). Microparticle interactions with organisms may lead to the reduction in foraging behaviour, malnutrition, and physical blockages of the gastro-intestinal tract (Graham and Thompson, 2009; Ebrahimbabaie et al., 2022; Sun et al., 2022a). These impacts might also be enhanced if the fibres have absorbed pharmaceutical, agricultural and industrial chemicals found in WWTPs and untreated freshwater (Jiang et al., 2021; Ebrahimbabaie et al., 2022).

Whilst much of the focus has been on aquatic biota interactions with synthetic microplastics (Dris et al., 2018; Sun et al., 2022a), some biotic interactions with cellulose-based fibres have been identified. Lusher et al. (2013) found that the majority of microparticle polymers ingested by fish within the English Channel were rayon-based. Remy et al. (2015) identified viscose fibres within the gut content of macroinvertebrates on the Mediterranean coast. Cotton fibres were identified within the stomachs of the Clupeiformes fish (Collard et al., 2015). Jamieson et al. (2019) reported the biotic ingestion of cellulose, rayon and lyocell fibres by deep-sea Lysianassoidea amphipods in the Pacific Ocean. Fibre ingestion also represents a potential biological pollutant pathway to higher trophic levels and other ecosystems (Lusher et al., 2013;

Remy et al., 2015). Evidence of ingestion of cellulosic fibres by biota (other than microorganisms) is however still lacking in freshwaters (Dris et al., 2018).

Physical ingestion and digestion can also contribute to the continued fragmentation of materials that may either remain in an organism's digestive tract or be excreted back into an aquatic environment (Straub et al., 2017; Dawson et al., 2018). This has been identified in a lab-based study by Mateos-Cárdenas et al. (2020), of freshwater amphipods, *Gammarus duebeni*, which ingested PE microparticles, physically fragmenting them into smaller size orders in process. This fragmentation may be continued and catalysed by gastrointestinal enzymatic action (i.e. from amylase, cellulase, esterase, protease and lipase etc.) within the organism (Song et al., 2020; Duan et al., 2021). A follow-up study was conducted by the authors which found a greater abundance of ingested cellulose fibres than PE by *Gammarus duebeni* (Mateos-Cárdenas et al., 2021), although it presented no clear ecotoxicological impacts or fragmentation and excretion behaviour.

2.3.4 Interacting degradation mechanisms and end products entering natural environments

Understanding the behaviour of these different degradation mechanisms within the environment is made difficult by the often interacting and overlapping nature of these processes. Nevertheless, this is key to better understand and quantify the overall environmental fate and extent of risk from cellulose based wipes.

For example, in freshwater systems, photodegradation mechanisms that cause embrittlement on the polymer surface simultaneously promote the rate of mechanical fragmentation by physical abrasion (Song et al., 2017; Duan et al., 2021). Photo-oxidation processes within the surface of a polymer, can speed up the rate of microbial biodegradation and enzymatic hydrolysis (Filiciotto and Rothenberg, 2021). Biological ingestion can alter the physicochemical properties of polymers, promoting fragments and fibres, and if excreted, can influence their river transport and subsequent mechanical fragmentation behaviour (Windsor et al., 2019a). Physical fragmentation and the formation of crack defects can increase the surface area for microbial colonisation and for enzymatic hydrolysis reactions to take place (Zambrano et al., 2020b; Ngyuen et al., 2021). Abiotic degradation mechanisms that cause

chain scission near the polymer surface may result in the formation of oxygen-containing groups, increasing its hydrophilicity and thus improving its biodegradation potential (Gewert et al., 2015; Ebrahimbabaie et al., 2022).

Hydroxyl radical-induced degradation is a common but important molecular breakdown subprocess. It occurs within polymers during exposure to a range of different chemical degradation mechanisms, such as photodegradation, oxidative-degradation, biodegradation, and hydrolytic degradation (Shao et al., 2018; Tian et al., 2019; Shao et al., 2020; Zhang et al., 2021a; Luo et al., 2022). In essence, highly reactive radicals attack the carbon backbone of the polymer, causing further chain scission and crosslinking processes to occur, and they play an important role in cellulose degradation. They can initiate hydrogen cleavage of the C–H bonds on cellulose's pyranose rings in the presence of oxygen and cause subsequent chain scissoring of the β (1 → 4) glycosidic bonds (Haskins and Hogsed, 1950). They can also form peroxyl radicals that interact with cellulose photolytically leading to further radical formation and molecular breakdown (Duan et al., 2021). As hydroxyl-radical degradation is caused by various chemical breakdown processes found in aquatic environments (e.g. abiotic hydrolysis and photodegradation), theoretically, it is expected to occur within cellulose-based wet wipes throughout their wastewater and freshwater transport.

Overall, the likely end-products of biodegradable wet wipe entering natural aquatic environments are the result of five main degradation mechanisms discussed in Sections 2.3.1–3. These end-products may consist of the following components: 1) Fibres of varying sizes, which occur due to physical fragmentation processes along the domestic disposal pathway, and biotic ingestion in the downstream environment; 2) Molecular breakdown products (e.g. oligomers, monomers, additives, and metabolites) generated through hydrolytic, ultraviolet, and microbial-induced degradation mechanisms at different stages of the wastewater disposal pathway (See Fig. 2.4); and 3) Swollen wet wipe fibres and their undegraded amalgamations, particularly observed in sewer systems and when exposed to water.

2.4 Variables controlling degradation in laboratory or natural water environments

2.4.1 Oxygen

Environmental oxygen levels can play a significant role in the extent of all previously mentioned degradation mechanisms within aquatic environments (Duan et al., 2021; Issac and Kandasubramanian, 2021). To date, however, research on the anaerobic degradation of microplastics (non-degradable and biodegradable) within the environment is scarce (Ebrahimbabaie et al., 2022). The presence of oxygen can alter the metabolic pathway of microbial action to polymers and may partially influence thermal degradation (Duan et al., 2021). In oxygen-rich environments, microorganisms use polymers as an energy source and produce carbon dioxide and water byproducts in addition to heat, whilst in anaerobic conditions, microbes produce methane gases and biomass with less thermal energy produced (Bátori et al., 2018).

WWTPs use both aerobic and anaerobic microbial conditions for biodegradation as an effective secondary treatment for removing organic matter within sewage systems (AEAL, 2009), with anaerobic microbial action commonly occurring within sewage sludge biosolids. Biodegradation is potentially more limited within unconditioned freshwater environments because there is a less diverse range of microbial communities available (Zambrano et al., 2020b). Oxygen levels within freshwater can also be changed by chemically toxic contaminants, often stemming from wastewater effluent and agriculture (Jamee and Siddique, 2019), which may have an influence on the rate of environmental degradation processes.

2.4.2 Water

Water itself can have varying impacts on environmental degradation processes. Photodegradation in freshwaters may be limited by light scattering on the water surface (Duan et al., 2021), although the extent of this hindrance would most likely depend on polymer buoyancy and the depth of the water source. Water plays an important role in influencing biodegradation, by impacting the types of microorganism communities available (including algae, bacteria, fungi, protozoa etc.), their polymer colonisation and ability to form biofilm,

and the rate of enzymatic hydrolysis (Yuan et al., 2020). Water content makes up a large proportion of wet wipes (>90 %), potentially promoting microbial colonisation and biofilm formation within aquatic environments (Salama et al., 2021).

Polysaccharides such as cellulose are generally hydrophilic in nature due to the high presence of hydroxyl groups, and as such, tend to react strongly to hydrolysis mechanisms from aquatic environments, especially within less organised amorphous regions (Karamanlioglu et al., 2017). However, as cellulose fibres are molecularly strong-bonded, water molecules are only capable of breaking already weakened bonds from other environmental degradation mechanisms (Khazraji and Robert, 2013). Nevertheless, cellulose-based wipes have been reported to easily tear and fragment when agitated in water (Ó Briain et al., 2020).

2.4.3 Temperature

Specific temperature conditions are important to many environmental degradation processes, but no more so than in biotic mechanisms. The biodegradation of biopolymers, as found in standardised industrial composting environments, partly relies on high temperature conditions (55 °C) (Liao and Chen, 2021) which are seldom found in the natural environment. Freshwater systems can vary in temperature by season and geography but usually range anywhere between 2 and 24 °C. WWTPs typically operate at slightly lower temperatures for biological filtration processes, and at slightly higher temperatures around 31 °C for aerobic biological treatment and 37.5 °C for anaerobic sludge digestion (Alisawi, 2020). Higher temperatures not only promote thermal degradation of the molecular bonds but also stimulate greater microbial and enzymatic activity (Duan et al., 2021; Luo et al. 2022), leading to a higher rate of hydrolytic breakdown in the polymer backbone (Kale et al., 2007). Zambrano et al. (2020a) found cellulosic cotton textile fabrics to shed more microfibres at higher temperatures during home laundering experiments. Research on cotton strip bioassays employed in freshwater streams have also identified temperature as a significant factor influencing cellulosic biodegradation (Griffiths and Tiegs, 2016).

2.4.4 pH level

The pH level of the receiving waters can also regulate degradation. For example, physical fragmentation of biodegradable polymers is reported faster in seawater compared to

freshwaters, as a result of higher rates of hydrolysis linked to less acid conditions (Wei et al., 2021). However, hydrolytic degradation can occur in both pH extremes (Elsawy et al., 2017), and depolymerisation of cellulose is commonly achieved under both acidic and alkaline conditions (Väisänen et al., 2021a), with glycosidic bond cleavage possible under acidic conditions and general hydroxyl radical-induced degradation purporting to be enhanced by both (Shao et al., 2018; Shao et al., 2020). Microparticle absorption behaviour in aquatic environments is known to vary with pH, with consequences for pollutant transport, and this could also apply to cellulose (Xu et al., 2018; Tirkey and Upadhyay, 2021; Sun et al., 2022b).

2.4.5 Organic matter, nutrients, and contaminants

Manufactured particles in aquatic systems can act as a vector for various toxic/nontoxic chemical elements, such as metal ions and natural organic matter, that could influence the surface morphology and degradation mechanisms (Li et al., 2018; Zhou et al., 2020). For example, microparticles in freshwaters are often covered in dissolved organic matter (i.e. humic acid) that carry negative ionic charge and interactive oxygen-containing functional groups, which may alter their surface properties and polymer degradation reactions (Chen et al., 2018; Sun et al., 2022b), particularly photodegradation since organic matter presents highly abundant chromophores that can act as photosensitisers (Duan et al., 2021).

The concentration of organic matter could also influence the abundance and variety of microbial communities that colonise onto wet wipes (Windsor et al., 2019a). For example, for microparticles, high concentrations of nutrients and organic matter within wastewater environments have been found to increase microbial activity, improving carbon and nitrogen mineralisation and bio-assimilation, and enzymatic degradation (Tiegs et al., 2013; Colas et al., 2019; Zambrano et al., 2020b; Schell et al., 2022). Furthermore, it is likely that cellulosic fibres that are degraded into their glucose units could also in turn provide nutrients that promote microbial colonisation and biofilm formation (Salama et al., 2021).

2.4.6 Summary of expected degradation behaviours

Based on the theorised mechanisms discussed in Section 2.3, and their likelihood within real-life environmental conditions highlighted in Section 2.4, physical fragmentation is expected to be the predominant degradation process throughout the disposal pathway of a flushed

biodegradable wet wipe. This process may occur independently, through physical interactions during transport, or in conjunction with physico-chemical breakdown mechanisms. Of the expected molecular degradation mechanisms, it seems abiotic hydrolysis is likely to be the most widespread, due to the interactions that flushed wet wipes will have with water throughout their transport. However, initial fragmentation and other molecular degradation processes are required for this to become effective. Moreover, based on the analysis in Sections 2.3 and 2.4, it seems that biochemical degradation is likely to be an effective mechanism for the molecular breakdown of cellulosic wet wipes in aquatic environments, especially within sewer systems and WWTPs where the most diverse range of microbial communities are likely to be found. Ultimately, the extent of these degradation processes in biodegradable wet wipes will be influenced by their specific fibre composition and physicochemical properties.

2.5 Conclusion

This chapter asked whether flushed wet wipes labelled as biodegradable really degraded during their passage through wastewater systems and into freshwater environments. Whilst mostly cellulose based, the findings indicated that these wipes often consist of a combination of bio-based and synthetic materials, including various chemical additives that are highly likely to limit their environmental degradation.

Given the current lack of research on the degradation mechanisms involved, five likely aquatic degradation mechanisms for cellulosic wet wipes were identified: photodegradation, abiotic hydrolysis, physical fragmentation, biophysical ingestion, and biochemical microbial degradation. Of these mechanisms, fragmentation and biochemical degradation were likely to be the most dominant in real-world conditions but the extent of the latter process is still limited. It was thus demonstrated that cellulosic wet wipes are likely to contribute significantly to microfibre pollution in aquatic environments due to limited molecular breakdown and significant physical fragmentation processes during transit from toilet disposal to aquatic environments. Consequently, and despite being marketed as “biodegradable” and “flushable”, these products are likely to persist in the environment and pose a significant threat to aquatic ecosystems. Lack of international regulation for biodegradable and flushable wet wipes will no doubt compound current consumer confusion around appropriate disposal methods.

To advance our understanding of the environmental impact of wet wipes and to support better decision-making for their management and regulation, the following research directions are proposed:

1) Conduct more field research to better understand the sources, abundance, and degradation mechanisms involved in biodegradable wet wipes and their microfibres within wastewater, sewage sludge, and freshwater environments.

2) Investigate the role of textile additives applied during wet wipe manufacturing, which may significantly influence the degree of environmental degradation and harm.

3) Apply a wider spatio-temporal context that captures the transport pathways of wet wipes and their fibres, and their possible degradation processes along this transport. This context should consider wider life cycle assessments of the product, from raw material source to disposal and final environmental fate.

4) Consider the biophysical interactions of wet wipes and their fibres in a wider ecological context, assessing the transference capability and risk of ingested bio-based microfibres to higher trophic levels within and beyond freshwater ecosystems.

5) Investigate further the environmental variables that influence physical and chemical breakdown, particularly for cellulosic fibres and wet wipes.

6) Standardise field sampling methodologies, including common units of measurement, chemical identification, and sampling techniques, to promote easier and more effective data communication and comparable results across research.

Chapter 3

Predicting Flushed Wet Wipe Emissions Into Rivers

A version of this chapter is available online as:

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In this chapter, Thomas Allison, Benjamin Ward, Isabelle Durance, and Michael Harbottle designed the concept and methodology. Thomas Allison collected, processed, analysed, and plotted the data. Thomas Allison drafted and edited the manuscript following review and comments from all authors.

Abstract

Flushed wet wipes pose a significant pollution risk to river systems at both macro and micro levels. However, the pathways linking their emissions to environmental contamination remain poorly understood. Here, emissions-based mathematical modelling was integrated with existing data on wet wipe disposal and microfibre generation to predict the quantity of emissions entering river systems and the transport pathways involved. Results indicate that wastewater pathways, including sewer overflows, wastewater treatment plants, and agricultural runoff, are major conduits for these pollutants. Despite advanced wastewater treatment, substantial microfibre emissions still enter the environment. Extrapolating modelled estimates to larger scales reveals wet wipe pollution as an international issue requiring urgent attention, with up to 180 and 1,200 tonnes annually entering UK and EU rivers, respectively, from flushed plastic and cellulosic wipes. This research offers a comprehensive modelling framework applicable to various wastewater pollutants, providing valuable insights for policymakers and the water industry. Improved data on wet wipe disposal, fate, and spatially distributed wastewater systems are necessary to pinpoint their environmental risks more accurately.

3 Predicting Flushed Wet Wipe Emissions into Rivers

3.1 Introduction

Driven by current hygiene standards and consumer convenience (Shruti et al., 2021), the prevalence of wet wipes and their improper disposal threatens river systems (Ó Briain et al., 2020). When flushed down the toilet, these wipes navigate wastewater systems, either resisting degradation due to their material strength and aggregation properties or physically breaking down into large volumes of microfibres (Pantoja-Munoz et al., 2018; Durukan & Karadagli, 2019; Atasagun & Bhat, 2020). Undegraded or partly degraded 'solid' wet wipes are notorious for blocking sewers and causing overflow, which lead to raw sewage spilling into rivers (Drinkwater & Moy, 2017; Giakoumis & Voulvoulis, 2023). Furthermore, wet wipe microfibres, mainly composed of plastic or cellulose, can be ingested by aquatic wildlife, act as vectors for surrounding pollutants, or release harmful chemical additives (McCoy et al., 2020; Ó Briain et al., 2020; Allison et al., 2023).

Despite widespread public knowledge and regulatory advisories against the flushing of wet wipes, improper disposal remains a persistent issue. To thoroughly assess the environmental risks created by these flushed wipes, it is important to first know the quantities likely to enter river systems. Studies estimating solid wet wipe emissions to wastewaters and microfibre generation under wastewater conditions provide valuable first insights to understanding the environmental fate of wet wipes (Lee et al., 2021; Kwon et al., 2022; UKWIR, 2022). However, they focus on the initial disposal stage of wipes into wastewaters. Lack of quantified understanding of the 'journey' of each wet wipe flushed down the toilet precludes any quantification of wet wipes emissions to rivers, and ultimately to seas and oceans. Preventing the flushing of wet wipes is a key mitigation strategy, but understanding their transport through wastewater systems is essential for developing further targeted solutions. This chapter aims to contribute to that effort.

Emission-based mathematical models, which estimate the input, output, and transport pathways of specific pollutants within a system, have proven effective in quantifying plastic emissions to rivers from wastewaters (Nizzetto et al., 2016; Siegfried et al., 2017; van Wijnen et al., 2019; Kawecki & Nowack, 2020). However, no models have specifically focused on wet

wipes or their microfibres, despite significant numbers entering wastewaters and their potential environmental impacts.

This chapter presents the first comprehensive model to quantify likely wet wipe emissions to rivers by integrating available data on flushed wet wipe disposal and their microfibre generation behaviour with emission-based mathematical modelling. In turn, the following steps were undertaken: 1) an emission model was parameterised to trace the entire 'journey' of flushed wet wipes from wastewater systems to rivers; 2) the main entry points of flushed wet wipe into rivers were identified and their predicted contributions quantified; 3) the modelling framework was applied to estimate wet wipe emissions across both UK and EU rivers; and 4) the impact of wet wipe pollution was contextualised by comparing their river emissions with those of laundry microfibres. This work provides the first large-scale quantification of wet wipe pollution, revealing the key pathways and the environmental hazards associated with both solid and microfibre forms of wet wipes.

3.2 Materials and methods

3.2.1 Overview of the emission model

The wet wipe model was developed by integrating microplastic emissions modelling with experimental models of wet wipe microfibre generation in wastewater systems. The emissions approach identifies point-source mass inputs of micropollutants through a system into the environment, while the experimental models simulate how different wet wipe materials behave in simulated wastewater systems, focusing on their disposal, microfibre shedding, and transport dynamics. This integrated model was used to identify likely wastewater transport pathways and controlling parameters for two emissions scenarios: flushed solid wet wipes and fragmented wet wipe microfibres (Fig. 3.1). In both scenarios, focus was placed on wet wipes made with either plastic-based or cellulose-based fibres to understand the broader impacts created by commonly flushed wet wipes.

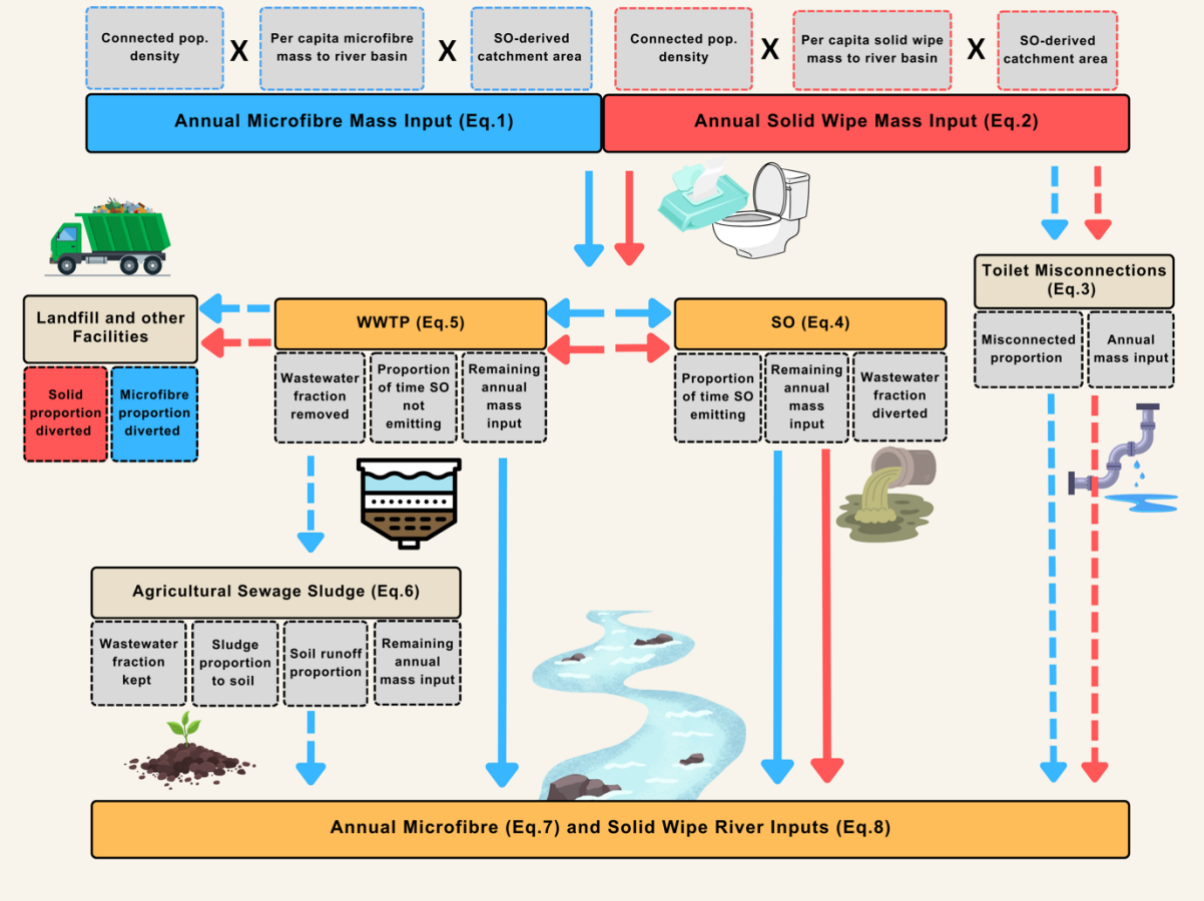

Fig. 3.1. Wastewater Emission Pathways and Parameter Inputs. Colour schemes represent modelled microfibre (blue) and solid wipe (red) emissions, with pathways categorised by lowest (orange; solid arrows) and greatest (beige; dashed arrows) uncertainty. Parameter inputs for each pathway are shown in grey and detailed in the methods, with corresponding equations attached to their respective pathways.

3.2.2 Study catchments

Wastewater systems in the model were based on local sewer overflow (SO) data. SOs refer to any spills from the sewer system and wastewater treatment plants (WWTPs) when capacity is exceeded. As visualised in Fig. 3.2, SO operational data was analysed from two UK river sub-catchments within larger catchments that vary in urbanisation: a semi-urban sub-catchment of the River Taff (83 km^2 area; 1,223 inhabitants/km^2, calculated using a weighted average of SO catchment areas; 26 SOs) and a rural sub-catchment of the River Wye (392 km^2 area; 311 inhabitants/km^2; 18 SOs).

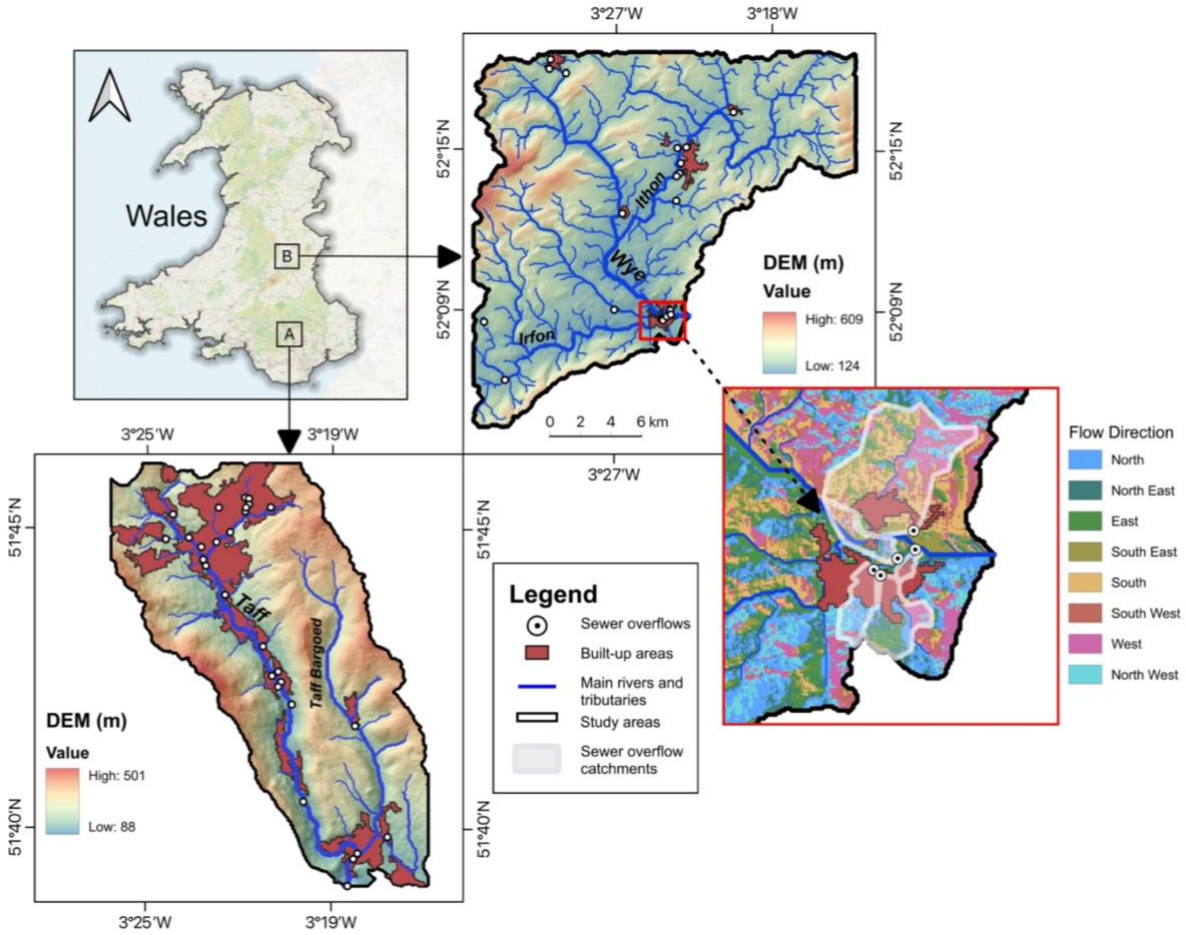

Fig. 3.2. The two sub-catchment study sites: A) Taff sub-catchment, B) Wye sub-catchment, showing publicly available sewer overflow locations, an example of delineated overflow drainage areas, surrounding built-up areas, and main rivers and tributaries. Delineated drainage areas were essential for applying the emissions model to any geographical area by identifying populations connected to each overflow. Map generated using the QGIS 3.36 software. OSM data provided by © *OpenStreetMap contributors* URL: https://www.openstreetmap.org/.

Due to the lack of data directly connecting populations to SOs, a method was developed to delineate SO drainage areas using local elevation data (SRTM, 2013) and flow direction modelling in QGIS version 3.26.0 (See Fig. 2). This approach was essential for estimating wet wipe emissions from connected populations into wastewater systems. SO drainage areas were assumed to be corresponded to the natural wastewater catchments, and this was validated using topographical data in QGIS. Population data for the catchments was sourced from Lower-layer Super Output Areas (LSOAs), which provide local demographic statistics (Ministry of Housing, 2019), while urban areas within these boundaries were identified using Corine Land Cover geospatial data (Copernicus, 2018).

3.2.3 Estimation of wet wipe mass inputs

UK annual mass inputs for plastic and cellulosic solid wet wipes (SG_{cap}) and their microfibres (MFG_{cap}) were estimated based on several assumptions. Annual UK wet wipe consumption is 11 billion, with 90% plastic and 10% assumed cellulosic (Water UK, 2022; BBC, 2023). Of these, about 2.5 billion wipes (23%) are flushed annually (UKWIR, 2022), equating to an average 33.2 plastic wipes and 3.69 cellulosic wipes flushed per person, based on the 2023 UK population of 67.78 million (United Nations, 2023).

For microfibre generation (MFG_{cap}), three case scenarios were used – best, worst, and average – based on mass ranges from Kwon et al. (2022). The best-case represents the lower bound, worst-case the upper bound, and average-case the mean (Table 3.1). Wet wipes were categorised into three types: 1) natural cellulosic fibres, 2) regenerated cellulosic fibres, and 3) plastic fibres, ranked by their shedding potential in simulated wastewaters (Kwon et al., 2022). 'Non-natural' fibres were treated as regenerated cellulosic fibres based on Zambrano et al.'s (2020) similar findings.

With the growing use and frequent misclassification of regenerated cellulose in wet wipes (Allison et al. 2023), a 10% share for regenerated fibres beyond the current 90:10 split was added to account for likely overlap or underrepresentation in existing estimates while preserving plastic and assumed natural fibre proportions. All microfibres were assumed to be released upon initial wastewater entry, simplifying the model to focus on estimating total microfibre load without modelling degradation during transport.

Microfibre inputs were based on an average wet wipe mass of 4.75 g (Durukan and Karadagli, 2019) (Table 3.1). Solid wet wipe mass inputs (SG_{cap}) were calculated by multiplying the number (#) of flushed wet wipes by their average mass. Using these experimental datasets (Durukan & Karadagli, 2019; Kwon et al., 2022), microfibre masses were also converted into counts, yielding an average of 2,603,000 microfibres per natural wipe, 132,050 per regenerated wipe, and 13,965 per plastic wipe released into wastewater.

Table 3.1. Microfibre generation data for wet wipes under wastewater conditions adapted into the modelling system from existing literature. Values not in brackets represent mean (average-case) scenarios, while the lower bound and upper bound values in brackets represent best- and worst-case scenarios, respectively.

Fibre type	Microfibre generation (#/g wipe)[a]	Mass generation (mg/g wipe) [a]	Total microfibre generation (#/wipe)*	Total mass generation (g/wipe)*
Natural	548,000 (163,000 – 933,000)	28 (16-40)	2,603,000 (774,250 – 4,431,750)	0.133 (0.076 – 0.19)
Regenerated†	27,800 (15,000 – 40,600)	3.6 (0.4 – 6.8)	132,050 (71,250 – 192,850)	0.0171 (0.0019 – 0.0323)
Plastic	2,940 (710 – 5170)	0.73 (0.24 – 1.22)	13,965 (3,373 – 24,558)	0.0034 (0.0011 – 0.0058)

[a] Values derived from Kwon et al (2022).
* Average wipe mass when wet of 4.75 g derived from Durukan and Karadagli (2019).
† Originally non-natural but relabelled as regenerated based on Zambrano et al. (2020).

3.2.4 Point source inputs to wastewater

Wet wipe inputs to rivers via wastewater systems in the model depend on several wastewater parameters, including per capita input of solid wet wipes and microfibres, SO catchment area size, and population density. Full population connectivity to wastewater systems was assumed while accounting for sewer misconnections. The input of wet wipe microfibres into wastewater systems via toilet flushing is calculated as:

$$WWi_{wmf} = PDenCon \times MFG_{cap} \times SOA \quad (3.1)$$

where WWi_{wmf} is the total mass of wet wipe microfibres entering wastewater systems from each sub-catchment (g/y); $PDenCon$ is the population density connected to the sewage system within the SO catchments (capita/km^2); MFG_{cap} is the estimated annual mass release of wet wipe microfibres in wastewater systems per person (g/capita/y); and SOA is the wastewater catchment area derived from SOs (km^2).

Similarly, the input of solid wet wipes is calculated as:

$$WWi_{ws} = PDenCon \times SG_{cap} \times SOA \quad (3.2)$$

where WWi_{ws} is the total mass of solid wet wipes entering wastewater systems from each sub-catchment (g/y); and SG_{cap} is the estimated annual mass input of solid wet wipes in wastewater systems per person (g/capita/y).

To calculate $PDenCon$, geomorphological drainage catchments were delineated for each SO and estimated population using several methods. In straightforward cases, the LSOA population was multiplied by the SO catchment area (SOA). For more complex regions with overlapping built-up areas or varied population densities, adjustments were made as follows for accuracy. If large catchments inflated LSOA estimates, the portion of built-up area around each SO was used to refine the population estimate. Where no built-up area existed, the default LSOA density was used. In catchments entirely within built-up areas, the LSOA population density was applied. When SO catchments overlapped multiple LSOAs, the population density was averaged.

3.2.5 Wastewater pathways into rivers

The emissions model parameterised three primary wastewater point sources to rivers: toilet misconnections, SOs, and WWTPs, along with diffuse sources through agricultural sewage sludge and soil runoff, and landfills as environmental sinks

Toilet misconnection inputs to rivers ($MISC_i$) were adapted from Ellis and Butler (2015), who identified an annual misconnection rate of 3% in England and Wales, of which 8% were linked to toilets. The annual mass input of wet wipes and microfibres entering rivers via misconnected toilets from each sub-catchment (g/y) was calculated as:

$$MISC_i = PMisc \times \ WWi_{wmf} \ or \ WWI_{wws} \quad (3.3)$$

where $PMisc$ is the annual UK toilet misconnection proportion (0.0024).

Annual river input from SOs during their periods of activity (SO_i) was proportionally modelled using 2021 UK wastewater monitoring data on SO operation hours. A constant flow of wet wipes during SO activity and 50% diversion rate to rivers was assumed, with the remainder

sent to WWTPs, in line with Jones et al. (2024, preprint). Therefore, the SO input to rivers in each sub-catchment was calculated as:

$$SO_i = \frac{WWi_{wmf} \ or \ WWI_{wws} \times PTime_{ON}}{2} \quad (3.4)$$

where, $Ptime_{ON}$ is the yearly operational proportion of a SO (0-1).

To estimate wet wipe microfibres entering rivers in each sub-catchment after wastewater treatment ($WWTP_{ti}$), average removal rates were used for microplastic and microfibres reported in the literature (Table 3.2). This approach enabled the modelling of the fraction of microfibres that escape removal under different levels of treatment. For solid wet wipes, complete removal by mesh screens or blockage maintenance was assumed, with the waste diverted to landfills. Microfibre input to rivers post-treatment (g/y) was calculated as:

$$WWTP_{ti} = (WWi_{wmf} - SO_i) \times 1 - Ptime_{ON} \times WWrem_{ti} \quad (3.5)$$

where, SO_i, in this case, represents wet wipes bypassing treatment during SO operation, and $WWrem_{ti}$ represents the inefficiency of microfibre removal for different treatment levels (0-1).

Table 3.2. Wastewater treatment efficiencies previously reported for microplastic pollutants in the surrounding literature.

Study	WWTP location	Population Equivalent	Pollutant type	Removal efficiency
Baresel and Olshammar (2019)	WWTP compilation, Baltic Sea Region	N/A	Microplastic	85% (Primary), 90% (Secondary), 98% (Tertiary)
Carr, et al. (2016)	8 WWTPs, Southern California, USA	N/A	Microplastic (fragments and fibres)	99.9% (Secondary), 99.9% (Tertiary)
Conley, et al. (2019)	Plum Island, South Carolina, USA;	180,000	Fibres	97.2% (Secondary)
	Rifle Range Road, South Carolina, USA;	53,000		80.2% (Secondary)
	Center Street, South Carolina, USA	32,000		83.7% (Secondary)

Edo, et al. (2020)	Henares River, Madrid, Spain	300,000	Microplastic (fragments and fibres) Cellulosic fibres	93.7% (Primary)
Gies, et al. (2018)	River Fraser, British Columbia, Canada.	1,300,000	Microplastic (fibres)	92.8% (Primary) 98.4% (Secondary)
Horton, et al. (2020)	4 unnamed UK WWTPs	126,425 average	Microplastic	99.8% average (Secondary)
	4 unnamed UK WWTPs	137,250 average		99.8% average (Tertiary)
Jiang, et al. (2022)	4 WWTPs, Ningbo, China	N/A	Microplastic (Fragments, Fibres, Films, Foams and Pellet) and Cellulose Fibres	95% average (Tertiary)
Lares, et al. (2018)	Kenkäveronnie-mi, Mikkeli, Finland	N/A	Microplastic (particles and fibres)	99.4% (Primary and Secondary)
Magnusson and Norén (2014)	Långeviksverket, Lysekil, Sweden	14,000	Microplastic (fibres, fragments and flakes)	99.9% (Secondary)
Michielssen, et al. (2016)	Detroit Michigan, USA	2,357,666	Microplastic and Cellulosic particles (fibres, fragments, microbeads and paint chips)	84.1% (Primary), 93.8% (Secondary)
	Northfield, Michigan, USA	9,909		88.4% (Primary), 89.8% (Secondary), 97.2% (Tertiary)
Murphy, et al. (2016)	River Clyde, Glasgow, UK	650,000	Microplastic (flakes, fibres, films, beads and foams)	78.34% (Primary), 98.41% (Secondary)
Talvitie, et al. (2017a)	Viikinmäki, Helsinki, Finland	800,000	Microplastic (fibres, fragments, flakes, films and spheres) Cellulosic fibres	97% (Primary), 99% (Secondary and Tertiary)
Zhang, et al. (2021)	WWTP Compilation and estimation	100,000	Microplastic (fibres)	95% average (Tertiary)

$WWrem_{ti}$ categorises three wastewater treatment types based on microplastics research: 1) Primary treatment, including basic physical processes like screening, sedimentation, and skimming (AEAL, 2009); 2) Secondary treatment, involving biological treatment and solid clarification (Michielssen et al., 2016; Murphy et al., 2016); and 3) Tertiary treatment, using advanced filtration technologies such as bioreactors and sand filtration. These treatment types

were applied to the best-case (tertiary), worst-case (primary) and average-case (secondary) emission scenarios, with the latter based on its common usage in the UK and EU (Kawecki & Nowack, 2020; Table 3.2). Average treatment efficiencies derived from the literature – 88.48% for primary, 94.58% for secondary, and 97.7% for tertiary – were converted into fractional inefficiencies (0-1) for calculations.

Based on the existing literature (Gies et al., 2018; Lares et al., 2018; Schell et al., 2022; Zhang et al., 2022), it was assumed that most fibres removed during treatment are eventually directed to sewage sludge facilities. Based on UK data (Lofty et al., 2022), an average 68% of sludge was applied to agricultural soils in our model, with the remaining 32% sent to landfills.

The model estimated microfibre transport from agricultural land as sewage sludge to rivers via soil runoff ($AgrSS_i$), assuming 25% soil retention for microfibres, and 75% transported to rivers. This was based on theory regarding heavier than water particle runoff from rivers and estimated microplastic retention in agricultural soils, reported to range from 10-40% (Nizzetto et al., 2016; Norling et al., 2024). Therefore, agricultural runoff to rivers was calculated as:

$$AgrSS_i = WWi_{wmf} \times WWrem \times P_{SS} \times P_{SR} \quad (3.6)$$

Where P_{SS} is the proportion of microfibres in sewage sludge exported to agricultural soils (0-1); and P_{SR} is the proportion of microfibre soil runoff to rivers (0-1).

3.2.6 River input from wastewater pathways

The model calculates the total annual mass of solid wet wipes (Ri_{WS}) and microfibres (Ri_{WMF}) entering rivers from the two study sub-catchments (g/y). This input is expressed for the microfibre and solid wet wipe scenarios, respectively, as:

$$Ri_{wmf} = \Sigma(MISC_i + WWTP_{ti} + CSO_i + AgrSS_i) \quad (3.7)$$

$$Ri_{ws} = \Sigma(MISC_i + CSO_i) \quad (3.8)$$

3.2.7 Model limitations

This study represents an initial attempt to model wet wipe emissions through wastewater systems, and thus, has inherent uncertainties. Due to the limited data on wet wipe disposal and fate in wastewater and river systems, conservative assumptions were used to construct parameters, acknowledging the inability to fully capture all variations and complexities. Confounding factors in microfibre scenarios were partly mitigated by using likely case-scenarios as upper and lower confidence bounds. Further limitations are discussed in the results and discussion sections. Other key limitations are discussed here.

It was assumed that all flushed wet wipe emissions enter the wastewater system. However, wipes that cause blockages are removed during maintenance and reallocated to the landfills. The framework only considers fragmentation processes due to limited data on biochemical and biophysical degradation processes in wet wipes (but see Allison et al. 2023 for a review). While the model provides insight into flushed wet wipe transport to rivers, downstream factors beyond the study's scope may influence the fate of emissions in rivers and other ecosystems (Besseling et al., 2017; Siegfried et al., 2017; van Wijnen et al., 2019; Kawecki & Nowack, 2020). In addition, the model applies particle deposition principles largely based on studies of fragments and spherical particles, whereas fibres exhibit distinct transport behaviours that are not fully captured by density-based assumptions (Schenkel et al. 2023).

3.2.8 Applying the model to laundry microfibres

To validate the wet wipe emission model, it was extended to assess laundry microfibres, a better-studied wastewater pollutant, to benchmark and enhance the robustness of our estimates. Using data from Vassilenko et al. (2021) on plastic (0.161 ± 0.173 g/kg/wash) and cellulosic (0.165 ± 0.44 g/kg/wash) fibres, new parameters were introduced to estimate annual inputs to wastewater systems per UK individual. These included an average household size of 2.36 individuals (Office for National Statistics, 2023), 260 laundry loads per household per year (Office for National Statistics, 2016), and a standard 6 kg wash load. All other parameters remained consistent with the wet wipe microfibre scenarios. The large standard deviation for plastic fibres reflects high variability in shedding across different textile types.

3.3 Results

3.3.1 Emission pathways and transport dynamics of wet wipes

To inform transport mechanisms, the scenarios were first modelled from individual inputs to wastewater systems, through interconnected pathways, to their eventual fate in rivers (See Fig. 3.1). Emission pathways and parameters were assigned based on prior studies of wastewater pollutants and emissions modelling (Murphy et al., 2016; Nizzetto et al., 2016; Siegfried et al., 2017; van Wijnen et al., 2019; Van den Berg et al., 2020; Di Nunno et al., 2021; Schell et al., 2022). Three sets of pathways were selected: 1) point sources directly transporting wet wipe materials to rivers (toilet-derived sewer misconnections, sewer overflows (SOs), and wastewater treatment plant (WWTP) effluents); 2) diffuse sources to rivers (agriculturally applied sewage sludge and soil runoff); and 3) terrestrial sinks, including landfill facilities and the retention of pollutants in agricultural soils (Fig. 3.3). The transport of wet wipe materials through these pathways are assumed to be size-dependent, with microfibres traversing all pathways due to their small size, and solid wipes being completely removed before or at the WWTP entry stage and directed to landfill and other facilities.

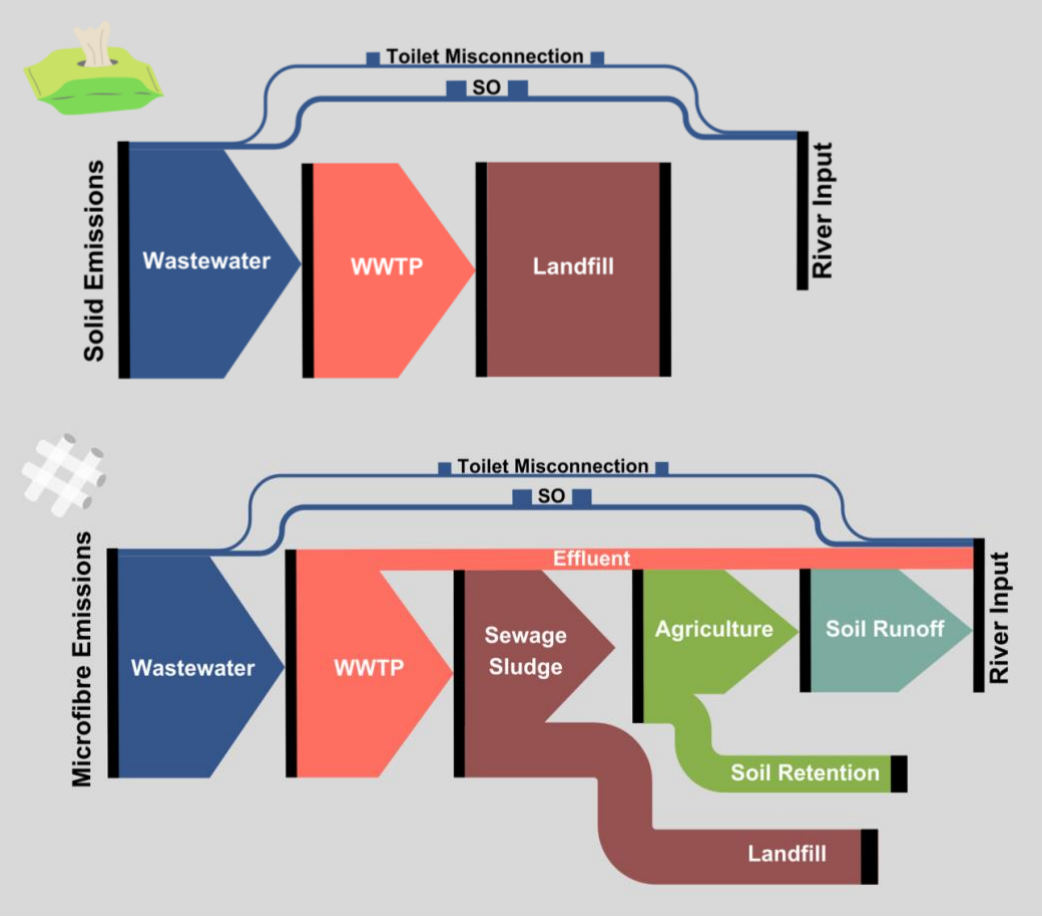

Fig. 3.3. Conceptual framework for wet wipe emissions through wastewater systems illustrating likely wastewater transport and comparative quantities through each pathway.

3.3.2 Contributions from wastewater pathways

Per capita contributions of wet wipe materials to wastewater systems in the UK are substantial. Plastic wipes dominate solid emissions, with individuals contributing an estimated average of 33 plastic wipes annually, compared to 4 cellulosic wipes. This translates to 157.7 g and 17.53 g of plastic and cellulosic wipes per person, respectively. In the case of microfibre emissions, a countertrend is observed. Based on the fibre generation findings of Kwon et al. (2022), natural microfibres are predominant, with an average UK individual contributing 0.49 g [mean] (0.28 - 0.7 g [range]) of natural fibres, 0.06 g (0.03-0.12 g) of regenerated fibres, and 0.11 g (0.04 - 0.19 g) of plastic fibres annually to wastewaters. On average, this equates to 268,520 natural fibres, 1,668 regenerated fibres, and 323 plastic fibres per person annually.

Scaled up to the Taff, representing our densely populated sub-catchment (78-3042 capita/km^2), a substantial wastewater input of 7,054 kg and 784 kg of solid plastic and cellulosic wipes was observed. This is equal to approximately 1.5 million plastic and 165,000 cellulosic wipes flushed annually. For microfibre emissions, natural fibres constitute the highest annual wastewater input, ranging between 12.5 kg and 31.4 kg (mean = 21.9 kg) in the Taff (Fig. 3.4).

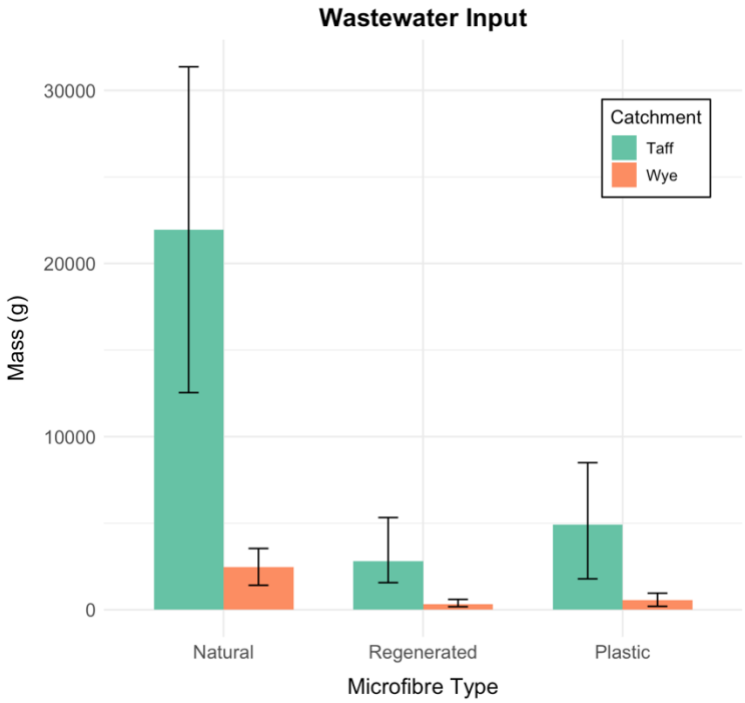

Fig. 3.4. Annual input of microfibres from different types of wet wipes to wastewater systems in our two study catchments. Error bars indicate scenarios of low and high microfibre mass generation.

WWTPs are the primary recipients of solid wet wipes and microfibres from wastewater systems, playing a crucial role in their environmental fate. Almost 99% of solid wet wipe emissions are transported to WWTPs, then directed to landfill and other facilities, serving as environmental sinks. This flow also accounts for potential wet wipe sewer blockage maintenance. For example, in the Taff, plastic and cellulosic wipes annually contribute 6,963,579 g and 774,074 g to environmental sinks.

The entry of microfibre emissions into WWTPs and their release as effluent varies greatly based on the different scenarios of filtration treatment (See Methods for details). These various treatment-based scenarios for annual microfibre emissions to effluent are shown in Fig. 3.5. Despite overall high treatment efficiency, the significant microfibre input to effluent highlights the importance of enhancing treatment strategies to mitigate microfibre pollution in rivers.

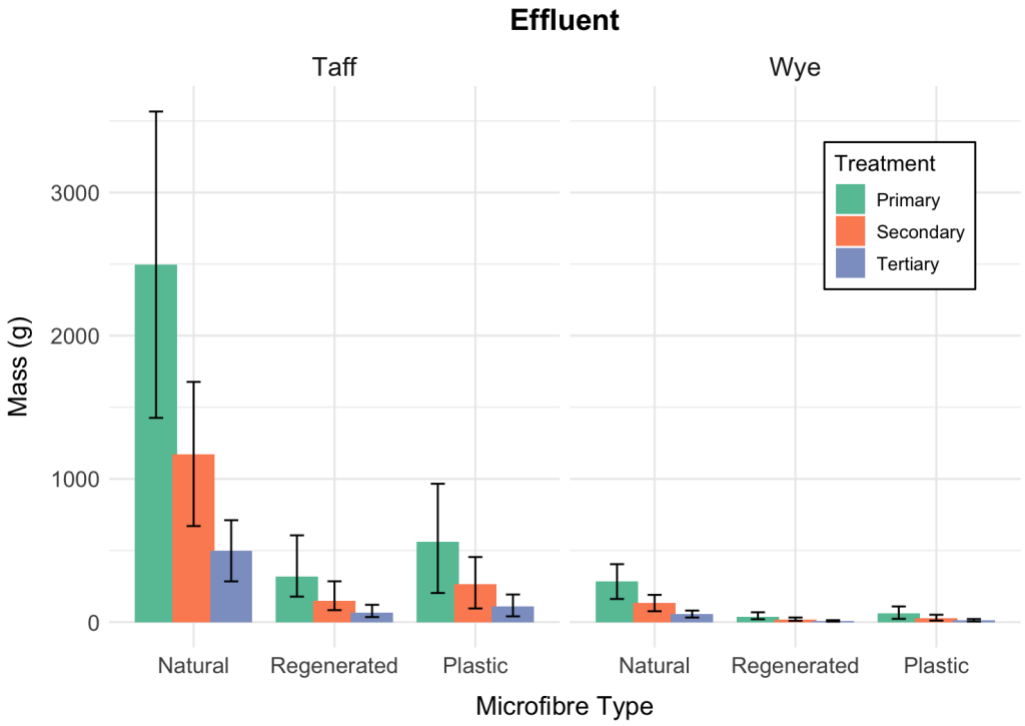

Fig. 3.5. Annual input scenarios of wet wipe microfibres to WWTP effluents in our study catchments under varying levels of filtration treatment. Error bars indicate additional scenarios of low and high microfibre mass generation.

Toilet misconnections and SOs both contribute to solid wet wipe and microfibre emissions in rivers but are often overlooked in wastewater pollution analyses. Toilet misconnections in the model divert about 0.24% of wastewater emissions to rivers annually. While modest, this diversion is substantial for solid wipe pollution, with estimated annual emissions of 14,813 g of plastic and 1,647 g of cellulosic wipes to the Taff through this pathway. However, misconnections are the least important pathway for microfibre transport in our model (Fig. 3.6).

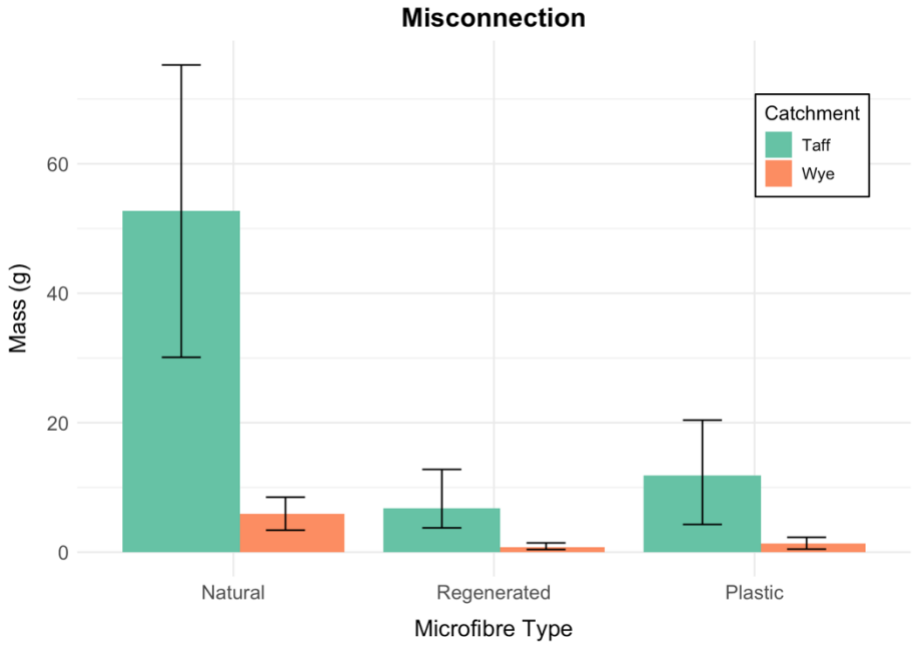

Fig. 3.6. Annual input of wet wipe microfibres to rivers through toilet misconnections. Error bars indicate scenarios for low and high microfibre mass generation.

In comparison, SOs are more significant contributors, particularly for solid wipes, where they input 5.1 times more emissions directly to rivers. Interestingly, the active duration of SO spills has little effect on emissions. Even with less variability in operational hours in the Taff sub-catchment (0-584 hours per year) compared to the Wye sub-catchment (0-2027 hours per year), river emissions from both wet wipe scenarios are considerably higher in the former study site, underscoring emissions volume as the key factor influencing input. Microfibre emissions to rivers via SOs, though more than from toilet misconnections, are still marginal compared to other pathways (Fig. 3.7).

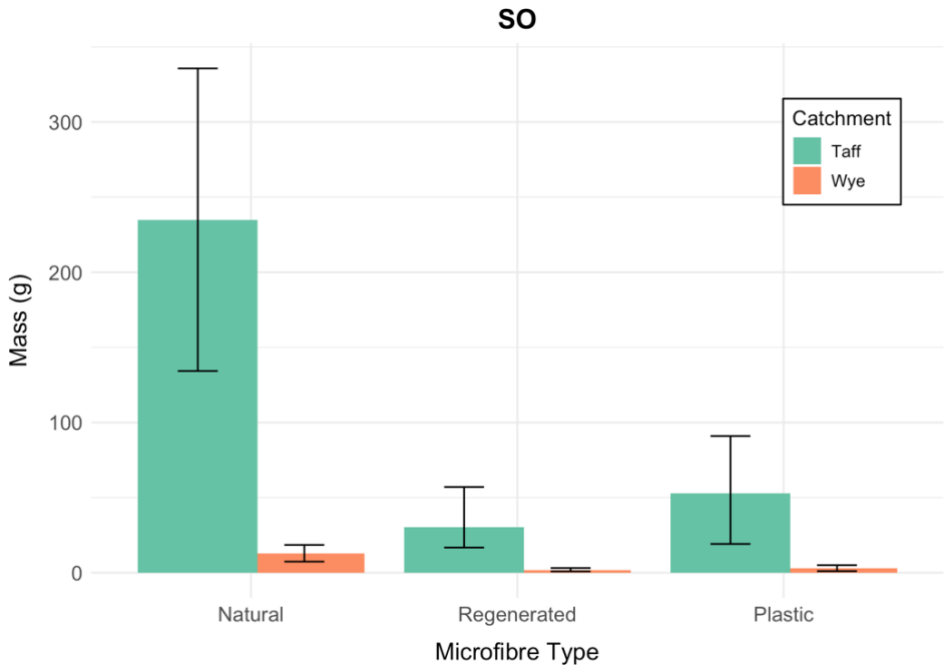

Fig. 3.7. Annual input of wet wipe microfibres to rivers through SOs. Error bars indicate scenarios for low and high microfibre mass generation.

Sewage sludge filtered out of WWTPs is the primary pathway for transporting wet wipe microfibres in the model. These microfibres are directed to agricultural soils or landfill sites, depending on wastewater filtration levels and average UK sewage sludge diversion percentages, outlined in the Methods section. Microfibre emissions are highest in the post-tertiary treatment scenario, accounting for about 66% to agricultural soils and 31% to landfill facilities of the total average emissions entering the wastewater system (Fig. 3.8).

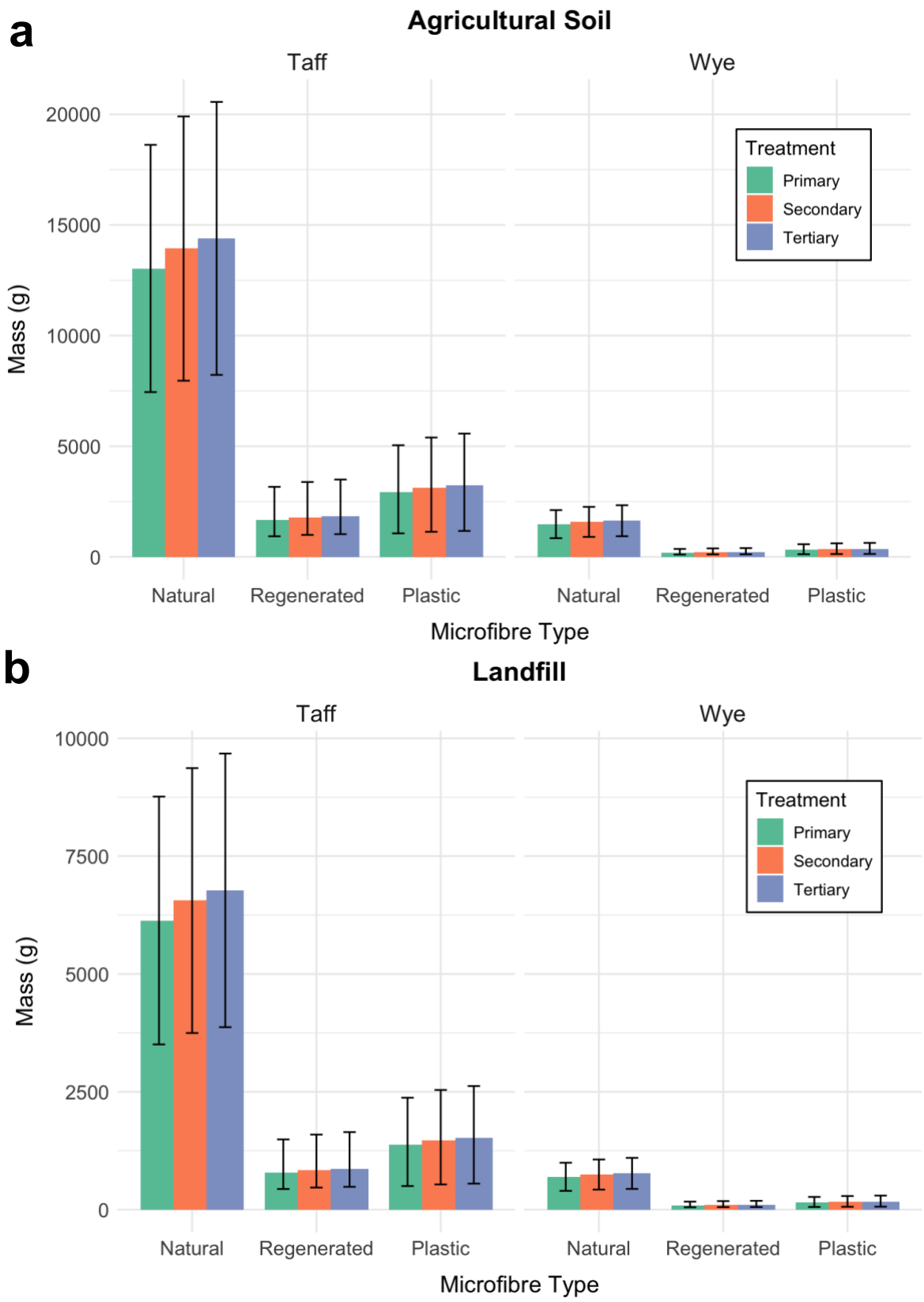

Fig. 3.8. Annual input of sewage sludge-derived wet wipe microfibres to a) agricultural soils and b) landfills in our study catchments. Error bars indicate scenarios for low and high microfibre mass generation. Colours indicate microfibre scenarios under different levels of wastewater treated sludge.

Soil runoff was found to be the main transport pathway for microfibre emissions to enter rivers in the model (Fig. 3.9). This pathway is distinguished from the average proportions of microfibres retained in the soil, which was conservatively assumed to hold a quarter of all emissions within agricultural soils. However, in reality, soil retention is likely to be a temporary environmental sink, and therefore, annual microfibre emissions from agricultural soils into rivers may be even greater.

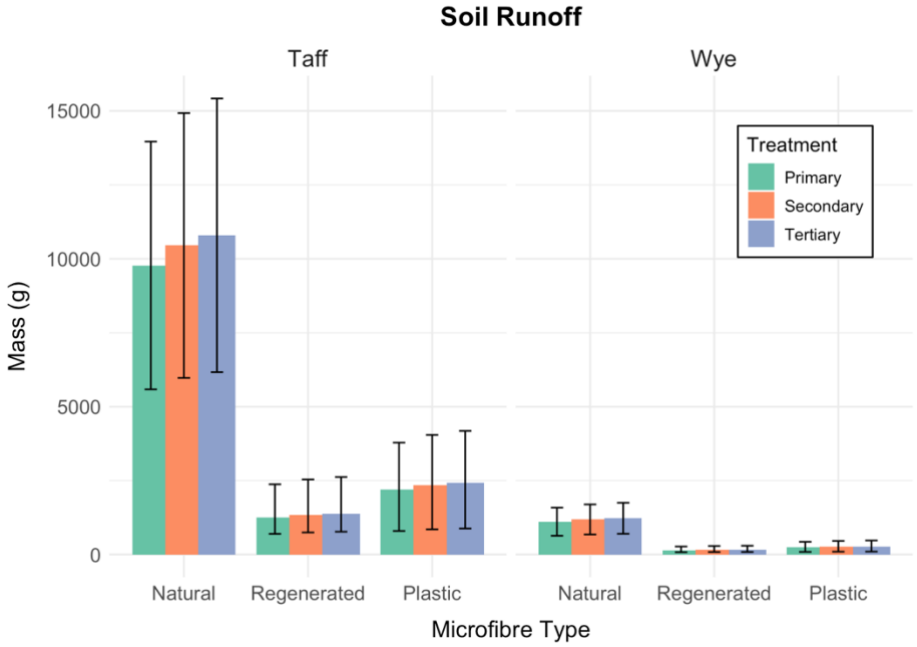

Fig. 3.9. Annual input of wet wipe microfibres to rivers through soil runoff. Error bars indicate scenarios for low and high microfibre mass generation. Colours indicate microfibre scenarios under different levels of wastewater treated sludge.

To further illustrate the annual emission contributions through each of the wastewater pathways discussed here for both the Taff and Wye sub-catchments, conceptual frameworks for solid wet wipes (Fig. 3.10 and 3.11) and microfibres (Fig. 3.12 and 3.13) have been provided. These figures represent average-case scenarios (mean emission input with secondary treatment) and outline the key transport pathways and their respective contributions to river inputs.

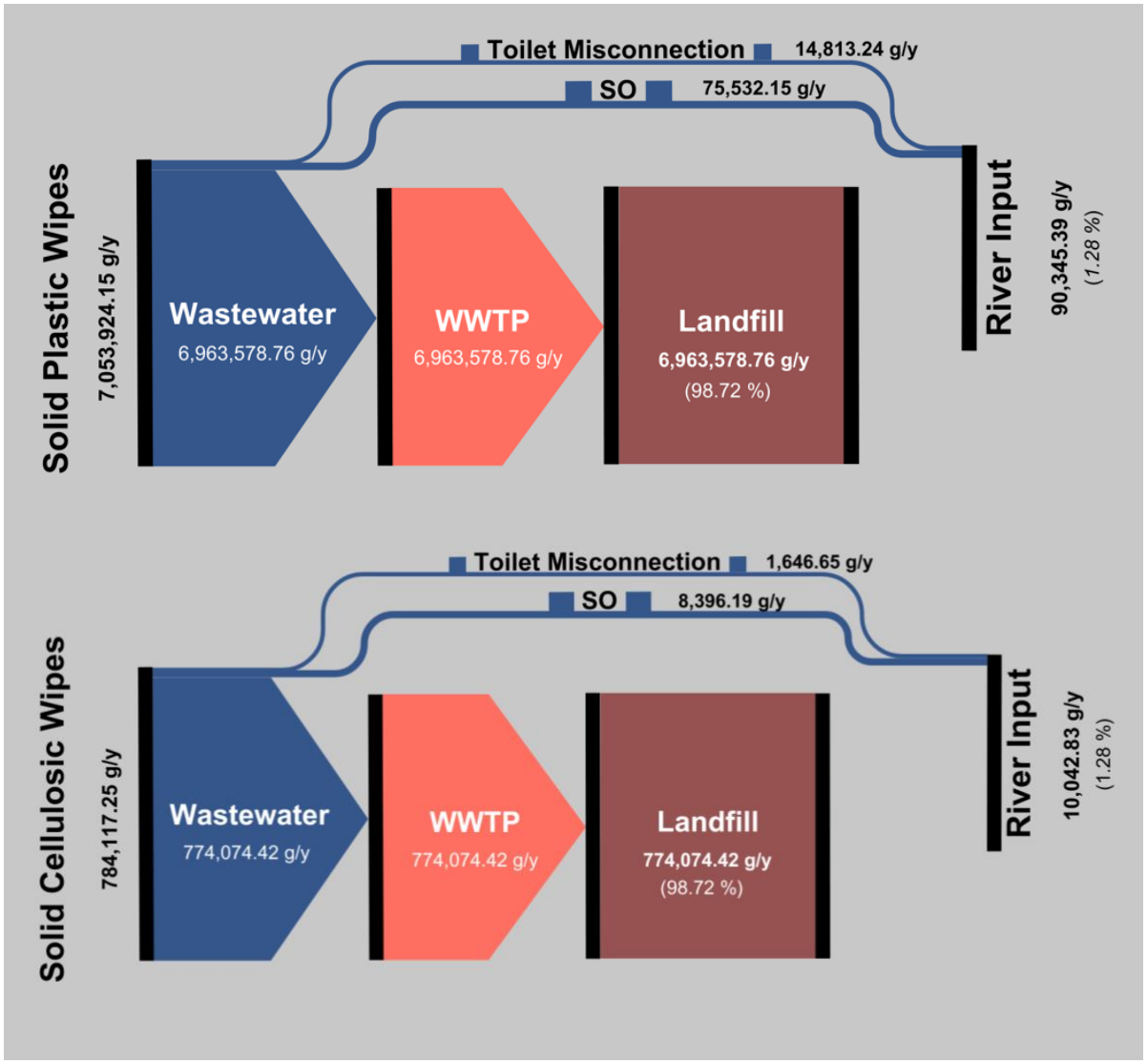

Fig. 3.10. Conceptual framework of solid wet wipe emissions through wastewater systems in the Taff sub-catchment. Percentages represent each pathway's share of total emissions.

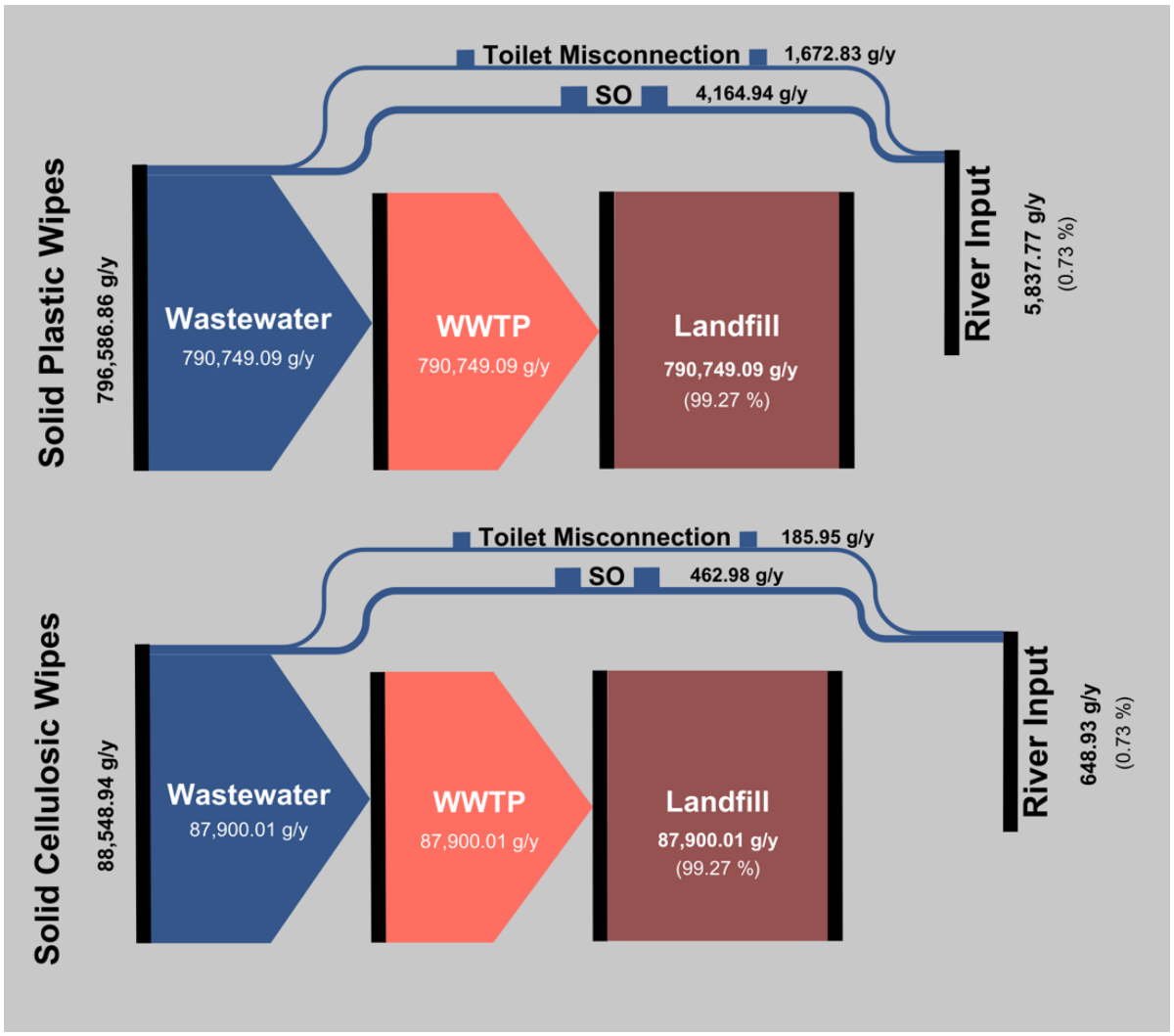

Fig. 3.11. Conceptual framework of solid wet wipe emissions through wastewater systems in the Wye sub-catchment. Percentages represent each pathway's share of total emissions.

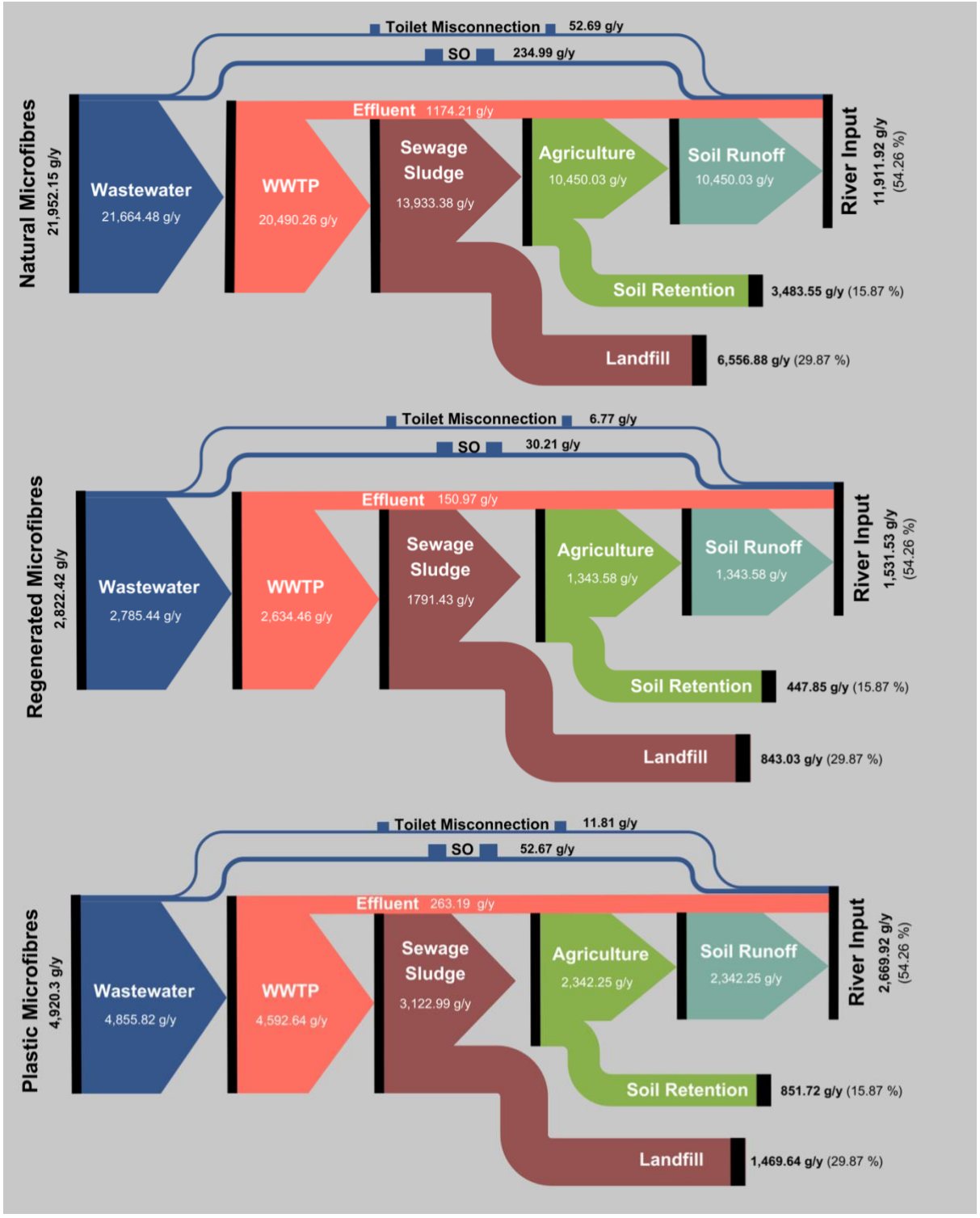

Fig. 3.12. Conceptual framework of wet wipe microfibre emissions through wastewater systems in the Taff sub-catchment based on our average case scenario (mean emission input, secondary treatment). Percentages represent each pathway's share of total emissions.

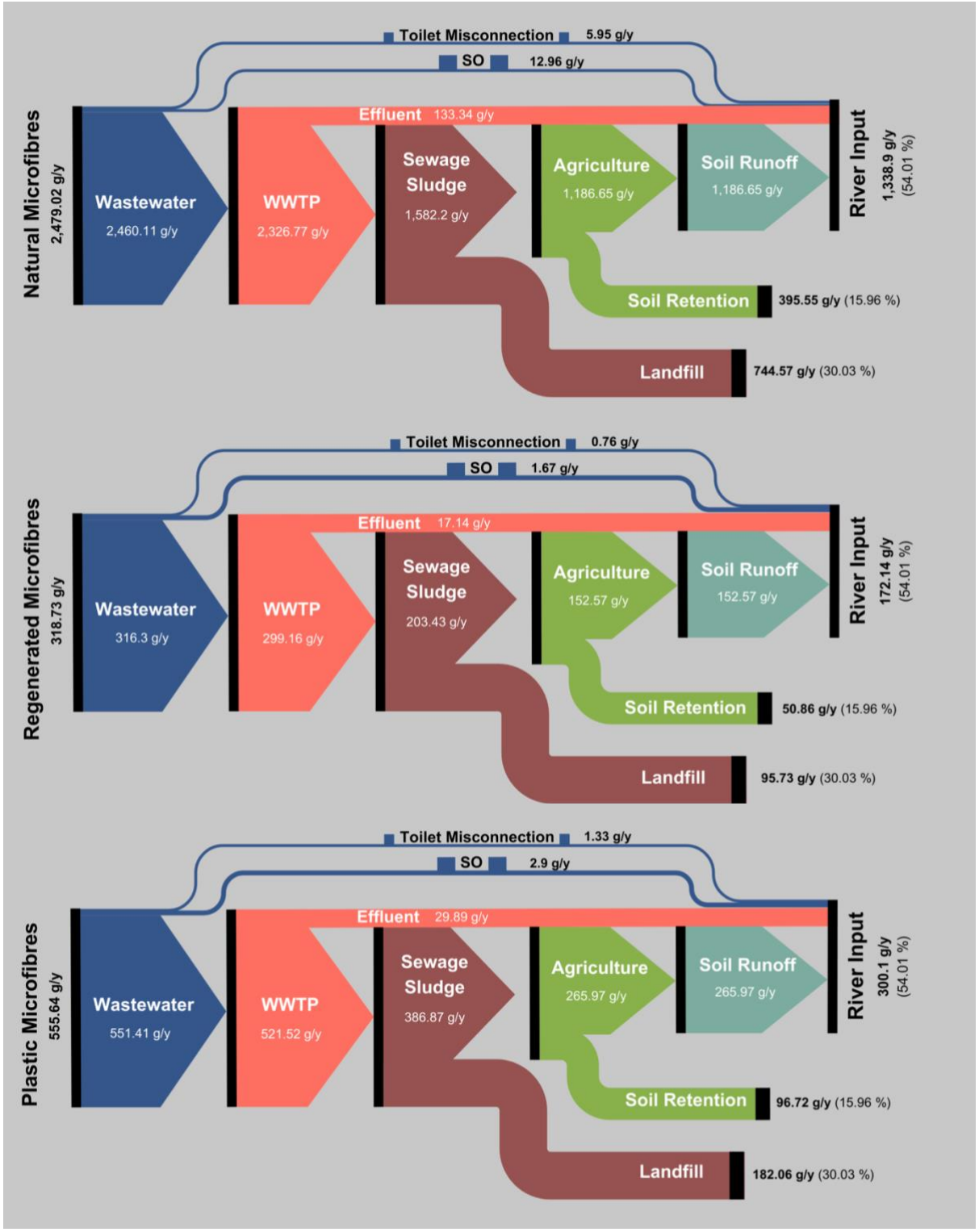

Fig. 3.13. Conceptual framework of wet wipe microfibre emissions through wastewater systems in the Wye sub-catchment based on our average case scenario (mean emission input, secondary treatment). Percentages represent each pathway's share of total emissions.

3.3.3 Emission scenarios to rivers

Modelling wet wipe and microfibre emissions into rivers provides crucial insights into their potential environmental risks. In solid emissions scenarios, plastic wipes significantly outweigh cellulosic wipes in river influx, constituting a small fraction (1.28%) of total emissions into the wastewater model system but raising concerns for freshwater ecosystems. For example, annual plastic wipe input to rivers in the Taff sub-catchment total approximately 90 kg (equivalent to 19,020 wipes), while cellulosic wipes contribute 10 kg (equivalent to 2,114 wipes), roughly translating to 2 g of plastic wipes and 0.23 g of cellulosic wipes per person annually.

Table 3.3 details the wet wipe microfibre export to the Taff and Wye sub-catchments, where natural microfibres account for 74% of total mass emissions to rivers (See also Fig. 3.14 for a full breakdown by microfibre type). The combined annual microfibre input to rivers is around 16kg for the Taff and 1.8 kg for the Wye, assuming average microfibre production and secondary treatment. Across all scenarios, combined inputs could range from 8.5 to 25.7 kg for the Taff and 0.9 to 2.9 kg for the Wye. The breakdown of this river input and contributions from each pathway for the average-case scenario is visualised in Fig. 3.15. Converted to volume of microfibres (see Methods), the combined river input equates to approximately 6.57 x 10^9 and 7.39 x 10^8 microfibres per year on average, with substantial variability, depending on available wastewater treatment and microfibre generation rates (See Table 3.3).

Table 3.3. Emission export of wet wipe microfibres to the two study sub-catchments. Mean values represent mean microfibre generation and secondary treatment scenarios. Predicted lower and upper ranges are derived from emission scenarios with low microfibre generation (L) and tertiary treatment (T) to high microfibre generation (H) with primary treatment (P).

Site	Wet wipe microfibre type	Mean mass microfibre input (g/y)	Microfibre mass range (g/y)	Mean microfibre input (#/y)	Microfibre range (#/y)
Taff	Natural	11,912	6,718 (L;T) – 17,942 (H;P)	6.53×10^{9}	1.10×10^{9} (L;T) – 1.67×10^{10} (H;P)
Taff	Regenerated	1,531	827 (L;T) – 3050 (H;P)	4.26×10^{7}	1.24×10^{7} (L;T) – 1.24×10^{8} (H;P)
Taff	Plastic	2,670	944 (L;T) – 4,862 (H;P)	7.85×10^{6}	6.7×10^{5} (L;T) – 2.51×10^{7} (H;P)
Wye	Natural	1,339	744 (L;T) – 2,018 (H;P)	7.34×10^{8}	1.21×10^{8} (L;T) – 1.88×10^{9} (H;P)
Wye	Regenerated	172	93 (L;T) – 343 (H;P)	4.78×10^{6}	1.4×10^{6} (L;T) – 1.39×10^{7} (H;P)
Wye	Plastic	300	106 (L;T) – 547 (H;P)	8.82×10^{5}	7.53×10^{4} (L;T) – 2.83×10^{6} (H;P)

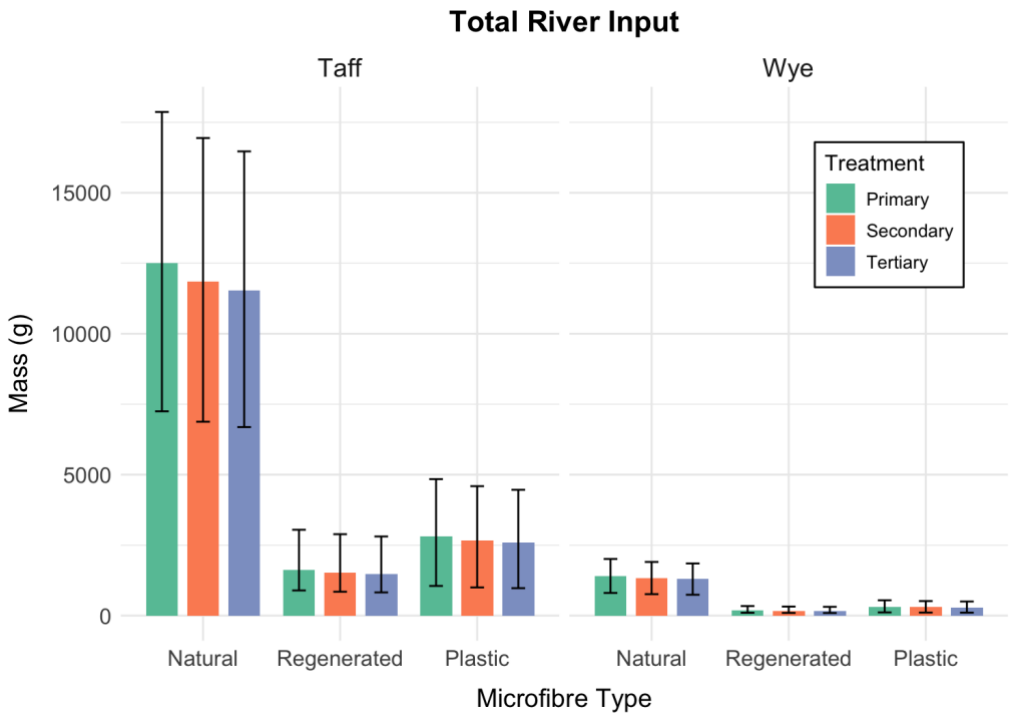

Fig. 3.14. Total annual input of wet wipe microfibres to rivers. Error bars indicate scenarios for low and high microfibre mass generation. Colours indicate microfibre scenarios under different levels of wastewater treatment.

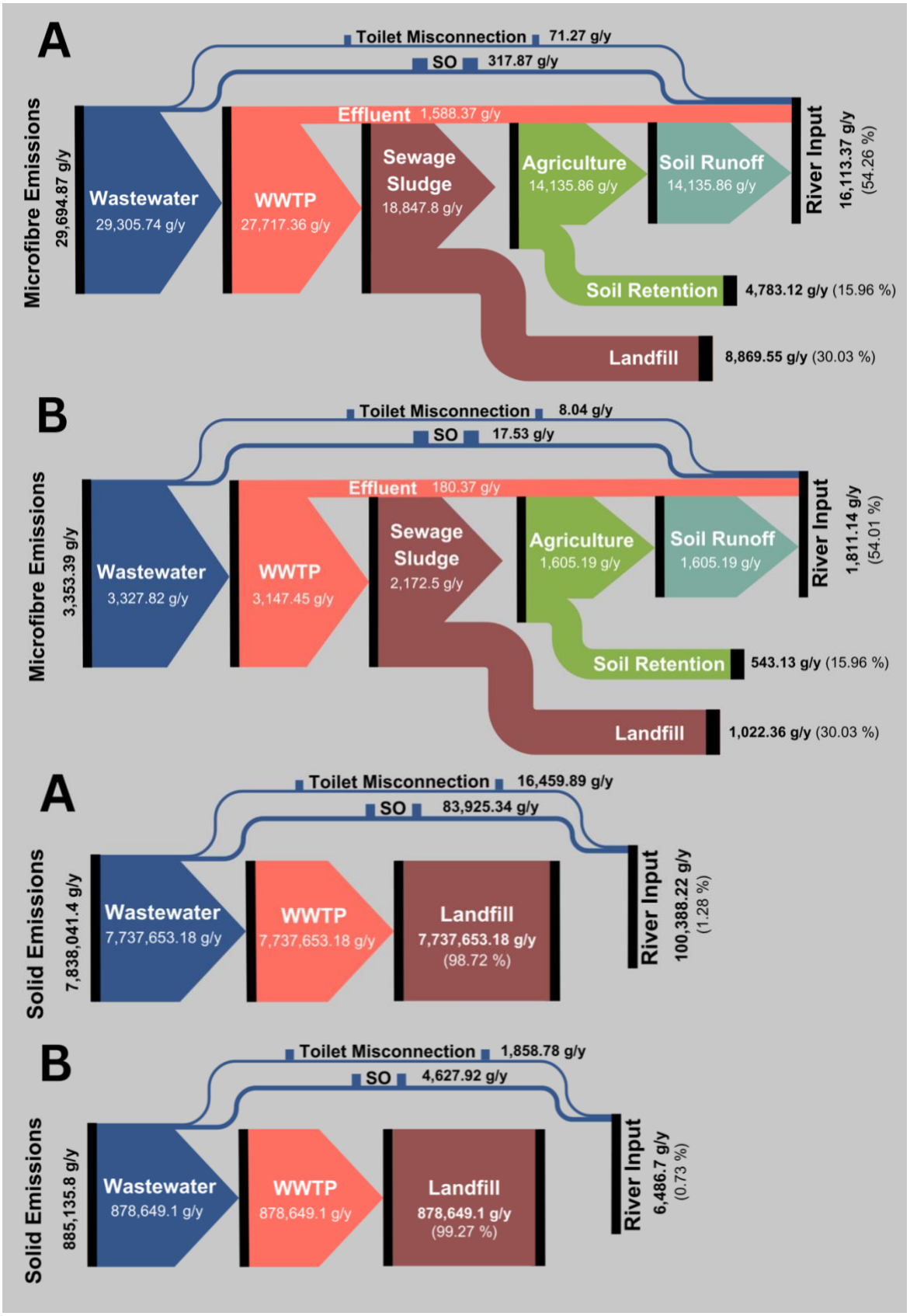

Fig. 3.15. Conceptual framework of annual wet wipe emissions through wastewater systems in the Taff (A) and Wye (B) sub-catchments based on average case scenarios (mean emission input, secondary treatment). Percentages represent each pathway's share of total emissions. Unit = g/y.

3.3.4 Scaling up the modelling framework

Scaling up the model from the local (our study sites) to the national and international scales reveals important insights for wet wipe pollution to rivers (Fig. 3.16). Extended to the UK national level, the model predicts annual inputs of 10,816 t (0.16 kg per person) from solid plastic wipes and 1,202 t (0.018 kg per person) from cellulosic wipes to wastewater systems. With refined parameters, including an average UK SO spill rate of 2.6% (Giakoumis & Voulvoulis, 2023), and a population of 67.78 million, the annual solid mass input to rivers is estimated as approximately 163.3 t (2.4g per person) for plastic wipes and 18.2 t (0.27 g per person) for cellulosic wipes.

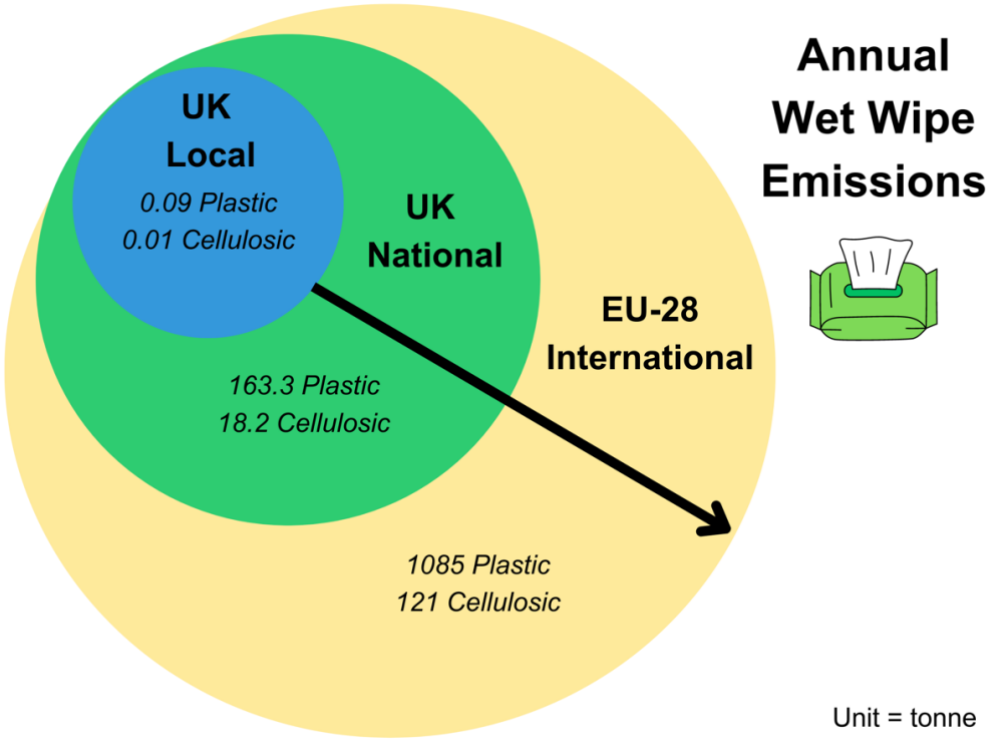

Fig. 3.16. Annual solid wet wipe emissions to rivers modelled across spatial scales. UK Local emissions represent the Taff sub-catchment. UK National and EU-28 International are based on refined model parameters highlighted in the results. Emission inputs are based on mean values, with unit measurements in tonnes.

Expanding to the EU-28 level, the model projects yearly contributions of up to 85,757 t (168 g per person) from solid plastic wipes and 9,529 t (18.6 g per person) from cellulosic wipes to wastewater systems. Within this, 1085 t of plastic wipes (2.12 g per person) and 121 t (0.24 g per person) of non-plastic wipes are estimated to enter European rivers annually. EU-level estimates were based on updated parameters such as an annual consumption of 68 billion wet wipes in the EU-28 from a population of 511 million (Cabrera & Garcia, 2019), an average flushing probability of 29.5% (Kawecki & Nowack, 2019), an average EU-28 SO spill rate of 1.97% (Quaranta et al., 2022), and a toilet misconnection rate of 0.28% (Ellis & Butler, 2015).

3.3.5 Model comparisons with laundry microfibres

Applying laundry microfibre generation data from Vassilenko et al. (2021) into the model reveals important comparisons that help to validate the modelling system. Model input values for natural and plastic laundry microfibres show considerable but variable annual emission rates, with per individual wastewater inputs of 109.1 g (± 29.08) and 106 g (± 114.35), respectively. Mean laundry input rates to wastewater vastly exceed all wet wipe microfibre scenarios but are somewhat lower than solid plastic wipes. For instance, projected over the Taff sub-catchment, natural and plastic laundry microfibres could annually contribute 4,878 (± 1,300) kg and 4,760 (± 5,115) kg to wastewaters, compared to 7,054 kg from solid plastic wipes annually. As total mass emissions to wastewater, the modelled wet wipe inputs (solid and fibres) were slightly less than those from laundry (natural and plastic), representing around 82% of their annual emissions.

In rivers, emissions from each laundry fibre scenario are roughly 28 times greater than solid plastic wipes on average. For instance, modelled natural and plastic laundry microfibres annually contribute an average 2,570 kg and 2,510 kg to rivers in the Taff sub-catchment, equating to 101.13 g and 98.77 g per person, respectively. Combined wet wipe mass emissions to rivers are approximately 2.3% of those contributed by total laundry fibres. These river input values and comparisons are based on average-case scenarios with secondary treatment. From the model assumptions, annual river inputs to the Taff sub-catchment for laundry microfibres could range between 1,850 kg (best-case scenario; tertiary treatment) and 3,450 kg (worst-case; primary treatment) for natural laundry fibres, and between zero (due to variability that results

in a negative lower bound) and 5,510 kg (worst-case scenario; primary treatment) for plastic laundry fibres.

3.4 Discussion

3.4.1 A comprehensive framework for wet wipe emissions

This study sought to unravel the complex dynamics of flushed wet wipes in wastewater systems. By integrating microplastic modelling with experimental microfibre generation data, a framework was developed to reveal and quantify the pathways of wet wipe emissions into rivers. The model identified nine pathways through which wet wipe emissions, both solid and microfibre, are transported, categorised into point sources, diffuse sources, and terrestrial sinks. These pathways include: 1) wastewater to WWTP, 2) WWTP to effluent, 3) WWTP to sewage sludge, 4) sewage sludge to landfill, 5) sewage sludge to agriculture, 6) soil retention in agriculture, 7) agricultural soil runoff to rivers, 8) toilet misconnections to rivers, and 9) SOs to rivers.

The model accounted for size-dependent transport behaviour: microfibres traverse all pathways, while solid wet wipes are largely removed or enter rivers at earlier stages. By parameterising these pathways using real-world data and assumptions justified by surrounding literature, the model ensured practical and relevant emission scenarios, allowing for a more accurate representation of the complexity of wet wipe transport in wastewater systems.

3.4.2 Challenges and uncertainties in data and model validation

The lack of comprehensive national data on wet wipe pollutants limited direct model validation. As detailed in the Methods section, empirical data for parameters were used such as wet wipe consumption, flushing rates, microfibre generation, and transport pathways when available. Where empirical data were unavailable, reasonable assumptions were made based on analogous pollutants and conservative estimates. Scenario modelling with best, worst, and average cases helped account for variability in wastewater systems and from spatio-temporal and environmental conditions (Kooi et al., 2018). Although this provides a strong baseline,

improving accuracy will require future regional and temporal data collection to reduce reliance on assumptions, as well as greater market share insights for wet wipe consumption.

3.4.3 Key emission pathways to rivers and the role of wastewater systems

The findings highlight WWTPs, SOs, and agricultural runoff as the main pathways for wet wipe emissions into rivers, with overlooked contributors such as toilet misconnections also playing a role. Agricultural runoff was the dominant pathway for microfibre emissions, accounting for 83-93% in the Taff sub-catchment. This aligns with prior studies (Nizzetto et al., 2016; Norling et al., 2024) but may be more on the conservative side, as reported runoff values have exceeded 99% elsewhere (Crossman et al., 2020). This is possibly due to the unique morphology of microfibres which increases their retention in soils (Zubris & Richards, 2005; Schell et al., 2022).

WWTPs also play a crucial role in wet wipe transport. Despite significant microfibre removal, large quantities still enter effluent and agricultural sludge. Natural microfibres contributed an average of 643 million fibres (14,375 per person) annually through effluent in the Taff and 73 million (14,452 per person) in the Wye, consistent with previous findings (Mason et al., 2016; Murphy et al., 2016). Solid wet wipes are directed to landfills, with an estimated 10,708 t of plastic and 1,190 t of cellulosic wipes entering UK landfills annually. This contribution does not include solid wet wipes disposed of through municipal waste streams. Given this scale, the findings underscore the need for effective waste management strategies at these environmental sinks to prevent remobilisation and further pollution risks from solid wet wipes (Shruti et al., 2021; Zhang et al., 2021b; Hu et al., 2022).

SOs contribute significantly to solid wet wipes in rivers, accounting for 77.5% of total emissions on average. This aligns with Kawecki and Nowack's (2020) findings on macro and microplastic emissions (80-95%), when also considering the toilet misconnection pathway in this study. However, unlike studies that report higher SO contributions for microplastics (Schernewski et al., 2020; Schernewski et al., 2021), the model showed SOs contributing only 1-2% of annual microfibre river emissions on average, consistent with Baresel and Olshammar (2019). These discrepancies may stem from regional differences in rainfall, wastewater infrastructure, urbanisation, and microplastic sources. Seasonal variation also affects SO

activity (Schernewski et al., 2020), with higher overflow frequencies during wetter months likely increasing wet wipe emissions. A fixed 50% diversion rule for SOs was applied based on limited data (Jones et al., 2024, in print), potentially simplifying spatio-temporal overflow dynamics compared to the higher-resolution methods of Schernewski et al. (2021). Nonetheless, the findings align with broader pollution analyses and highlight the need for more region-specific data to better understand SO impacts and spill rates.

Toilet misconnections are often overlooked as transport pathways for pollutants, yet the findings show that they can contribute up to 16% of all solid wet wipe emissions to rivers. Future studies should further explore this pathway to better understand its role in pollutant transport.

Per capita input estimates for plastic wipes indicate a significant risk to wastewater and river systems. These estimates are roughly four times lower than those in Spence et al. (2016), possibly explained by the unspecified materials compositions also considered in their study. Conversely, the plastic estimates exceed those from Swiss river systems (Kawecki & Nowack, 2019), which suggest an annual 0.45 g per person based on an assumed population of 8.8 million (Worldometer, 2023). While their study assumes a flushing probability between 0.6% and 46%, aligning with this current study's average assumption of 23%, discrepancies in river inputs may result from variations in wipe mass, their exclusive focus on SO pathways, and differences in SO infrastructure and operational periods.

The model also highlights the potential environmental risks posed by solid wipes and their microfibres in river systems. Although they account for a smaller fraction of total mass inputs, plastic wipes are the dominant solid wipe material and still present a significant risk to aquatic environments. Once in rivers, plastic wipes may resist chemical degradation but fragment due to material defects and physical interactions (Enfrin et al., 2020; Ó Briain et al., 2020), contributing to persistent microfibre loads (Hu et al., 2022).

The high-volume of solid wipes is also expected to correlate with global occurrences of sewer blockages, which have serious operational and ecological consequences (Durukan & Karadagli, 2019; Lee et al., 2021; Allison et al., 2023). Wet wipes are responsible for up to 90% of sewer blockages (Drinkwater & Moy, 2017), increasing the likelihood of sewer overflows

and the release of untreated wastewater and associated pollutants into rivers. However, the extent of this relationship remains debated (Giakoumis & Voulvoulis, 2023).

3.4.4 Extrapolating wet wipe emissions to wider spatial scales

Refinement of model parameters enabled the extension of solid wet wipe emissions analysis to both national and international scales, focusing specifically on the UK and EU-28. Quantifications revealed substantial solid inputs to wastewater systems, particularly from plastic wipes, driven by factors such as annual wet wipe consumption rates and flushing probabilities at each scale. Updated parameters for average SO spills further highlight their critical role as transport pathways for solid wet wipes into rivers.

These EU-level extrapolations are intended to illustrate the potential scale of solid wet wipe emissions to rivers rather than provide precise fibre quantifications. Variability in wastewater treatment and sludge disposal to agricultural land across Europe, ranging from 0.3% to 89% (Lofty et al. 2022), highlights the challenge of applying uniform modelling to such a diverse region. Although this extrapolation focuses on solid wipes entering rivers via SOs and misconnections, these findings underscore the broad environmental impact of wet wipe emissions across multiple regions.

3.4.5 Comparing wet wipe emissions with laundry microfibres

To contextualise and validate the model, wet wipe emissions were compared with laundry-derived microfibres using wastewater release data from Vassilenko et al. (2021). Laundry emissions contributed more microfibres to rivers, especially natural fibres, consistent with previous studies (Zambrano et al., 2019). Finnish data also supported the findings on natural fibre emissions, although their plastic fibre emissions reported were significantly lower than the projections presented in this study (Sillanpää & Sainio, 2017). Despite the lower proportion of cellulosic textiles in Vassilenko et al.'s (2021) study, they showed similar fibre shedding to plastic textiles, indicating a higher shedding propensity for cellulosic materials a pattern also observed for wet wipes (Kwon et al. 2022). The high variability in plastic fibre shedding across different textile types suggests that, in worst-case scenarios, plastic laundry fibres could be the greatest contributors to river pollution in our modelling system.

Solid plastic wipes contribute more mass to wastewaters than either natural or plastic laundry fibres individually. However, this contrasts for their river emissions, as laundry microfibres can bypass wastewater treatment and enter rivers (Miller et al., 2017; Talvitie et al., 2017b). Detailed comparisons of laundry microfibres are limited due to a lack of mass quantification. However, the model's per capita laundry microfibre inputs to wastewater are approximately 30 times higher than those reported by Hartline et al. (2016) for synthetic laundry microfibres, while their estimates align more closely with the wet wipe emission rates in this study.

While laundry fibres contribute substantially more microfibre mass to rivers annually, the environmental implications of wet wipe pollution are equally important to consider. Intact wet wipes reaching waterbodies can degrade into vast amounts of microfibres (Ó Briain et al., 2020; Lee et al., 2021; Hu et al., 2022), exacerbating existing microfibre pollution and increasing ecological risks. Furthermore, the conservative assumptions in this study regarding laundry emissions, particularly for plastic microfibres, may lead to overestimation, potentially underrepresenting their comparability to wet wipes. The shift among manufacturers and consumers toward 'biodegradable' wet wipes is also likely to intensify cellulosic (both natural and regenerated) microfibre pollution in aquatic environments (Hadley et al., 2023).

3.5 Conclusion

This study presents the first comprehensive quantification of wet wipe emissions and microfibre release through wastewater systems into rivers. By integrating experimental microfibre generation data with microplastic modelling approaches, a model was developed that tracks the transport dynamics of both solid wet wipes and their microfibres. Applied to two sub-catchments with varying degrees of urbanisation – the Taff and the Wye – annual emissions of solid wet wipes to these rivers were estimated at approximately 100 kg and 6.49 kg, respectively. Across all modelled microfibre scenarios, total annual river emissions may range from 9.4 - 28.8 kg between the Taff and Wye sub-catchments, or approximately 1.23×10^9 - 1.89×10^{10} microfibres per year.

A key innovation of this study is the development of a geo-spatial method to delineate SO drainage areas using topographical and hydrological data. This approach allowed for the

connection of local populations to wastewater systems, enabling more precise, and spatially-specific estimates of emissions to wastewater systems.

The model identifies SOs, WWTPs, and particularly agricultural runoff from sewage sludge as critical pathways through which wet wipe microfibres enter rivers. Despite generally advanced wastewater treatment technologies in place, substantial volumes of flushed microfibres could still reach agricultural land via sewage sludge. Combined with emissions from undegraded wipes during SO periods, this poses a significant pollution risk to aquatic ecosystems.

Additionally, the model demonstrates adaptability to other pollutant types, such as laundry microfibres, and across various geographical scales, providing insights into the environmental impact of wet wipes. While this study establishes a strong foundation, future work is encouraged to focus on empirical data collection and region-specific modelling to refine the parameters and emission estimates presented here, and to develop targeted strategies to mitigate wet wipe pollution in aquatic systems.

Chapter 4

Degradation of Cellulose-Based Wet Wipes in River Environments

A version of this chapter has been submitted for publication as:

Allison, T., Ward, B.D., Harbottle, M. and Durance, I. Cellulose-Based Wet Wipes Undergo Limited Degradation in River Environments (In prep).

In this chapter, Thomas Allison, Benjamin Ward, Michael Harbottle, and Isabelle Durance designed the experiment. Thomas Allison conducted the experiment, processed, analysed, and plotted the data. Thomas Allison drafted and edited the manuscript following review and comments from all authors.

Abstract

The environmental fate of cellulose-based “biodegradable” wet wipes in freshwater ecosystems remains poorly understood, despite growing market demand and legislative shifts banning plastic-containing alternatives. This study evaluated the degradation behaviour of two commercially available biodegradable wet wipe brands in upland stream mesocosms mimicking real-world river conditions. Using tensile strength loss (TSL) as the primary degradation metric, wipe degradation was compared across varied pH, temperature, nutrient, and light regimes, alongside cotton strip controls. Results revealed that although degradation rates varied by material and environmental context, both wet wipe brands persisted in river systems, with Brand A degrading ~50% faster than Brand B and nearly twice as fast as cotton controls. Degradation was significantly influenced by pH, temperature, and total dissolved solids, but not by wipe positioning in the water column (hyporheic, submerged, surface) or microbial biomass alone. Temperature-adjusted TSL (% per degree day) emerged as the most robust degradation metric, suggesting initial physical disintegration preceded microbial breakdown. These findings challenge current biodegradability claims and highlight the need for regulatory testing under environmentally relevant freshwater conditions to ensure truly biodegradable wet wipe products.

4 Degradation of Cellulose-Based Wet Wipes in River Environments

4.1 Introduction

Growing awareness of the environmental impacts of flushing plastic-containing wet wipes has driven the development of bio-based, biodegradable alternatives. However, despite being marketed as biodegradable or dispersible, many of these products persist in wastewater and river systems, contributing to environmental pollution (Joksimovic et al., 2020; Ó Briain et al., 2020; Harter et al., 2021; Choudhuri et al., 2024; Kachef, 2024; Bach et al., 2025). One key reason for this is the persistence of low-degradable synthetic fibres in supposedly biodegradable or flushable wet wipes (Khan et al., 2021; Allison et al., 2023). Yet, even bio-based wipes made from cellulosic materials (e.g. viscose, lyocell, or cotton) can persist in aquatic environments – both as macro-pollutants and as microfibres, which they shed in vast amounts (Ó Briain et al., 2020; Kwon et al., 2022; Hadley et al., 2023; Allison et al., 2025; Bach et al., 2025). Despite growing evidence of their pollution in aquatic systems, little is known about their actual degradation behaviour in river environments or the environmental factors controlling this process. This presents a critical knowledge gap, especially in the UK, where recent legislation banning plastic-containing wet wipes has rapidly shifted the market toward these cellulosic alternatives (DEFRA, 2024a).

The absence of a universal definition or legally binding standard for biodegradability complicates the issue further. Voluntary biodegradation standards developed by ISO, EN, and ASTM differ in criteria and rely on laboratory tests conducted under controlled conditions that do not reflect real-world aquatic environments (Kale et al., 2007; Mitchell, 2019; Zambrano et al., 2020a; Liao and Chen, 2021). While some cellulosic wipes meet biodegradation standards such as OK compost HOME (TÜV AUSTRIA) and ISO 24855, these tests are typically conducted under aerobic composting conditions rather than in riverine environments where wipes often accumulate. Consequently, it remains unclear whether these standards provide an accurate representation of biodegradation in natural freshwaters. Addressing this uncertainty is crucial to determining whether biodegradable wet wipes offer genuine environmental benefits or simply shift pollution to a different material form.

Cellulose degradation is a key ecological process in rivers for decomposing organic matter like leaf litter and can thus offer insights into the biodegradation of cellulose-based wet wipes (Burdon et al., 2020; Carballeira et al., 2020). Microorganisms play a central role in biodegradation, both initiating and sustaining breakdown. They colonise cellulose surfaces and produce extracellular cellulase enzymes, hydrolysing cellulose into simpler, more palatable compounds for invertebrate shredders to process further (Baudoin et al., 2008; Polman et al., 2021). A range of microbes contribute to cellulose degradation, including fungi (e.g. *Trichoderma*, *Penicillium*, and *Fusarium* spp.*)* and bacteria (e.g. *Bacillus, Pseudomonas, Streptomyces, and Clostridium* spp.) (Salama et al., 2021; Ziklo et al., 2024). Typically, fungi dominate the early stages of microbial breakdown (Baudoin et al., 2008; Langhans et al., 2008), while bacterial communities become more important over time as community diversity and abundance increase (Burdon et al., 2020; Hayer et al., 2022).

A range of environmental factors influence microbial cellulose degradation in rivers. Warmer stream temperatures enhance microbial metabolism and enzymatic activity (Yue et al., 2016; Burdon et al., 2020; Li et al., 2023), while circumneutral pH levels optimise cellulolytic (particularly fungi) microbial diversity and cellulase activity, with acidic or alkaline extremes inhibiting these processes (Pye et al., 2012; Li et al., 2023). Adequate dissolved oxygen and moderate nutrient availability (e.g. nitrogen and phosphorus) support microbial biomass and hydrolytic activity, although nutrient extremes can destabilise microbial communities (Tiegs et al., 2013; Chauvet et al., 2016). Sunlight availability further stimulates phototrophic microbial activity (Southwell et al., 2020; Blackman et al., 2024). Interactions between these factors often amplify their effects on cellulose biodegradation too. For example, cellulolytic degradation can be accelerated by increased nutrient enrichment combined with warmer temperatures (Burdon et al., 2020), while physical fragmentation increases surface area for microbial colonisation and action (Zambrano et al., 2020a).

Cotton strip bioassays – standardised cellulose textiles – provide a valuable proxy for studying cellulose biodegradation in rivers. These textiles share key similarities with leaf litter, including their predominantly cellulosic composition, ability to host similar microbial communities, and sensitivity to environmental conditions such as pH, temperature, and nutrient availability (Slocum et al., 2009; Tiegs et al., 2009; Tiegs et al., 2013; Colas et al., 2019). These findings suggest two key points: 1) cellulose-based textiles, such as wet wipes, may degrade in rivers in

a manner analogous to cotton strips and leaf litter, and 2) microbial activity is central to understanding the environmental fate of biodegradable wet wipes.

In this chapter, the degradation behaviour of cellulose-based 'biodegradable' wet wipes in rivers was examined. Based on existing knowledge of cellulosic cotton strips, it was hypothesised that: 1) greater microbial activity, represented as epilithic microbial biomass, will drive higher degradation rates of cellulosic wet wipes in rivers; 2) variations in freshwater physico-chemical conditions, such as acidity, temperature, sunlight and nutrient availability, will significantly influence degradation rates; and 3) interactions between biological and physico-chemical factors will shape overall degradation outcomes. To test these hypotheses, experiments were conducted under controlled mesocosm conditions, comparing the behaviour of biodegradable wet wipes to cotton strips.

4.2 Methods

4.2.1 Study area

To assess the degradation of cellulosic wet wipes under conditions that closely mimic natural river systems, upland stream mesocosms were utilised at the Llyn Brianne Observatory, Wales, UK, between May and July 2024. Unlike microcosm-based lab experiments, which often lack hydrological complexity and ecological realism (Stewart et al., 2013), these field mesocosms provided a controlled yet dynamic environment. Crucially, these mesocosms were positioned along a natural acidification gradient, allowing for the examination of pH variation effects on degradation while maintaining controlled flow conditions. This design isolated the direct effects of key biological and environmental variables influencing wet wipe degradation within realistic river systems.

The study area, characterised extensively over a 43-year span (Weatherley and Ormerod, 1987; Ormerod and Durance, 2009; Pye et al., 2023), has a temperate maritime climate with stream temperatures ranging from 0-16 °C, a mean annual precipitation of ~1900 mm, and solar radiation levels of 7.85 MJ m^{-2} d^{-1}.

To minimise natural variability in degradation, two sets of replicate mesocosms were used (Fig. 4.1), each fed by first-order streams across an acid-base gradient. Two mesocosms are fed by

streams draining sheep-grazed moorland catchments with typical pH levels between 6.8 and 7.2 (L6-Carpenter and L7-Davies), while two are fed by streams draining regularly logged conifer catchments with pH levels estimated between 5.3 and 5.8 (L3-Hanwell and L8-Sidaway). Each mesocosm shares physicochemical and ecological characteristics with its source stream, enabling controlled comparisons that reflect the environmental diversity of the UK uplands (Seymour et al., 2018). Each stream mesocosm consists of three channels (20 m x 0.2 m x 0.2 m) with a mixed gravel and cobble substrate and water sourced directly from adjacent headwater streams.

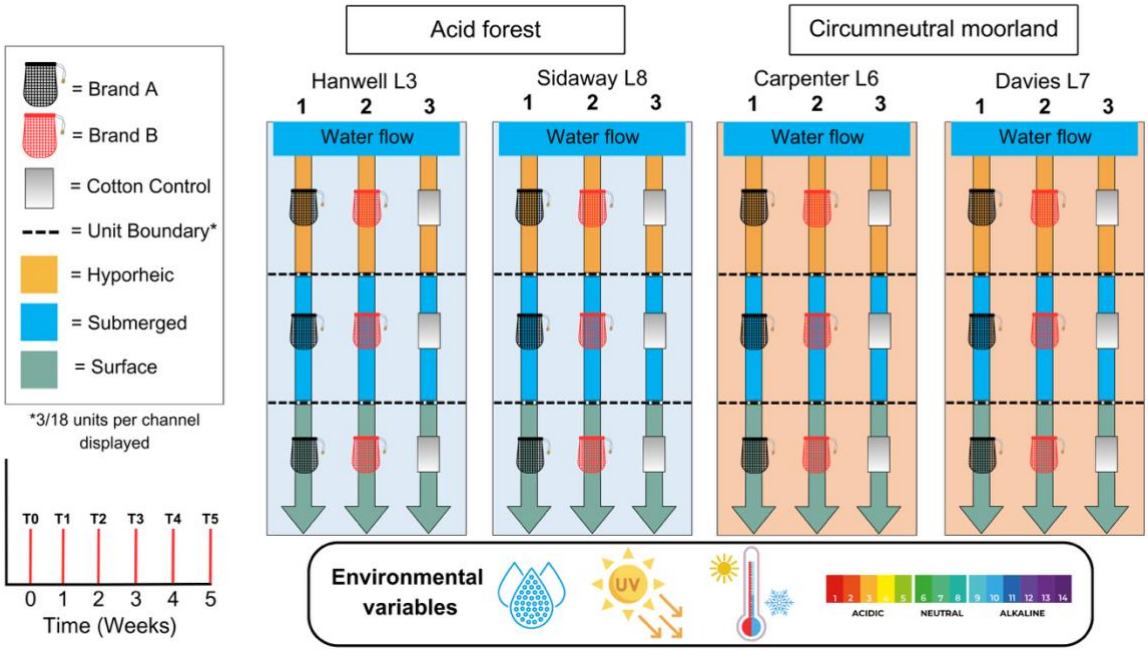

Fig. 4.1. Overview of the mesocosm design, including environmental variables under investigation and the study period with weekly subset collection points. Each mesocosm is represented with their associated names. Dashed lines indicate each 1 m channel unit section (only the first 3 units are displayed here). Channel colours (yellow, blue, green) represent the different environmental treatments in each unit. Background colours (blue and orange) signify the pH gradient of the mesocosms.

4.2.2 Environmental treatments

To simulate typical conditions where improperly disposed wet wipes may end up, three treatments were introduced: hyporheic, submerged, and surface. In the hyporheic treatment, samples were embedded in the channel substrate, replicating wipes buried in riverbeds or

riparian zones that experience dynamic exchanges between stream and groundwater. The submerged treatment placed samples fully below the water surface, while the surface treatment held samples just above the water, simulating wipes caught on riparian vegetation with intermittent contact to water. Simultaneously, each treatment represents a distinct ecological niche with unique environmental and hydrological conditions likely to shape microbial community composition, diversity, and functional potential (Ouyang et al., 2020; Wang et al., 2023).

Each channel consists of 18 usable units (only the first three are shown in Fig. 4.1 for simplicity) and is categorised by its position relative to the headwater source: proximal (channel 1), middle (2), and distal (3). Treatments were systematically applied across these units within each channel, ensuring that each treatment had six replicates distributed along the entire channel length.

To monitor environmental factors affecting degradation, temperature and light was measured hourly using automated data loggers (HOBO Pendant® Temp/Light). Two loggers per channel were positioned randomly using stratified sampling to capture spatial and treatment-based variations (24 loggers across four mesocosms), but due to malfunctions, data were excluded from two of these loggers. Throughout the study period, pH and total dissolved solids (TDS) was monitored weekly with a handheld tester (HANNA instruments® pH/EC/TDS Combo Tester, HI98129). Values were in line with the 40 years of monitoring records at those sites.

4.2.3 Decomposition bioassays

Wet wipes labelled as biodegradable and composed of 100% bio-based fibres were selected from two different brands (referred to as Brand A and B) for the degradation analysis. Labelled additive categories in Brand A included moisturisers, anti-inflammatory agents, antimicrobial preservatives, antioxidants, soothing botanical extracts, and pH buffer. Brand B also contained similar ingredients, but included emollients, surfactants and emulsifiers.

Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy (Shimadzu IR Affinity-1) confirmed the bio-based composition of these two wipe brands against cellulose reference spectra. However, decisive classification between cellulose types

(e.g., natural vs regenerated) was not possible due to the inherent difficulty of spectral discrimination (Saito et al., 2021), particularly in materials containing various chemical additives. FTIR spectra, and data comparisons from Geminiani et al. (2022), seemed to indicate that Brand A was predominantly (if not completely) natural cellulose-derived, and Brand B a mixed composition of natural and regenerated cellulose. For this FTIR analysis, spectra from wipe samples (n = 10 per brand) were collected over a broad spectral range (400 – 4,000 cm^{-1}) at a resolution of 4 cm^{-1} from an average of 20 sample scans. The Happ-Genzel function in absorbance mode enhanced accuracy, and baseline correction and normalisation improved interpretation of spectral peaks.

Cotton strips served as control assays, chosen for their high cellulosic content (> 95%), sensitivity to environmental conditions, as well as their convenience in assessing microbial activity (Tiegs et al., 2013). Cotton strips were prepared by cutting unprimed, 100% natural cotton canvas (12 oz heavyweight, Discount Fabrics LTD, UK) into 8 x 2.5 cm rectangles, following established protocols (Tiegs et al., 2013; Colas et al., 2019). Strip edges were frayed (>3 mm) to prevent unravelling and handling was limited to strip edges to preserve tensile strength.

Brand A and B wet wipes and cotton controls were deployed in mesocosms. Wipes were deployed in their original sizes (Brand A = 200 x 160 mm; Brand B = 180 x 165 mm) to replicate natural freshwater pollution, but additional samples were cut to match control strip dimensions for tensile testing. This setup also allowed standardised comparisons across sample types.

Each mesocosm received 18 intact wipes and 18 cut strips from Brands A and B, distributed equally across channels 1 and 2, respectively, with only control strips in channel 3. This ensured exposure across treatments and spatial coverage, deploying a total of 72 intact wipes and 72 strips per brand, alongside 72 controls, for five weeks. Samples were placed in zip-lock mesh bags (11 x 15 cm, 100 µm mesh aperture, iQuatics, https://www.iquaticsonline.co.uk/), a standard method for isolating microbial biodegradation from invertebrate interference (Tiegs et al., 2007; Pye et al., 2012). A specific focus on microbial-driven biodegradation mechanisms in biodegradable wet wipes was allowed by this approach, while potential confounding effects from invertebrate activity were minimised.

Weekly, subsets from each channel were collected, covering all treatment and channel segments. Collected samples were sealed in zip-lock plastic bags (1 L, 24 cm x 17 cm) with 5g desiccant, transported to the lab chilled and in the dark to minimise ambient environmental degradation. In the lab, samples were gently rinsed with deionised water, dried at 40°C for 24 hours, and stored in desiccators until analysis.

4.2.4 Degradation measurements

Wet wipe degradation was assessed using mass loss and tensile strength loss (TSL), as these have been shown to reliably reflect microbial activity and cellulose breakdown in aquatic environments (Tiegs et al., 2013; Colas et al., 2019; Carballeira et al., 2020; Read et al., 2024). Initial wet and dry masses of reference wipes and strips (n = 20) were recorded to the nearest 0.1 mg (Ohaus Pioneer). For weekly subsets, mass loss (%) was calculated by subtracting each dry sample weight from its corresponding reference weight. Tensile strength was measured for wet wipes and control strips using a Zwick/Roell Z050 tensile testing machine with self-tightening roller grips. Strips were pulled at a fixed rate of 20 cm/min (preload = 1 N, preload speed = 5 cm/min, initial grip-to-grip separation = 11.61 cm), and each strip's maximum tensile strength was recorded.

To quantify TSL over time, the percentage of initial tensile strength lost per day of incubation was calculated, following a linear degradation model from established methods (Tiegs et al., 2013; Colas et al., 2019; Tiegs et al., 2019). TSL was determined using the formula:

$$TSL = \left[1 - \left(\frac{Tensile\ Strength_{treatment\ strips}}{Tensile\ Strength_{reference\ strips}}\right)\right] \times \frac{100}{Incubation\ Time} \quad (4.1)$$

where $Tensile\ Strength_{treatment\ strips}$ is the maximum tensile strength of field-incubated strips, $Tensile\ Strength_{reference\ strips}$ is the mean tensile strength of 10 non-incubated reference strips (per wipe brand and cotton controls), and incubation time is the number of days the strips were exposed in the field. To account for temperature differences, TSL was also expressed per degree-day, with incubation time replaced by cumulative degree-days (Tiegs et al., 2013). Degree-days was calculated by summing mean daily temperatures in each mesocosm channel (> 0 °C).

4.2.5 Microbial biomass

As a proxy for microbial activity, biofilm biomass samples were collected from each mesocosm channel (n = 24) at the end of the 5-week study period. Duplicate unglazed terracotta tiles (15 cm x 25 cm x 5 cm) were randomly placed along each channel to capture spatial variations. Both sides of the tiles were scraped into 50 mL Corning tubes, following established biomass sampling protocols (Steinman et al., 2006), and samples were stored on ice in the dark for transport.

Using the ash-free dry mass (AFDM) method, a robust gravimetric estimate of biomass, microbial biomass was quantified and correlated with degradation rates to assess microbial colonisation and activity. Following Steinman et al. (2006), tile biomass samples were filtered onto pre-weighed glass microfibre filters (47 mm, VWR, UK) and dried at 80 °C for 24 hours to a constant weight. The dried samples were then oxidised in a muffle furnace at 500 °C for 1 hour, cooled in a desiccator, and reweighed. Based on Steinman et al. (2006), dry mass and AFDM were calculated as:

$$Dry\ Mass\ = \frac{(W_a - W_f)}{A_t} \tag{4.2}$$

$$AFDM\ = \frac{(W_a - W_{ash})}{A_t} \tag{4.3}$$

where W_a is the dried biomass weight on filter (mg) before ashing, W_f is the filter weight (mg), W_{ash} is the post-ashing weight on filter (mg), and A_t is the tile area (cm^2).

4.2.6 Statistical Analyses

Statistical analyses were conducted using R (version 4.3.1) (R Core Team, 2021). Metrics of mass loss (%) and tensile strength loss (% per day, % per degree day) were analysed to assess degradation in intact wipes and all strips.

Normality and homoscedasticity were verified through Shapiro-Wilk tests, Q-Q plots, and residual plots. Descriptive statistics (e.g., mean, standard deviation) were calculated with the '*psych'* package (Revelle, 2024). Data manipulation and visualisation were performed using the *tidyverse* package (Wickham et al., 2019), with plots arranged using '*gridExtra'* (Auguie and Antonov, 2017), '*ggstatsplot'* (Patil, 2021), and '*patchwork'* (Pedersen, 2024). Model selection followed a backward stepwise approach using Akaike Information Criterion (AIC) to optimise parsimony and fit when required.

To test for differences in material type, treatment type, mesocosms, and time on degradation metrics, Aligned Rank Transformation (ART) from the '*ARTool'* package was used (Kay et al., 2021), with ART post hoc tests for pairwise comparisons. ART was selected over traditional ANOVA or Kruskal-Wallis tests due to its suitability for nonparametric data in factorial designs and its ability to incorporate random effects for hierarchical data. Specifically, this method works by aligning values to remove the effects of other factors, ranking the aligned values, and then applying standard ANOVA procedures to the ranked data - allowing valid tests of main and interaction effects in nonparametric contexts.

To further examine relationships between key explanatory variables and degradation metrics correlation analyses were conducted, as were linear mixed models (GLMMs) from the '*glmmTMB*' package (Brooks et al., 2017), and generalised additive mixed models (GAMMs) from the '*mgcv'* package (Wood, 2011) to account for the hierarchical and spatio-temporal structure and complex interactions within the data. A summary of these different mixed model variables is provided (Table 4.1).

Table 4.1. Summary of final GLMM and GAMM structures used to explore both TSL degradation metrics (TSL % per day, TSL % per degree day) against key environmental variables and over time. Environmental variables were universally scaled to improve model convergence. Flume was used as a fixed effect only when investigating combined material effects, and was removed in material specific analyses. Smoothed effects in GAMMs are indicated by 's' proceeding the variable name.

Metric	Model type	Effects	Random effects
TSL % per day	GLMM	Mean_PH + Mean_AFDM + Mean_TDS + Mean_Temp + Mean_Light + Day + Flume	Day:Mesocosm
TSL % per degree day	GLMM	Mean_PH + Mean_AFDM + Mean_TDS + Mean_Temp + Mean_Light + Day + Flume	Day:Mesocosm
TSL % per day	GAMM	sMean_PH + sMean_AFDM + sMean_PPM + sMean_Temp + sMean_Light, + sDay + Flume	Mesocosm
TSL % per degree day	GAMM	sMean_PH + sMean_AFDM + sMean_PPM + sMean_Temp + sMean_Light, + sDay + Flume	Mesocosm

GLMMs used a Tweedie distribution with a log link function to manage zero-inflated data and overdispersion, while GAMMs used a Gamma distribution and log link due to the positive skew of the degradation metrics. Scales of the environmental variables in the GLMM and GAMM models were standardised to improve model performance and interpretations. Thin-plate regression splines and Restricted Maximum Likelihood (REML) improved GAMM flexibility and performance. Model assumptions in these mixed models were validated using residual diagnostics through the '*DHARMa'* package (Hartig, 2024) and the '*gam.check'* function.

In the dataset, approximately 9% (18 out of 202) of observations exhibited negative TSL, presenting extreme outliers. A sensitivity analysis was performed comparing GLMMs using the full dataset versus a dataset excluding these negatives. Fixed effects were consistent across models, while the model without negatives improved fit (lower AIC and dispersion). Therefore, negative TSL values were excluded in analyses to improve model accuracy.

4.3 Results

4.3.1 Mesocosm characteristics

Key environmental variables were measured across mesocosm channels throughout the study (Fig. 4.2). Mean pH levels reflected the natural acidification gradient, with the acidic forest sites L3 and L8 exhibiting values of 6.12 (± SD 0.15), and 5.71 (± 0.1), respectively, while the circumneutral moorland sites L6 and L7 had higher pH levels of 6.88 (± 0.04) and 6.99 (± 0.02). Total dissolved solids (TDS) values also varied among mesocosms, ranging from 18.63 mg/L (± 1.33) in L8 to 43 mg/L (± 3.35) in L7, indicating varying organic and inorganic material content across sites.

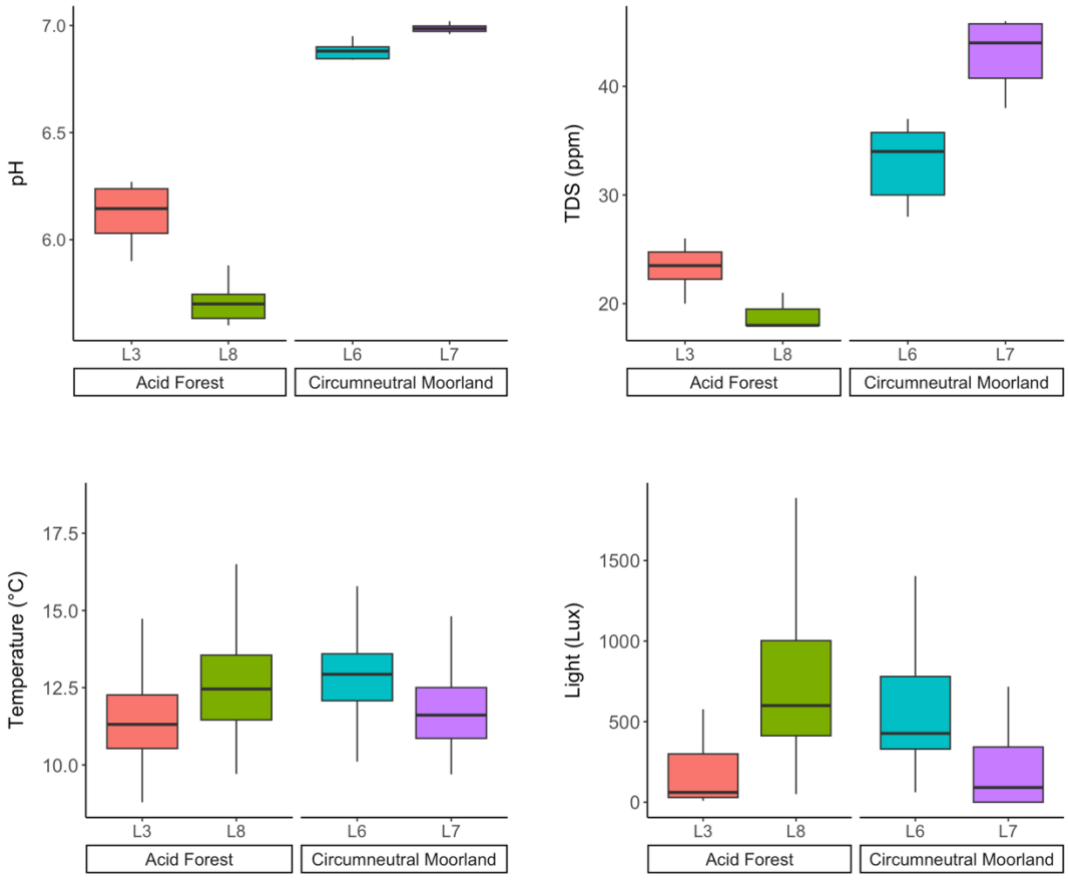

Fig. 4.2. Environmental variables from weekly sampling events (pH and TDS) and daily averaged temperature and light intensities for each mesocosm. Error bars indicate standard errors.

Temperature and light intensity were continuously monitored in each mesocosm channel throughout the study. Mean daily temperatures were relatively stable across mesocosms, ranging from 11.5 °C (± 1.49) in L3 to 13 °C (± 1.64) in L6. Light intensity, however, varied significantly, with L8 recording the highest average (709.7 lux ± 392.4), compared to L6 (580.2 lux ± 382.9), L7 (180.4 lux ± 218.6), and L3 (179.8 lux ± 201.7). This variation likely reflects differences in surrounding topography and vegetation, with hillside shading, tree cover, and orientation relative to the sun reducing light in L3, L6, and L7.

Temperature and light intensity varied significantly between the channels within mesocosms (Fig. 4.3). Temperature differences were most pronounced in L3 ($F_{2,68} = 33.65$, $p < 0.001$) and L7 ($F_{2,68} = 96.99$, $p < 0.001$), specifically for the Brand B channel in L3 and the Control channel in L7. Light intensity demonstrated even greater sensitivity to channel differences, particularly in L7 ($F_{2,68} = 543.92$, $p < 0.001$) and L8 ($F_{2,68} = 88.65$, $p < 0.001$), where the Control channel in L7 and Brand A channel in L8 had the most pronounced differences.

Fig. 4.3. Time series of averaged daily temperature and light intensities for each mesocosm and material type in their respective channels.

Microbial activity, measured as ash-free dry mass (AFDM) of biofilms collected from tiles, varied widely among mesocosms (Fig. 4.4). L8 had the highest mean AFDM (0.029 mg/cm² ± 0.009), suggesting greater biomass and potential for enhanced biodegradation, while L7 recorded the lowest (0.014 mg/cm² ± 0.004), reflecting limited biomass. Acidic forest mesocosms (L3, L8) generally showed higher AFDM than circumneutral moorland mesocosms (L6, L7).

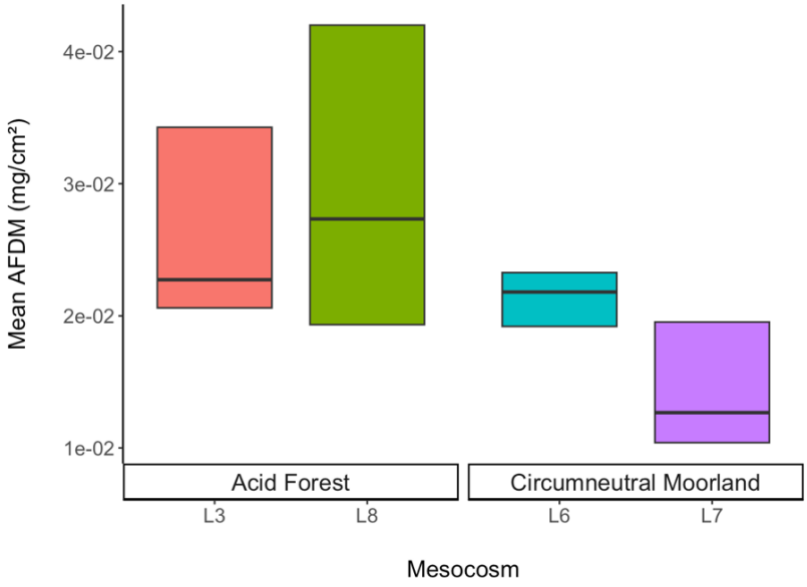

Fig. 4.4. Mean biofilm biomass across each mesocosm over the 5-week study period. Biomass is shown as standardised AFDM (mg/cm^2). Metrics are based on collected biofilms attached to tiles (n=3) in each mesocosm. Error bars represent standard deviation.

4.3.2 Material degradation

Wet wipe degradation was assessed by measuring tensile strength loss (TSL) as a proxy. Wet wipe and cotton control samples were cut into uniform strips, with non-incubated reference strips (n = 10 per material) used as baselines. The reference tensile strength (Fmax) was similar for both wet wipe brands: Brand A averaged 18.03 N ± 3.35, and Brand B averaged 17.76 N ± 0.89. In contrast, the cotton control strips exhibited a much higher mean tensile strength of 317.21 N ± 9.27 – approximately 18 times greater – highlighting their inherently stronger material structure. Initial reference strip dry masses were also recorded as baselines for mass loss, with Brand A averaging 0.094 g ± 0.01, Brand B at 0.122 g ± 0.007, and the cotton control at 0.746 g ± 0.026.

4.3.2.1 TSL metrics

TSL rates were measured using two metrics: TSL % per day (d^{-1}) and TSL % per degree day (dd^{-1}). Overall TSL rates varied widely across mesocosms, ranging from 0.02% to 7.18 % d^{-1} and -0.0003 to 0.21% dd^{-1} after removing extreme outliers primarily characterised by negative

percentage values (tensile strength gain). TSL rates varied between material types (d^{-1}, $F_{2,174}$ = 22.22, p = < 0.001; dd^{-1}, $F_{2,174}$ = 23.61, p < 0.001; Fig. 4.5a), with Brand A degrading the fastest for both metrics (ART post-hoc contrast test; p <0.001; mean ± SD: 2.03 ± 1.23 % d^{-1}; 0.09 ± 0.05% dd^{-1}), followed by Brand B (1.55 ± 1.35 % d^{-1}; 0.07 ± 0.05 % dd^{-1}) and cotton control strips (0.96 ± 0.87 % d^{-1}; 0.05 ± 0.04 % dd^{-1}).

TSL rates differed between mesocosms (d^{-1}, $F_{3,174}$ = 11.43, p < 0.001; dd^{-1}, $F_{3,174}$ = 13.47, p < 0.001; Fig. 4.5b). Specifically, pairwise contrasts revealed that circumneutral moorland sites L6 and L7 generally had higher degradation rates than acidic forest sites L3 and L8 (p < 0.05) although L6 and L8 differences were not statistically significant. Overall, L7 had the fastest degradation (1.97 ± 1.22 % d^{-1}; 0.09 ± 0.05 % dd^{-1}) and L3 the slowest (1.01 ± 1.15 % d^{-1}; 0.043 ± 0.038 % dd^{-1}).

To understand wet wipe degradation behaviour in different riverine zones, three experimental treatments were introduced in the mesocosms (hyporheic, submerged, surface). However, treatments did not significantly affect TSL metrics alone or between different material types.

TSL metrics across all materials demonstrated significant temporal trends in generalised linear mixed models (GLMMs). TSL % d^{-1} declined over time (β = −0.023, p <0.01), whereas TSL % dd^{-1} increased (β = 0.027, p < 0.001). LOESS predictions revealed non-linear temporal patterns - a rapid initial decline in TSL % d^{-1} over the first two weeks followed by stabilisation – while an inverse pattern was observed for TSL % dd^{-1} (Fig. 4.5c). Generalised additive mixed models (GAMMs) confirmed these non-linear findings for TSL % d^{-1} (edf = 1.9, F = 8.94, p < 0.001), however the effect of time became strictly linear and positively associated for TSL % dd^{-1} (edf = 1, F = 7.45, p < 0.01), supporting the GLMM findings.

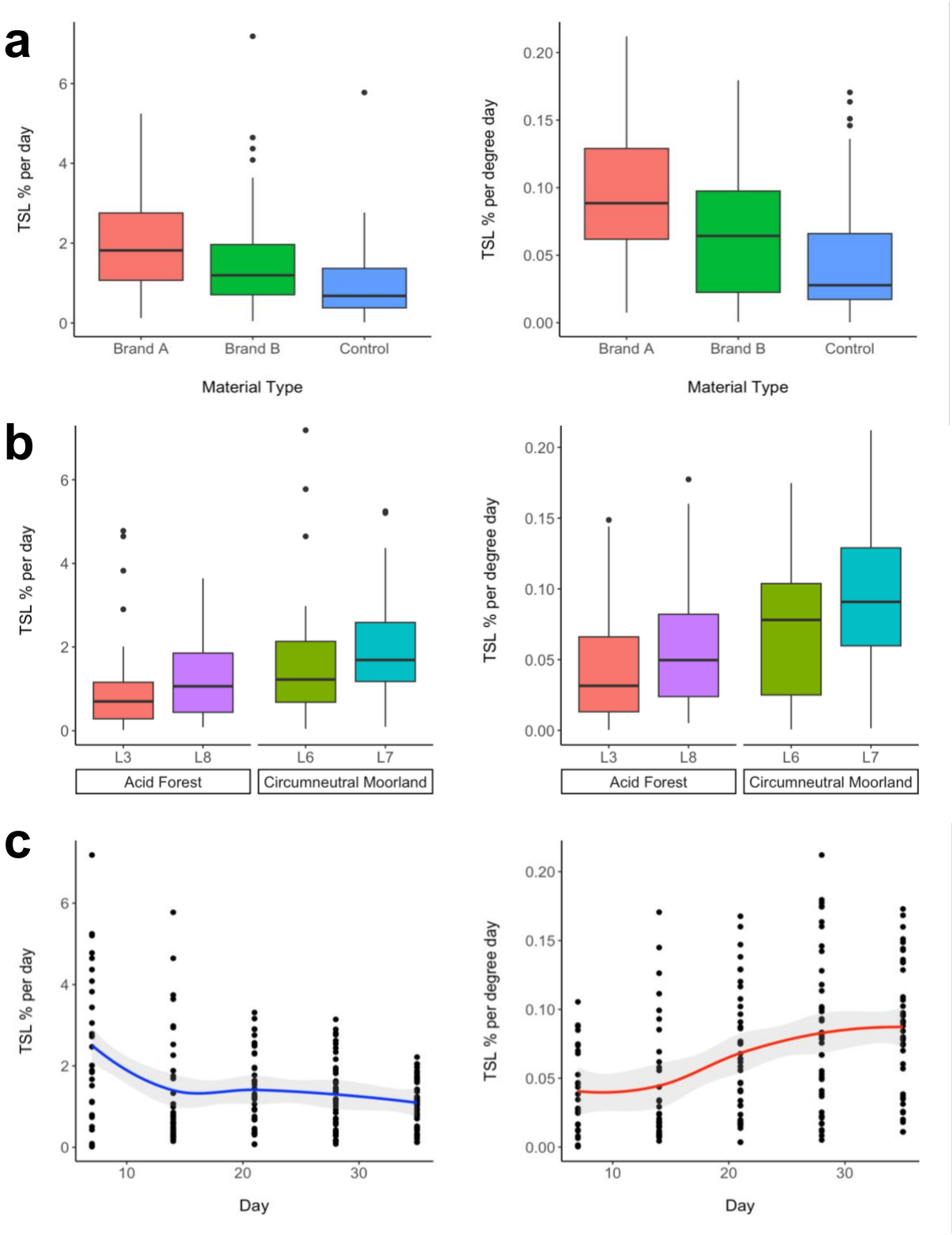

Fig. 4.5. TSL degradation metrics (TSL % per day and TSL % per degree day) for wet wipe and cotton control strips within the mesocosms. Boxplots of TSL metrics by a) material type and b) mesocosms, while c) shows non-linear LOESS regression trends of combined TSL rates over time.

Material-specific GAMMs helped clarify these temporal patterns (Fig. 4.6). In Brand A, TSL % d^{-1} showed a pronounced non-linear decline (edf = 1.9, F = 14.9, $p < 0.001$), mirroring the LOESS predictions, whereas the temperature-adjusted metric declined in a linear fashion but did not reach significance. Brand B also exhibited an early non-linear drop in TSL % d^{-1} (edf = 1.9, F = 5.2, $p < 0.05$), albeit less steep than Brand A, which began to increase again over the last week. However, this shifted to a non-linear increase once temperature was accounted for (edf = 1.7, F = 3.98, $p < 0.05$). By contrast, Cotton Controls showed no significant time effect in TSL % d^{-1} yet demonstrated a modest near-linear rise in the temperature-adjusted measure (edf = 1.0, F = 9.0, $p < 0.05$).

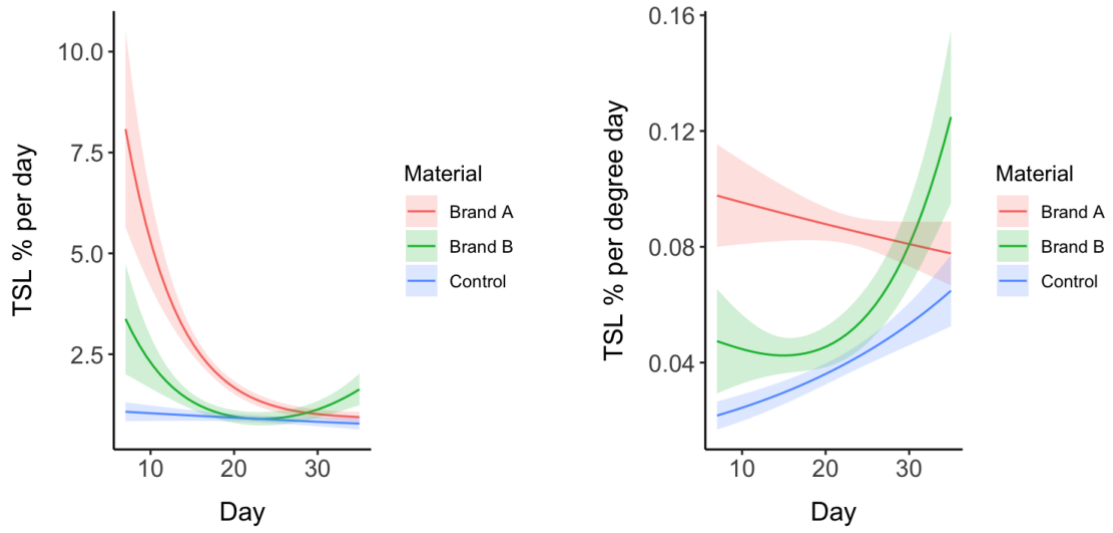

Fig. 4.6. GAMM predictions for material-specific degradation metrics (TSL % per day and TSL % per degree day) over time including standard errors.

Correlations between environmental variables and TSL metrics revealed distinct patterns. When all materials were combined, TSL rates increased only slightly with pH (r = 0.28, $p < 0.05$). However, material-specific analyses uncovered noticeable differences. For Brand A wipes, TSL increased moderately with pH (r = 0.57, $p < 0.05$), while temperature-adjusted TSL was also positively linked to pH (r = 0.41, $p < 0.05$) but also increased with temperature (r = 0.51, $p < 0.05$). In contrast, Brand B wipes exhibited stable degradation with no significant associations, while cotton control strips showed similar trends to Brand A – with TSL

increasing with pH ($r = 0.37$, $p < 0.05$) and temperature-adjusted TSL rising with temperature ($r = 0.38$, $p < 0.05$).

Across material types, environmental variables were interrelated. For instance, light intensity increased with temperature ($r = 0.23$, $p < 0.05$) and TDS ($r = 42$, $p < 0.05$), while pH decreased with both light ($r = -0.38$, $p < 0.05$) and biomass ($r = -0.6$, $p < 0.05$). These patterns suggest that co-occurring environmental conditions may indirectly drive degradation or at least set the stage for it.

GLMMs further elucidated the linear effects of environmental factors on degradation over time while accounting for spatial-temporal clustering using a meocosm:day random interaction. In combined-material models, higher pH (d^{-1}, $\beta = 0.29$, $p < 0.001$; dd^{-1}, $\beta = 0.36$, $p < 0.001$) and TDS (d^{-1}, $\beta = 0.15$, $p < 0.05$; dd^{-1}, $\beta = 0.20$, $p < 0.001$) were robust predictors of increased degradation, whereas biomass, temperature and light showed inconsistent effects. Material-specific GLMMs revealed that Brand A was primarily responsible for these trends: its degradation rates increased significantly with pH (d^{-1}, $\beta = 0.40$, $p < 0.05$; dd^{-1}, $\beta = 0.32$, $p < 0.01$) and TDS (d^{-1}, $\beta = 0.29$, $p < 0.01$; dd^{-1}, $\beta = 0.34$, $p < 0.001$), and further increased with temperature ($\beta = 0.32$, $p < 0.001$) when TSL was adjusted for temperature. In contrast, Brand B showed no significant environmental effects on TSL % d^{-1} , while its temperature-adjusted metric greatly increased with TDS ($\beta = 1.32$ $p < 0.05$), with only borderline influences of pH and biomass. Control samples exhibited no significant environmental effects on either metric.

Non-linear relationships were also explored using GAMMs but revealed linear environmental responses (edf = 1). Thus, GAMM results corroborated the GLMM findings.

4.3.2.2 Mass loss metric

Mass loss (%) was also measured as a proxy for degradation. However, organic debris and fine sediment particles were found to accumulate on both intact and strip samples over the study period. Although efforts were made to clean samples of aquatic debris, the compact fibre structure of the wipes prevented complete debris removal without compromising sample integrity. Therefore, there were instances of negative mass loss, or mass gain, complicating its reliability as a degradation indicator, and which was then excluded from the analysis. For

interest, overall mass loss rates in strips ranged from -52.98 to 42.52 % (mean = -0.03 ± 15.3 %), while for intact wipes, mass loss rates ranged from -33.01 to 34.72 % (mean = 5.57 ± 15.09 %). By material type, this debris accumulation was greatest on average for Brand A > Brand B > Cotton, likely the result of the different material porosities.

4.3.2.3 Intact wipe degradation

Over the 5-week study period, both Brand A and B wet wipes (full size and strips) remained intact within the acid forest mesocosms but less so in the circumneutral moorland mesocosms, where both wipe brands started to fragment and degrade from Week 3 onwards, particularly within L7 (Fig. 4.7). All wet wipes in contact with river water darkened and collected organic and inorganic materials in their structure over time. The influence of attached debris may have played a role in wet wipe breakdown, particularly in terms of physical fragmentation, which allows for greater degradation from physico-chemical breakdown processes.

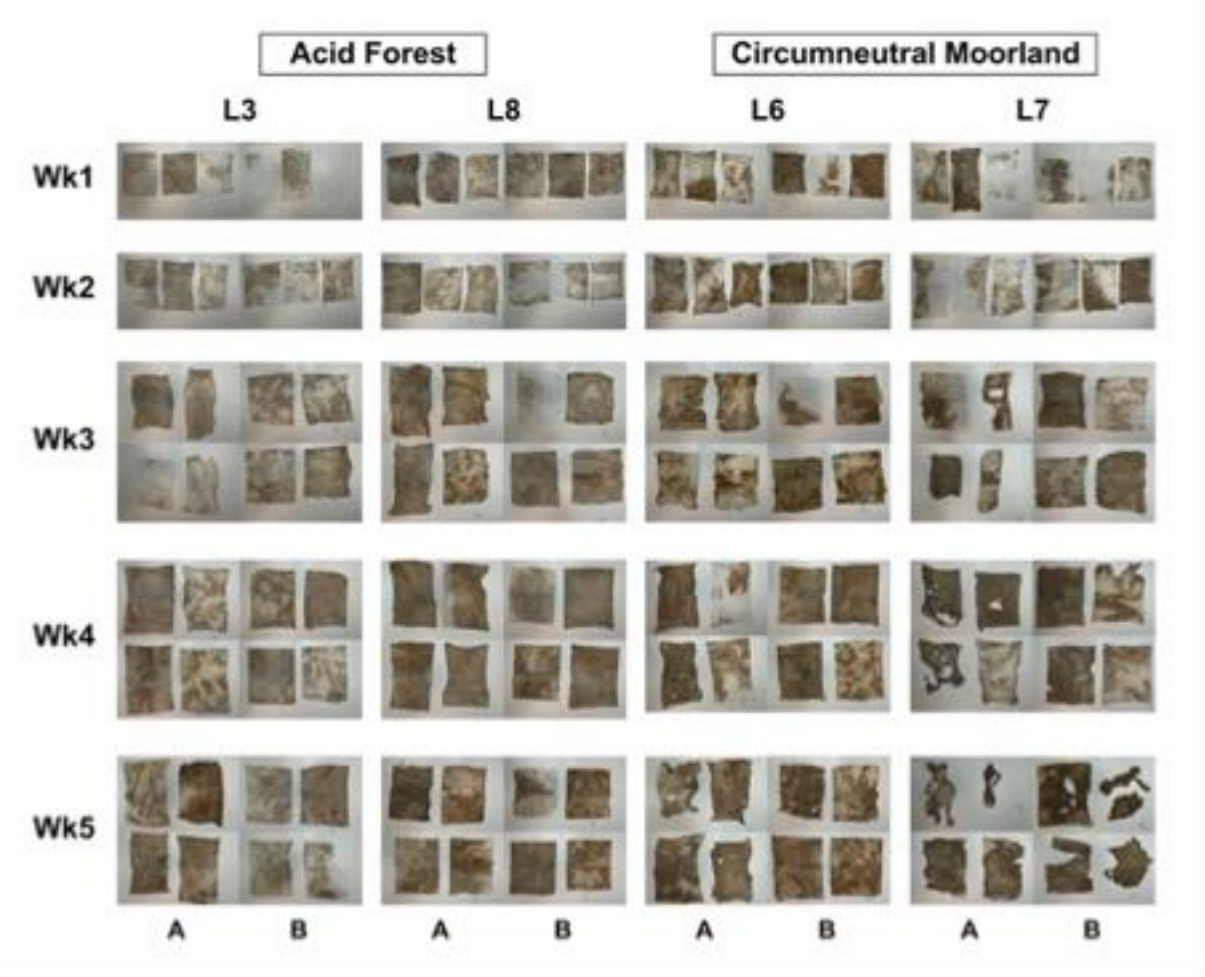

Fig. 4.7. Visual assessment of intact (Brand A and B) wet wipe degradation in each mesocosm across all treatment types. These intact wipe findings also mirror patterns found for their strip-sized versions.

4.4 Discussion

Despite the rising demand for cellulose-based biodegradable wet wipes, their degradation in natural freshwater systems remains poorly understood. This study assessed the degradability of cellulose-based wet wipes and identified key environmental drivers influencing their breakdown. These insights are critical for evaluating whether such alternatives genuinely mitigate environmental pollution or persist in freshwater environments like conventional plastic-containing wipes.

4.4.1 Degradation metrics

A major challenge in assessing cellulosic degradation is selecting a reliable, practical, and ecologically relevant metric. Prior studies using leaf litter and cotton strip bioassays have shown that both mass loss and tensile strength loss (TSL) can effectively quantify cellulose breakdown (Boulton and Boon, 1991; Tiegs et al., 2013; Griffiths and Tiegs, 2016; Tiegs et al., 2019; Blackman et al., 2024). However, in this study, mass loss was unreliable due to sediment entrapment and organic debris accumulation, an issue likely exacerbated by the high porosity and loosely arranged fibres of wet wipes (Durukan and Karadagli, 2019; Ziklo et al., 2024).

Among the tested metrics, temperature-adjusted TSL (% per degree day) provided more consistent degradation estimates across mesocosms, as it reduced the influence of temporal temperature variation and revealed clearer linear and non-linear relationships with temporal and environmental drivers. This aligns with previous research emphasising the importance of temperature-normalised decomposition rates for understanding additional environmental drivers of cellulose breakdown (Mancuso et al., 2023; Blackman et al., 2024). However, TSL captures wet wipe degradation as a whole without distinguishing between biological, chemical or physical drivers of degradation.

4.4.2 Degradation across material types

Degradation rates for both TSL metrics varied consistently by material, following the pattern Brand A > Brand B > Cotton. On average, Brand A degraded ~50% faster than Brand B and nearly twice as fast as cotton strips. However, despite being marketed as biodegradable, both wet wipe brands remained largely intact after five weeks, with only partial breakdown observed – namely for Brand A in circumneutral mesocosms.

Both wet wipe brands exhibited an initial rapid decline in raw TSL, followed by relative stabilisation, whereas cotton strips degraded steadily but more slowly throughout the study. The faster raw TSL decline of wet wipes compared to cotton controls aligns with expectations, as their non-woven, porous, and loosely entangled structure likely enhanced fibre fragmentation, microbial colonisation, and enzymatic biodegradation (Colas et al., 2019; Kwon et al., 2022).

However, when adjusted for temperature (TSL % dd^{-1}), degradation rates generally followed an inverse pattern, suggesting that thermal exposure significantly influenced degradation trajectories. Despite its steep early decline in raw TSL, Brand A's temperature-adjusted TSL remained stable, indicating that its initial degradation was largely driven by physical fibre loss rather than microbial activity. In contrast, Brand B's TSL % dd^{-1} gradually increased, suggesting that its degradation became more temperature-sensitive over time. Meanwhile, cotton strips exhibited minimal change in raw TSL but a steady increase when temperature adjusted, reinforcing the notion that their degradation was more reliant on thermal exposure and its environmental and biological conditioning over time.

FTIR analysis indicated that Brand A was predominantly, if not completely, composed of natural cellulose, while Brand B contained a blend of natural and regenerated cellulose. Therefore, differences in cellulosic wipe degradation rates and behaviour over time may reflect contrasting fibre compositions and physico-chemical properties.

Regenerated cellulose fibres (e.g., viscose, lyocell) typically have lower crystallinity, and are more hydrophilic and biodegradable than natural cellulose (Park et al., 2004; Zambrano et al., 2020c; You et al., 2021). However, surface morphology also plays a key role - natural cellulose fibres often have a ribbon-like structure that increases surface area for microbial colonisation (Ziklo et al., 2024), which can enhance overall biodegradation. Consistently, previous studies show that natural fibre-containing wipes and textiles exhibit higher TSL and aquatic biodegradation rates than regenerated cellulose alternatives (Durukan and Karadagli, 2019; Zambrano et al., 2020b; Kwon et al., 2023; Smith et al., 2024).

Microfibre shedding may also explain the temporal degradation patterns. Natural fibre-based wet wipes tend to shed many microfibres in water, more so than regenerated and plastic alternatives due to their irregular fibre morphology and weaker inter-fibre bonding (Kwon et al., 2022; Li et al., 2022; Allison et al., 2025). In Brand A, this could account for the steep initial decline in TSL % d^{-1}, as natural fibres were rapidly lost through mechanical fragmentation rather than temperature-dependent biological or chemical processes. Because this early fibre loss is likely independent of temperature, the corresponding temperature-adjusted TSL (% dd^{-1}) remained relatively stable – capturing little additional degradation beyond that early shedding. As the more labile natural fibres were lost, the remaining material – possibly composed of more structurally bound natural fibres or instead regenerated fibres,

resisted further breakdown, further slowing degradation over time. In contrast, Brand B, which likely contains a blend of regenerated and natural cellulose, showed a more gradual decline in tensile strength, consistent with less microfibre shedding in early stages.

Both wet wipe brands contained antimicrobial preservatives (e.g., benzoic acid, phenoxyethanol, potassium sorbate, sodium benzoate), chemical additives commonly used in textiles to suppress bacterial and fungal growth (Windler et al., 2013; Tawiah et al., 2016; Malis et al., 2019). These compounds may have helped delay microbial colonisation, supporting the idea that initial degradation in wet wipes was dominated by physical disintegration rather than microbial activity. However, since these additives are loosely bound, they likely leach out soon after immersion, reducing their long-term influence on reduced degradation rates (Sait et al., 2021). The delayed increase in Brand B's temperature-adjusted degradation could suggest that its degradation was initially inhibited but intensified once additives had leached.

4.4.3 Environmental and biological drivers of degradation

In this study, pH, total dissolved solids (TDS), and temperature emerged as the strongest environmental predictors of cellulosic material degradation, particularly for Brand A wipes. However, direct light effects and biofilm biomass, as a surrogate for biological activity, were minimal.

pH strongly influenced degradation patterns over time, with significantly higher TSL rates in circumneutral mesocosms (L6, L7) than in acid forest mesocosms (L3, L8). This aligns with research showing that acid conditions suppress cellulolytic microbial activity, inhibiting leaf-litter and cotton strip breakdown (Dangles and Chauvet, 2003; Dangles et al., 2004; Pye et al., 2012; Ferreira and Guérold, 2017; Colas et al., 2019). TDS was also positively correlated with degradation over time, suggesting that greater ionic and organic matter availability enhanced cellulose breakdown efficiency, consistent with previous findings (Boulton and Quinn, 2000; Gulis et al., 2006; Ferreira and Guérold, 2017). This effect may reflect the relatively low-nutrient conditions of upland streams, as excessive nutrient enrichment – common in agricultural or urban waters – can disrupt microbial communities and inhibit degradation (Woodward et al., 2012; Colas et al., 2019).

Despite the narrow temperature range (11.5 - 13 °C) across mesocosms, stream temperature influenced degradation rates in a material-dependent manner, becoming clearer over time when adjusted for cumulative thermal exposure. Brand A and cotton strips exhibited stronger temperature sensitivity, with temperature-adjusted TSL increasing with temperature, suggesting that thermal input enhanced degradation, likely via greater cellulolytic microbial availability which is known to accelerate with rising stream temperature (Griffiths and Tiegs, 2016; Yue et al., 2016). Brand B's raw TSL showed no clear correlation with temperature, but its temperature-adjusted TSL increased over time and was positively associated with TDS. This could suggest that chemical conditions, rather than temperature alone, played a greater role in its degradation. However, given the lack of a temperature effect on raw TSL, the observed increase in TSL % dd^{-1} may also reflect artefacts introduced by adjusting for temperature, rather than a true material-specific response.

The increase in temperature-adjusted TSL over time, particularly for Brand A and cotton strips, suggests a shift from early fibre loss to more temperature-dependent degradation processes, such as enzymatic or hydrolytic degradation. If microbial or molecular processes had driven early degradation, temperature-adjusted TSL would have initially declined rather than remain stable. Instead, as raw TSL stabilised and temperature-adjusted TSL increased, molecular degradation likely played a greater role. Temperature effects may have been more pronounced over seasonal and broader spatial gradients (Blackman et al., 2024), with a longer study duration – particularly through peak summer conditions – likely revealing stronger direct and indirect temperature-driven degradation effects (Mancuso et al., 2023).

Findings highlight that biofilm biomass, a proxy for biological activity, did not significantly influence degradation rates, but still revealed important and possibly explanatory insights. Epilithic biomass was low across all sites (0.014 - 0.029 mg/cm² on average) reflecting the low nutrient availability, cooler temperatures, and hydrological variability characteristic of upland streams (Anderson-Glenna et al., 2008; Zancarini et al., 2017). Biofilm biomass was higher in acid forest sites than in circumneutral moorland sites. This may reflect two known interacting mechanisms: 1) algal adaptations to acid, low-nutrient conditions, leading to their dominance in epilithic biofilm communities (Winterbourn et al., 1992); and 2) reduced invertebrate grazing pressure in acid sites, allowing greater accumulation of algal and microbial biomass compared to circumneutral sites, where invertebrate densities are typically higher (Ledger and Hildrew, 2008).

Light availability had no direct effect on degradation but likely influenced environmental conditions. Notably, light was positively correlated with temperature and varied between mesocosms, reflecting shading effects from surrounding vegetation and hillside cover, particularly in acid forest sites. Light was also positively associated with TDS, possibly reflecting increased primary production and organic matter exudation under higher light conditions (Isles et al., 2021). While lower light availability in forested streams has been linked to slower decomposition rates (Mancuso et al., 2023), its effect was likely overshadowed by stronger environmental drivers of pH and TDS in this study.

Average cotton strip TSL rates in this study (~0.96% per day and 0.05% per degree day) were comparable but generally slower than those reported in previous field studies (Tiegs et al., 2013; Griffiths and Tiegs, 2016; Jabiol et al., 2020; Hill et al., 2022). Notably, both Griffiths and Tiegs (2016) and Jabiol et al. (2020) conducted their assays in headwater streams, indicating that our slower degradation rates likely reflect more constrained conditions specific to the mesocosm environment. Unlike dynamic downstream or urban river systems, where higher flow, nutrient inputs, and microbial diversity enhance cellulose breakdown, these mesocosms reflected upland stream conditions, characterised by lower nutrient availability, acid waters, and lower organic matter inputs. This is reflected in the low AFDM values in this study, which indicate reduced biological activity across all sites and suggest nutrient limitation as a key constraint on biodegradation.

4.4.4 Mesocosm treatment effects on degradation

Three river treatments were tested – surface, submerged, and hyporheic – to simulate conditions where wet wipe accumulate and assess how environmental and biological conditions influence wet wipe degradation. Microbial biodegradation is often slower in riparian zones due to reduced moisture availability (Mancuso et al., 2023), while submerged conditions can enhance abiotic hydrolysis – a molecular degradation process via moisture interactions (Allison et al., 2023). However, contrary to expectations, no significant treatment-driven differences in TSL were observed across mesocosms or material types. Several factors may explain this result:

1. Hyporheic conditions may not have differed enough from submerged environments. The shallow, gravel-cobble substrate likely allowed high interstitial flow and oxygen penetration, preventing the reduced aerobic microbial activity seen in deeper, fine-sediment riverbeds (Boulton and Quinn, 2000). Similarly, Burrows et al. (2017) reported that in well-oxygenated, saturated hyporheic zones, cellulose breakdown rates can match or even exceed surface environments, supporting the findings from this study that microbial degradation remained similar across treatments in the mesocosms.
2. The study's short duration may have limited treatment-specific differences. It is posited that early degradation was likely dominated by physical fibre fragmentation rather than microbial breakdown, particularly in cooler, nutrient limited upland streams (Fig. 4.8). Therefore, longer periods may have been required to detect microbial-driven degradation differences across treatments.
3. Surface-exposed wipes, may have remained sufficiently moist, preventing desiccation. Occasional water contact is likely to have sustained sufficient physical fragmentation, microbial colonisation and enzymatic degradation, minimising differences from submerged, and possibly hyporheic treatments. Alternatively, while moisture and mechanical action (e.g. river flow) in aquatic conditions has been shown to enhance wet wipe microfibre shedding (Lee et al., 2021; Kwon et al., 2022), intermittent water contact may have accelerated wet wipe fibre shedding due to increased wetting and drying cycles (Li et al., 2022).

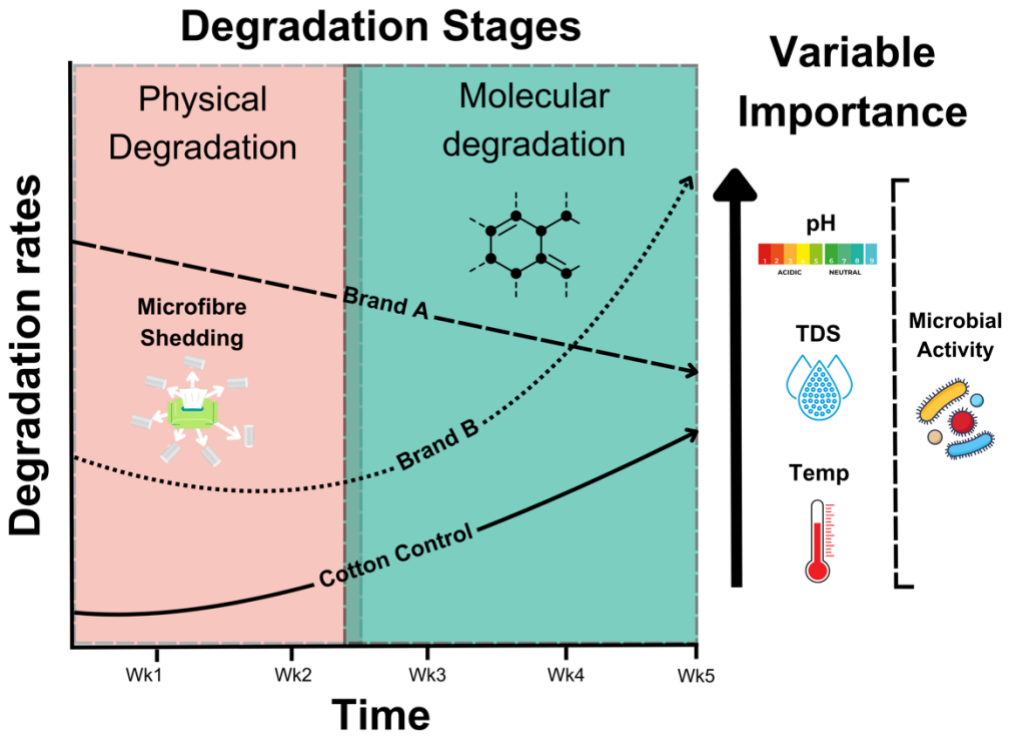

Fig. 4.8. Conceptual diagram highlighting observed degradation rates for each material type over time, including the likely degradation stages and the most important environmental variables influencing molecular degradation, which are all indirectly associated with microbial availability and activity.

4.5 Conclusion

This study is the first to systematically evaluate the degradation of cellulose-based biodegradable wet wipes in freshwater systems, using replicated, near-natural river mesocosms to identify the key physico-chemical drivers of breakdown. The findings show that biodegradable wet wipes can persist in river systems for over a month, creating short-term aquatic pollution risks through downstream transport. Tensile strength loss was also validated as a reliable proxy for assessing wet wipe degradation in the environment, though future research should integrate chemical analyses to better distinguish molecular and physical degradation mechanisms. Environmental conditions predominantly shaped degradation rates in the study, with warmer, circumneutral streams with higher total dissolved solids generally accelerating cellulose breakdown over time.

These insights raise key considerations for product design, biodegradability standards, and waste management strategies of biodegradable wet wipes. Biodegradability claims should be tested under real-world aquatic conditions, rather than controlled laboratory or unrealistic settings, to ensure wipes degrade efficiently in the environments where they most often accumulate. Additionally, early-stage fibre shedding highlights the need to balance structural integrity with biodegradability to prevent excessive microfibre pollution as a byproduct while maintaining effective breakdown.

Understanding the factors that enhance or hinder degradation can inform regulatory policies, promote genuinely biodegradable alternatives, and refine disposal guidelines. Future research should extend this work to longer timeframes, capture broader but relevant environmental conditions, and incorporate more-detailed molecular-level analyses to fully unravel the fate of biodegradable wet wipes in freshwater ecosystems.

Crucially, this study establishes a framework for testing wet wipe degradation in urban river environments, where wipes typically accumulate. This next phase of research will determine how real-world urban pollution, hydrodynamics, environmental and microbial conditions influence biodegradation, further refining understandings of the environmental fate of biodegradable wipes.

Chapter 5

In-Situ Degradation of Biodegradable Wet Wipes in Urban Rivers

In this chapter, Thomas Allison, Benjamin Ward, Michael Harbottle, and Isabelle Durance designed the experiment. Thomas Allison conducted the experiment, processed, analysed, and plotted the data. Thomas Allison drafted and edited the manuscript following review and comments from all authors.

Abstract

The environmental degradation and fate of cellulose-based “biodegradable” wet wipes under real-world conditions remain underexplored, particularly in urban freshwater systems where they are frequently discharged via toilet flushing. Building on the previous mesocosm study, this chapter assesses the degradation behaviour of two commercially available biodegradable wet wipe brands across ten urban rivers and streams in Cardiff, UK. Tensile strength loss (TSL) was used to assess degradation of wipes alongside cotton strip bioassays as ecological benchmarks. Degradation rates varied significantly by material composition, environmental conditions, microbial activity, and hydrodynamics. Wipes rich in natural cellulose degraded substantially faster than those dominated by regenerated cellulose or cotton bioassay controls. Key environmental influences on degradation included microbial biomass (AFDM), total dissolved solids (TDS), temperature, and river-level fluctuations, though exposure duration emerged as the dominant factor – suggesting complex interactions between physical and biological processes. Despite early-stage degradation, most wipe samples persisted after five weeks, challenging biodegradability claims. These findings highlight the potential ecological risks of persistent fibre pollution, underscoring the need for updated biodegradability standards and labelling that appropriately reflect real-world freshwater conditions, as well as greater scrutiny of plastic-free alternative products and their environmental fates.

5 In-Situ Degradation of Biodegradable Wet Wipes in Urban Rivers

5.1 Introduction

Cellulose-based wet wipes, usually marketed as biodegradable, are designed to break down readily in natural environments (see Chapter 2 for definitions and their manufacturing). Yet their degradation behaviour in freshwater systems – particularly urban rivers and streams – remains poorly understood. These environments frequently receive flushed wipes via sewage discharges (McGoran et al., 2017; Thames21, 2019; McCoy et al., 2020; Besley and Cassidy, 2022; Fortibuoni et al., 2025), but little is known about their real-time breakdown or environmental fate. Moreover, eco-friendly branding may inadvertently encourage flushing (Kachef, 2024; Kargar and Joksimovic, 2024), increasing their occurrence in aquatic systems from source to sea.

In these environments, cellulose-based wipes also shed large quantities of microfibres (Kwon et al., 2022) – a form of pollutant that travels through wastewater infrastructure and into receiving waters (UKWIR, 2022; Tserendorj et al., 2024; Bach et al., 2025). Both macro- and micro-scale pollution from these wipes can affect aquatic wildlife through ingestion, chemical additive leaching, or by transporting contaminants and pathogens found in wastewaters and rivers (Windsor et al., 2019; McCoy et al., 2020; Ó Briain et al., 2020; Zimmerman et al., 2020; Courtene-Jones et al., 2024; MacAulay et al., 2024; Smith et al., 2024), with potential risks to human health as well (e.g. Metcalf et al., 2024). These concerns underscore the need to quantify both the degradation and fate of biodegradable wipes under real-world conditions, in order to better understand their ecological and human health impacts.

Despite this, research on overall wet wipe degradation remains limited. Most existing studies focus on lab-based reconstructed wastewaters (Joksimovic et al., 2020; Lee et al., 2021; Kwon et al., 2022; Pedersen et al., 2022; Kargar and Joksimovic, 2024) or beach mesocosms (Metcalf et al., 2024). These studies largely assess physical degradation such as dispersibility or microfibre release, rather than microbial or molecular processes, and often report slow or minimal breakdown in cellulose-based wipes. Perdersen et al. (2022) found that cellulosic wipes retained over 93% of their structure after 48 hours under simulated wastewater

conditions, while Kwon et al. (2022) reported higher microfibre shedding from natural cellulose wipes than from regenerated or synthetic alternatives under similar experimental conditions. Some studies show that cellulosic wipes remain largely intact even under elevated flow velocities and turbulence in laboratory-scale sewer pipe models (Kargar and Joksimovic, 2024). In fact, despite 'flushable' labelling, many altogether fail disintegration tests such as the IWSFG's 'Slosh box' test (Joksimovic et al., 2020). Outside of wastewaters, Metcalf et al. (2024) documented the degradation of compostable wipes buried in beach sand, finding that commercially compostable and home-compostable wipes persisted for up to 8 and 15 weeks, respectively, with *E.coli* remaining viable on the latter for at least 14 weeks; however, the drivers of degradation were not examined.

A recent mesocosm study (Allison et al., in prep/Chapter 4) addressed this gap by simulating river conditions to explore wipe degradation in controlled field settings. Wipe degradation was attributed to both physical fibre loss – likely driven by continual flow and turbulence (Kargar and Joksimovic, 2024) – and environmental conditions such as temperature, pH, and total dissolved solids (TDS), which likely supported microbial activity. However, overall degradation remained limited over the five-week study period, possibly due to low microbial availability typical of the upland stream mesocosms used (Zancarini et al., 2017). These findings aligned with broader field-based evidence that microbial colonisation and enzymatic activity govern cellulose degradation in freshwater, shaped by water pH, light, temperature, and nutrient availability (Chauvet et al., 2016; Griffiths and Tiegs, 2016; Burdon et al., 2020; Pingram et al., 2020). Nonetheless, the mesocosm design did not account for the complexity of highly urban rivers - where wipes are more likely to accumulate - and where more variable water chemistry, hydrology, microbial assemblages, and pollution profiles may strongly influence their degradation dynamics (Paul and Meyer, 2001; Imberger et al., 2010; Burdon et al., 2020; Tiegs et al., 2024).

The mesocosm study also demonstrated the utility of tensile strength loss (TSL) as a proxy for cellulosic degradation. TSL is widely used in freshwater ecology via standardised cotton strip assays to track microbial and invertebrate activity and infer ecosystem functioning (Tiegs et al., 2013; Colas et al., 2019; Carballeira et al., 2020; Hill et al., 2022). Applied to wet wipes, TSL offers a practical, ecologically meaningful method to assess degradation in field conditions. However, TSL only quantifies overall structural loss, not the specific mechanisms

behind it – whether mechanical, chemical, or biological (see Chapter 2). Additional chemical or structural analyses are needed to differentiate among these degradation pathways.

This chapter investigates the degradation of cellulose-based biodegradable wet wipe brands in urban rivers and streams – environments where they are known to accumulate. Cotton strip assays were also deployed as ecological controls to benchmark degradation responses. Building on the mesocosm findings, this study examines how water chemistry, biological activity, and local environmental conditions influence wipe degradation under dynamic, real-world flow regimes. It aims to improve understandings on the fate of biodegradable alternatives to plastic wipes and assess the environmental risks they may pose in freshwater ecosystems.

5.2 Methods

5.2.1 Study area

To investigate the degradation of biodegradable wet wipes in environments where they commonly accumulate, samples were deployed in urban rivers and streams around Cardiff, Wales, UK. Cardiff spans 141 km^2 and had an estimated population of ~383,500 in 2023 (ONS, 2024). The city has a temperate maritime climate, with river temperatures ranging from 2-25°C (Farr et al., 2017), average solar radiation of 8.5 MJ/ m^2/day (Solargis, 2025), mean annual precipitation of ~1,203 mm, and mean annual air temperatures between 7.26 - 14.95°C (Met Office, 2025).

Ten urban sites were selected (Fig. 5.1), including three on the River Taff (upper, middle, and lower reaches), one on the River Ely, and six residential streams: Rhiwbina (upper and lower Rhydwaedlyd Brook), Lisvane (upper and lower Nant Fawr), and Pontprennau (upper and lower Nant Glandulais). Site selection was based on locations where wipes were likely to end up and where prior water pollution research had been conducted (Hadley et al., in prep), as well as accessibility, and efforts to minimise public interference. Sampling took place over five weeks from October to November 2024.

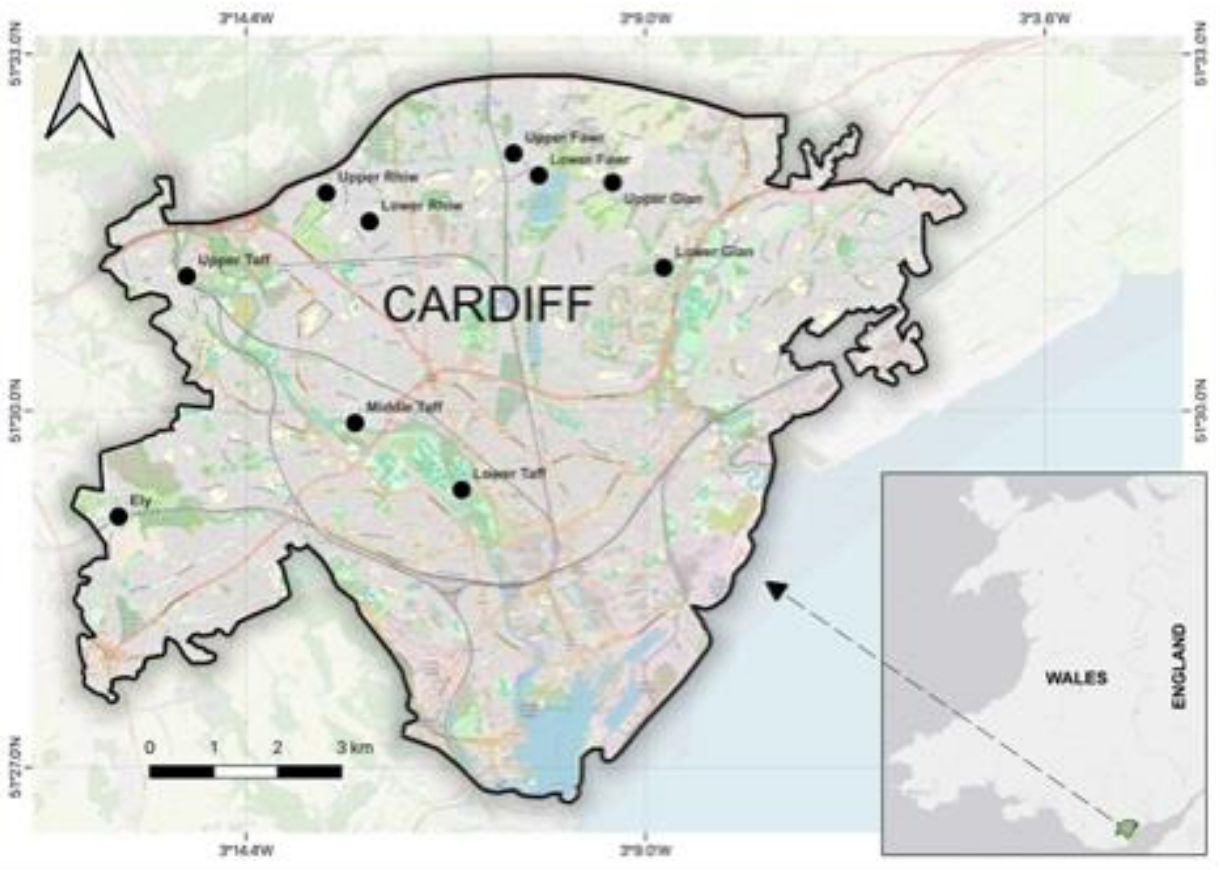

Fig. 5.1. Locations of urban river and stream study sites in Cardiff, Wales, UK. Rhiwbina and Glandulais sites are shown as abbreviations.

5.2.2 Materials and study design

Two commercial wet wipe brands (Brand A and Brand B), both marketed as biodegradable and composed of 100% bio-based fibres with similar additives (as stated on their packaging), were selected. Standardised cotton strips (8 x 2.5 cm) served as control bioassays due to their high cellulose content (> 95%) and established sensitivity to aquatic biodegradation (Tiegs et al., 2013). Wipes were tested in their original sizes (Brand A: 20 x 16 cm; Brand B: 18 x 16.5 cm) and as cut strips matching the cotton controls. Preparation methods are detailed in Allison et al. (in prep/Chapter 4).

At each site, five full-sized wipes and fifteen strips per brand were deployed, along with five cotton strips. All samples were individually enclosed in fine mesh bags (100 µm aperture, 11 x 15 cm; iQuatics), which prevented macroinvertebrate interference and allowed microbial

degradation to occur in isolation. Mesh bags were colour-coded with cable ties for identification, secured to bricks, submerged, and anchored to the streambed using metal pegs.

Samples were retrieved weekly over five intervals. Each collection included one full-sized wipe and three strips per brand, and one cotton control strip. Retrieved samples were sealed in 1 L zip-lock bags (24 x 17 cm) with 5 g desiccant, chilled, and kept in the dark to prevent further degradation during transport. In the laboratory, samples were rinsed with deionised water to remove debris, dried at 40 °C for 24 hours, and stored in desiccators until analysis.

Across all sites, 50 full wipes and 150 strips per brand were deployed, along with 50 cotton strips, enabling comparisons of degradation rates across materials and environmental conditions.

5.2.3 Quantifying tensile strength loss

To assess material degradation, tensile strength loss (TSL) was measured for sample strips as a proxy. Strips were mounted in the self-tightening roller grips of a tensiometer (Zwick/Roell Z050) and pulled at a constant rate of 20 cm/min (preload = 1 N; preload speed = 5 cm/min; initial grip separation = 11.61 cm) until failure.

Reference strength values were obtained from non-incubated strips (n = 10 per material), which underwent the same laboratory wetting and drying processes as incubated samples. Baseline maximum tensile strengths were similar for both wet wipe brands (Brand A: 18.03 N ± 3.35; Brand B: 17.76 N ± 0.89), while cotton strips, due to their woven structure, exhibited significantly higher strength (317.21 N ± 9.27).

TSL was calculated as the percentage of initial tensile strength lost per day of incubation, using a linear degradation model from established methods (Tiegs et al., 2013; Colas et al., 2019; Tiegs et al. 2019):

$$TSL = \left[1 - \left(\frac{Tensile\ Strength_{treatment\ strips}}{Tensile\ Strength_{reference\ strips}}\right)\right] \times \frac{100}{Incubation\ Time} \quad (5.1)$$

where Tensile $\text{Strength}_{\text{treatment strips}}$ is the maximum tensile strength of incubated strips, Tensile $\text{Strength}_{\text{reference strips}}$ is the mean tensile strength of the ten non-incubated reference strips for that respective material type, and incubation time is the number of days the strip was exposed for. To account for temperature variation across sites and sampling intervals, TSL was also expressed per degree-day. In this case, incubation time was replaced with cumulative degree-days, calculated by summing mean daily temperatures above 0 °C for each site.

5.2.4 Environmental variables

To monitor environmental drivers of degradation, pH and total dissolved solids (TDS) were recorded weekly at each site using a handheld test (HANNA instruments® pH/EC/TDS Combo Tester, HI98129), which was calibrated prior to each sampling event. Additionally, temperature and light intensity were measured hourly using automated data loggers (HOBO Pendant® Temp/Light). One logger was deployed per site, attached to the brick anchoring the sample mesh bags.

To characterise hydrological conditions at each site, both static and time-varying proxy metrics were incorporated. Mean river flow (m^3/s) was estimated using a standard hydrological equation for discharge:

$$Q = A\ x\ v \tag{5.2}$$

where Q is the mean flow rate (m^3/s), A is the cross-sectional area of the river or stream (m^2) derived from the product of measured width (m) and average depth (m), and v is the average flow velocity (m/s).

For the River Taff sites, recent mean flow data were obtained from the National River Flow Archive (NFRA; see https://nrfa.ceh.ac.uk) Station 57005 (Pontypridd). Although located upstream of Cardiff, this station's mean flow rate (21.175 m^3/s) was closely comparable to historical records from the decommissioned downstream Cardiff gauge at Tongwynlais (Station 57003; mean = 21.373 m^3/s between 1965-1972), justifying its use as a representative proxy. Site-specific velocities for the Upper, Middle, and Lower Taff were calculated by dividing the upstream mean flow by each site's measured cross-sectional area.

For the River Ely sites, long-term gauged flow data from NRFA Station 57009 (St Fagans) were divided by the field-measured cross-sectional area to derive average velocity. This Ely velocity (0.45 m/s) was then scaled proportionally for each urban stream site based on the ratio of its cross-sectional area relative to the Ely. This scaling assumed that smaller streams with lower cross-sectional area would exhibit proportionally reduced flow velocities, resulting in differentiated flow rates across stream sites while maintaining physical plausibility in the absence of direct gauging data.

To capture temporal hydrological variability, daily river stage height (m) was used as a proxy for discharge events. Continuous river level data were obtained from Natural Resources Wales monitoring stations on the Ely (St Fagans) and the Taff (Western Avenue; https://rivers-and-seas.naturalresources.wales/). Due to the absence of local monitoring infrastructure in urban streams, daily river levels from the Taff were assigned to all stream sites based on closer hydrological proximity. While these values do not represent absolute site-specific river levels, they provide a consistent, regionally relevant measure of rainfall-driven changes in flow across the study area during the monitoring period.

5.2.5 Microbial biomass determination

To assess microbial activity, a terracotta tile (12 x 25 x 5 cm) was deployed at each site (n = 10) to allow biofilm accumulation over the five-week period. At the final sampling point (Week 5), biofilm was scraped from both tile faces into 50 mL site-labelled Corning tubes, stored on ice, and kept in darkness during transport.

Microbial biomass was estimated gravimetrically using ash-free dry mass (AFDM). Biofilm samples were filtered, dried to constant weight, combusted in a muffle furnace, and reweighed. Calculations followed Steinman et al. (2006):

$$Dry\ Mass = \frac{(W_a - W_f)}{A_t} \quad (5.3)$$

$$AFDM = \frac{(W_a - W_{ash})}{A_t} \quad (5.4)$$

where W_a represents the dried biomass weight on the filter before ashing (mg), W_f is the filter weight alone (mg), W_{ash} is the biomass weight after ashing (mg), and A_t is the total tile area scraped (cm^2).

5.2.6 Scanning electron microscopy

To characterise wet wipe fibre composition and degradation patterns, scanning electron microscopy (SEM) was used on both pristine and incubated wipe samples. Samples were air-dried for 24 hours and mounted onto aluminium SEM stubs using conductive carbon tape. All samples were then gold-palladium coated using a Quorum Q150T ES sputter coater to improve conductivity and image resolution.

SEM imaging was conducted using a TESCAN MAIA-3 (Cardiff Catalysis Institute Electron Microscope Facility, UK). A 10.0 kV accelerating voltage was used and images were captured at multiple magnifications (ranging from 250× to 2,500×) to assess both fibre arrangement and fine-scale surface features such as cracking, fraying, and debris attachment.

To determine fibre composition and assess surface elemental profiles, energy-dispersive X-ray spectroscopy (EDS) analysis was conducted on selected pristine and degraded samples. EDS spectra were acquired using an Oxford Instruments X-Max 80^{NT} silicon drift detector (SDD), Energy Dispersive X-ray detector integrated with the SEM system. Analyses focused on identifying key elemental signals associated with cellulose-based fibres, additives, and environmental contamination.

5.2.7 Statistical analysis

All statistical analyses were conducted in R (version 4.3.1) (R Core Team, 2023). Data were checked for normality and homoscedasticity using Shapiro-Wilk tests, Q-Q/residual plots. Descriptive statistics were generated using the '*psych*' package (Revelle, 2024). Data manipulation and visualisation were performed with '*tidyverse'* (Wickham et al., 2019), '*gridExtra'* (Auguie and Antonov, 2017), '*ggstatsplot'* (Patil, 2021), and '*patchwork'* packages (Pedersen, 2024). The '*broom*' package was used to streamline regression data outputs (Robinson et al., 2025).

Observations with negative TSL values – interpreted as tensile strength gains – were treated as artefacts and removed (~3-4% of data). Additionally, extreme light intensity outliers (top 5% were excluded to improve model stability and clarity in visualisation.

To test for differences in datalogger variables (temperature and light) between sites, and in TSL rates between material types, sites, and timepoints, Aligned Rank Transformation (ART) ANOVA was used with the '*ARTool'* package (Kay et al., 2021), followed by ART-based post hoc pairwise comparisons. Square-root transformation was applied to TSL in material-based models to improve normality and residual behaviour. ART was selected for its robustness in factorial, non-parametric designs with nested or hierarchical structures.

To explore environmental influences on degradation, initial correlation matrices (combined and per material) were examined to identify independent associations between predictors and responses. A modelling workflow was then adapted from Hill et al. (2022). Environmental variables were scaled and screened for multicollinearity using variance inflation factors (VIFs) from the 'car' package (Fox and Weisberg, 2019), with a removal threshold of 7. This resulted in the exclusion of mean pH (VIF = 20.67), which was moderately to strongly correlated with AFDM, TDS, and flow rate. Outliers were removed where environmental values exceeded three times the interquartile range (IQR). Two response variables were modelled: raw TSL (% d) and temperature-adjusted TSL (% dd).

Each environmental predictor (mean temperature, light intensity, river level, flow rate, TDS, and biofilm AFDM) was fitted to four candidate model forms – linear, exponential, logarithmic, and quadratic – with lowest Akaike Information Criterion (AIC) used to identify the best fit. Exposure duration (Day) was included in additional models as an additive covariate to account for influences on degradation over time. The full model selection process was repeated for each material. R^2 and p-values from best fit models were extracted for model evaluation, with Benjamini-Hochberg (BH) correction applied to control for false discovery rate. Final plots used 95% confidence intervals and unscaled predictor values for interpretability.

All significant models (adjusted $p < 0.05$) underwent diagnostic checks, including residual normality (Shapiro–Wilk test) heteroscedasticity (Breusch–Pagan test), and influential outliers (Cook's distance > 4/n). Models were flagged based on assumption violations: "Mild Warning"

models with no or minor violations were retained, while “Review” models (e.g., with strong heteroscedasticity and/or ≥4 high-leverage points) were excluded from final outputs.

To investigate time effects on degradation independently, linear regressions were first fitted between exposure duration (Day) and each square-root transformed TSL metric, separately for each material. To account for potential non-linear trends and spatial autocorrelation, generalised additive mixed models (GAMMs) were then used with the ‘*mgcv*’ package (Wood, 2011). Each GAMM included a smooth term for Day (k value = 5) and a random intercept for Site. Effective degrees of freedom (edf) values were used to determine the complexity (non-linearity) of the smoothed terms. Residual diagnostics and basis dimension checks confirmed model validity and appropriate smooth complexity.

5.3 Findings

5.3.1 Environmental variation at urban sites

Water chemistry and environmental conditions varied across the ten urban river and stream sites (Table 5.1; Fig. 5.2) While mean pH remained relatively consistent (7.94 - 8.09), variability differed between sites – Upper Rhiwbina, for example, showed greater fluctuations (SD = 0.51) compared to most others (SD = 0.12 - 0.26). Mean total dissolved solids (TDS) ranged more widely (114 – 260 mg/L), with notably higher values in non-Taff stream sites (187 – 211 mg/L). Within the River Taff itself, TDS increased progressively downstream.

Table 5.1. Environmental and hydrological conditions measured across each urban river site (Mean ± SD). pH and total dissolved solids (TDS) were measured weekly while temperature and light were recorded hourly and converted to daily averages. Flow rate (mean; rounded) and water level (mean ± SD) were derived from available hydrological data (Ely and Taff sites) and proxies. Blank values reflect missing dataloggers at sites.

Site	**pH**	**TDS** (mg/L)	**Temperature** (°C)	**Light** (Lux)	**Water Level** (m)	**Flow rate** (m^3/s)
Ely	7.95 (0.12)	208.00 (38.65)	11.31 (1.55)	59.93 (72.01)	0.55 (0.4)	4.72
Upper Fawr	8.09 (0.22)	209.80 (22.39)	10.46 (1.65)	7.40 (8.74)	0.86 (0.54)	0.02
Lower Fawr	8.08 (0.26)	211.40 (20.31)	10.22 (1.58)	32.59 (55.69)	0.86 (0.54)	0.19
Upper Glandulais	8.02 (0.20)	200.00 (17.54)	10.63 (2.45)	31.29 (25.09)	0.86 (0.54)	0.02
Lower Glandulais	8.01 (0.15)	203.20 (26.04)	11.11 (1.75)	1.79 (4.42)	0.86 (0.54)	0.15
Upper Rhiwbina	7.95 (0.51)	204.80 (26.28)	–	–	0.86 (0.54)	0.04
Lower Rhiwbina	8.07 (0.29)	186.80 (32.46)	10.71 (1.01)	39.24 (65.93)	0.86 (0.54)	0.03
Upper Taff	8.01 (0.10)	127.80 (18.43)	–	–	0.86 (0.54)	19.97
Middle Taff	8.04 (0.13)	144.80 (32.62)	–	–	0.86 (0.54)	20.71
Lower Taff	7.94 (0.13)	161.60 (38.95)	10.77 (1.89)	34.70 (70.58)	0.86 (0.54)	22.46

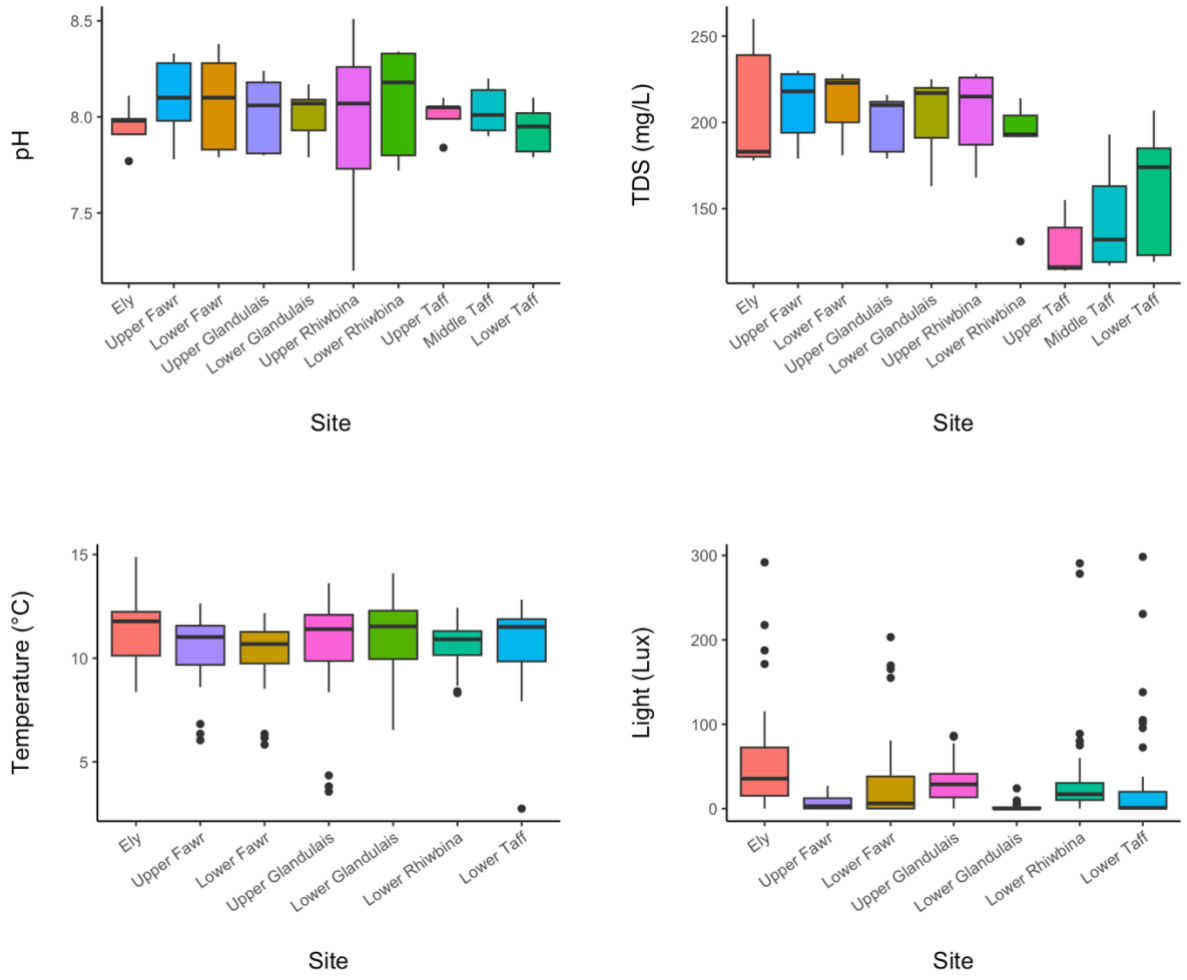

Fig. 5.2. Environmental variation of urban flowing water sites. Boxplots show pH, total dissolved solids (TDS), mean temperature, and mean light intensity across sites. Error bars indicate standard errors. Outliers are represented as black dots, to highlight the high degree of variability within sites, particularly for temperature and light.

Temperature and light intensity data were available for seven sites; the Upper and Middle Taff sites were excluded due to logger washouts after Week 1, and the Upper Rhiwbina logger was corrupted. Over the five-week study period, hourly measurements were aggregated into daily means. Temperature and light differed significantly between sites (ART test; $F_{6, 195} = 27.29$, $p < 0.001$; $F_{6, 196} = 17.62$ $p < 0.001$, respectively). Mean temperature was highest at Ely and lowest at Lower Fawr, while mean light intensity followed a similar pattern, with Ely again having the highest values, but Lower Glandulais the lowest (See Table 5.1). Over the study period, only mean temperatures followed a clear temporal pattern, generally decreasing over time, with the sharpest declines occurring between the 17th and 23rd of November.

Extreme outliers were present in the aggregated temperature and light datasets, even after filtering out light intensity values (> 300 lux) to improve modelling and interpretability. These outliers likely result from dynamic hydrological variability (e.g. fluctuating water depths) and organic debris accumulation, causing abrupt shifts between full shading and full exposure across the study period. These sensitivities highlight the limitations of logging accuracy in dynamic flowing waters.

Hydrological conditions also varied across the urban sites (Table 5.1). Mean flow rates spanned three orders of magnitude, from 0.02 m^3/s at Upper Glandulais to 22.46 m^3/s at Lower Taff, reflecting stark but expected contrasts between small residential streams and major river channels. The three Taff sites exhibited the highest flows (19.97 - 22.46 m^3/s), followed by the Ely (4.72 m^3/s), while all stream sites remained below 0.2 m^3/s. Mean daily water level, derived from river stage height across the monitoring period, remained constant across all non-Ely sites (mean = 0.86 ± 0.54 m), as a single Taff gauge time series was applied to approximate regional discharge fluctuations. However, distinct rainfall-induced discharge events were evident between Ely and Taff-based sites (Fig. 5.3), with the later showing more pronounced peaks, particularly around the 23 November.

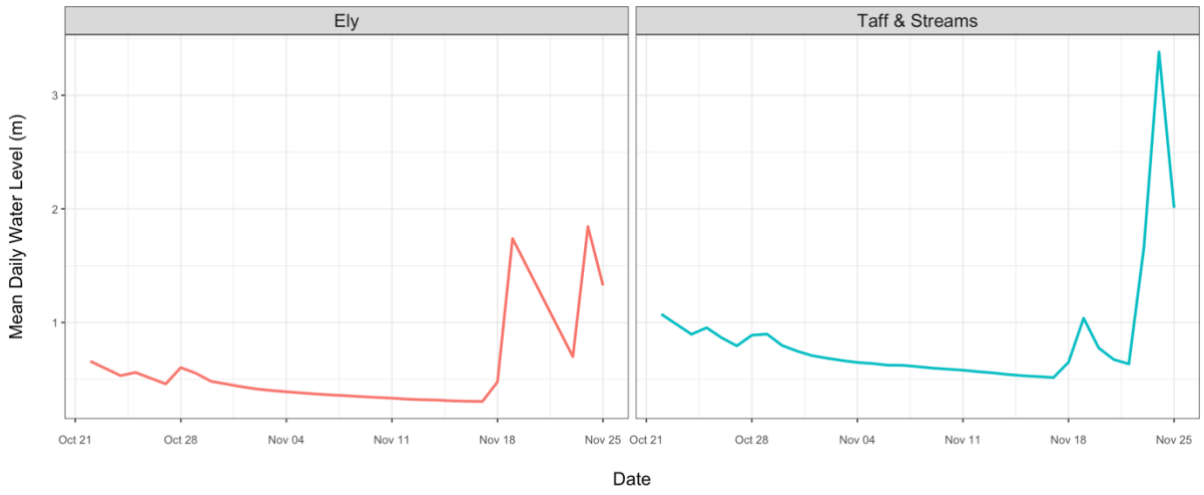

Fig. 5.3. Mean daily water levels at River Ely and Taff/stream sites over the 5-week study period.

Biofilm biomass, estimated via ash-free dry mass (AFDM), also varied across the seven sites with valid data (Fig. 5.4). Smaller streams such as Upper Fawr (0.107 mg/cm^2) and Lower Rhiwbina (0.099 mg/cm^2) supported the highest biomass, while the larger River Ely had the lowest (0.025 mg/cm^2).

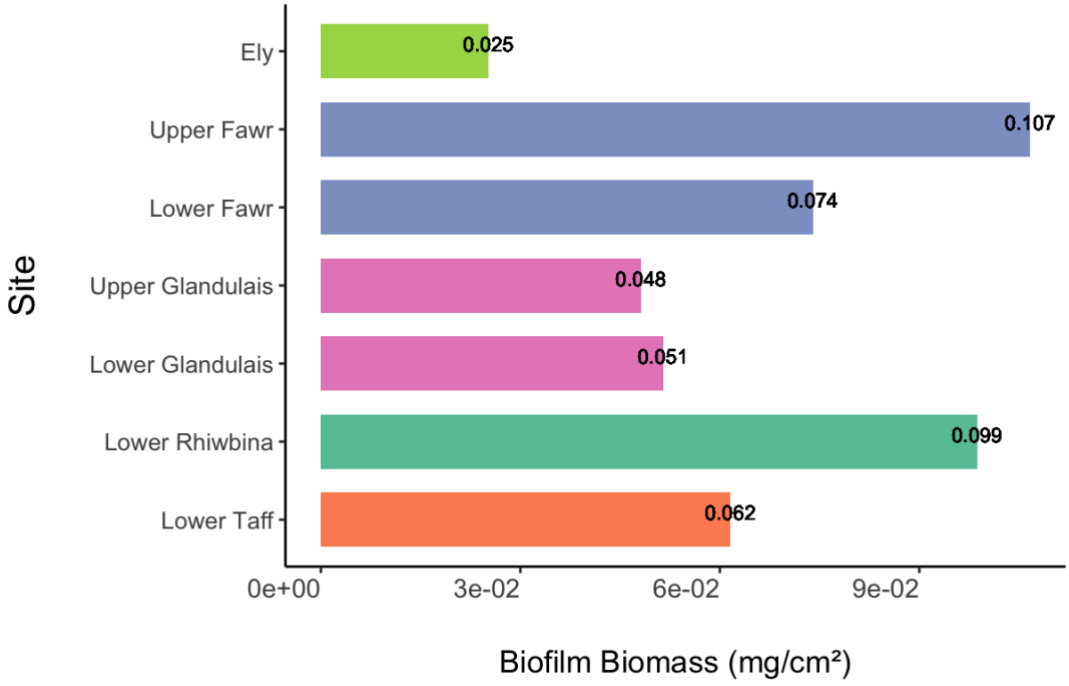

Fig. 5.4. Biofilm biomass across each site over the 5-week study period. Biomass is shown as standardised AFDM (mg/cm^2). Metrics are based on collected biofilms attached to tiles (n=1) in each urban flowing water site.

5.3.2 Tensile strength loss across urban flowing water sites

Material degradation was quantified as mean rates of tensile strength loss (TSL) per day (% d) and temperature-normalised TSL per degree day (% dd). Due to washouts, only Week 1 data were available for the Upper and Middle Taff sites. After removing negative values (interpreted as artefacts; 3.2% of data), TSL ranged from 0.019 - 13.33% d and 0.002 - 0.95% dd across all materials and sites. Material type had a significant effect on degradation rates (% d: $F_{2, 139} = 74.5$, $p < 0.001$; % dd: $F_{2, 112} = 57.76$, $p < 0.001$). Brand A wipes degraded fastest (6.69 ± 3.19 % d; 0.5 ± 0.23 % dd), followed by Brand B (3.12 ± 1.93 % d; 0.26 ± 0.17 % dd), and cotton

controls generally the slowest (2.22 ± 1.00 % d; 0.19 ± 0.09 % dd; Fig 5.5). Post-hoc ARTANOVA tests confirmed significant differences between Brand A and both Brand B and controls ($p < 0.001$ for all comparisons). Differences between Brand B and cotton were weaker but still statistically significant (% d: $p < 0.01$; % dd: $p < 0.05$).

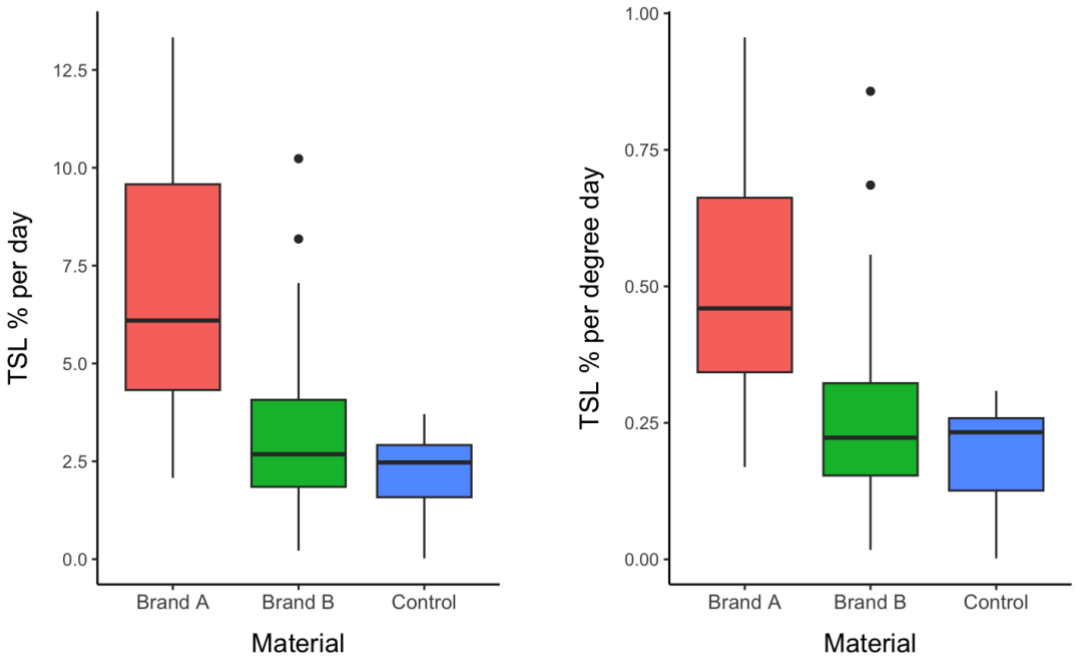

Fig. 5.5. Mean TSL degradation rates (TSL % per day and TSL % degree day) for each material type across all sites. Boxplots represent median and interquartile range.

Site-level differences in TSL rates were also significant (% d: $F_{9, 137} = 7.89$, $p < 0.001$; % dd: $F_{6, 111} = 11.38$, $p < 0.001$; Fig. 5.6). Lower Taff displayed the greater inter-site contrast, especially compared to Lower Fawr and Lower Glandulais (Post-hoc ART contrasts; all $p < 0.001$). Although the Middle and Upper Taff had the highest raw TSL rates overall, their results lacked statistical power - likely due to limited data and higher variability. Still, their similarity to Lower Taff suggests a consistent pattern of rapid degradation across the Taff sites.

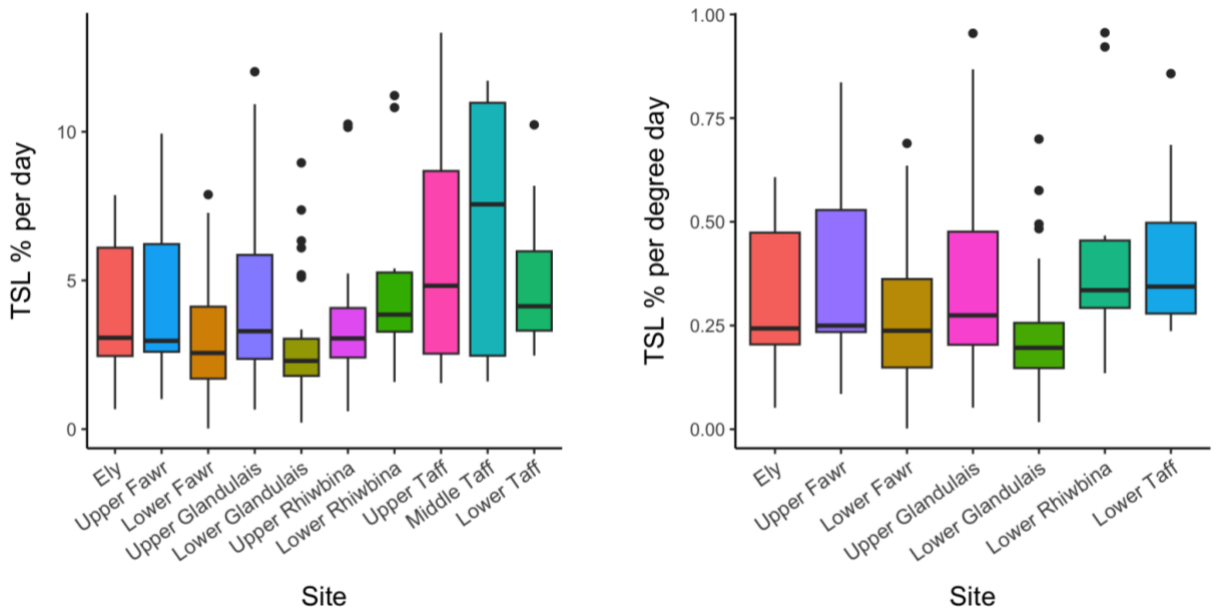

Fig. 5.6. Boxplot showing mean TSL rates (TSL % d and TSL % dd, respectively) across materials for each site. Boxplots represent median and interquartile range.

Between-material comparisons reinforced site-specific patterns in TSL rates (Fig. 5.7; Table 5.2). Brand A degraded fastest at Lower Rhiwbina (11.01 ± 0.28 % d; 0.94 ± 0.02% dd) and slowest at Lower Glandulais (4.51 ± 2.32 % d; 0.36 ± 0.18 % dd). Cotton controls showed a similar pattern, degrading fastest at Lower Rhiwbina (3.19 ± 0.39 % d; 0.28 ± 0.03% dd) and slowest at Lower Fawr (1.12 ± 0.73 % d; 0.10 ± 0.07 % dd). Brand B peaked at Lower Taff (6.57 ± 2.30 % d; 0.55 ± 0.19 % dd) and was slowest at Upper Glandulais (1.83 ± 1.06 % d; 0.15 ± 0.08 % dd). Notably, Brand A strips at Lower Taff fully degraded within the first week before collection, however, based on Brand B results, it is possible that the Lower Taff had the highest degradation patterns for all wet wipes. While numerous significant post-hoc contrasts were identified, the overarching trend across materials points to strong site-specific drivers influencing degradation rates.

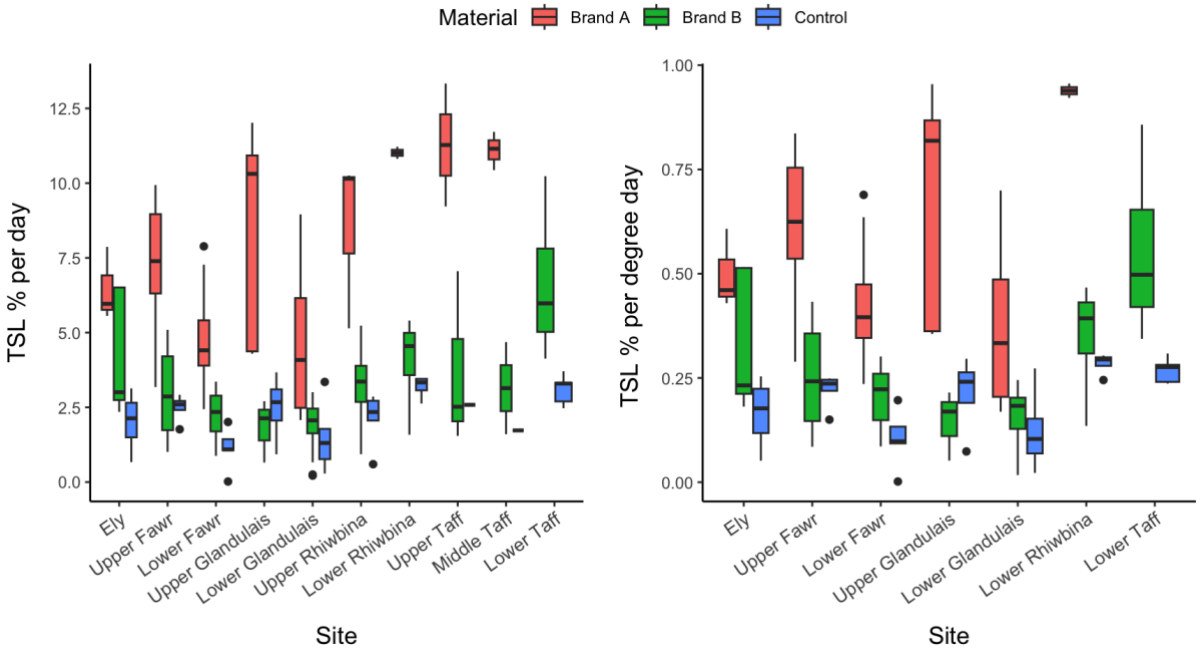

Fig. 5.7. Degradation rates (TSL % per day and TSL % per degree day) for each material type between sites with available data. For raw TSL, Upper and Middle Taff data represents Week 1 findings only and are shown for illustrative purposes alone. Sites with missing temperature data were excluded for temperature adjusted TSL.

Table 5.2. Raw (% per day) and temperature-adjusted (% per degree day) tensile strength loss rates (mean ± S.D.) for each material type at each site across the 5-week study period. NAs reflect missing dataloggers and TSL samples at specific sites.

Site	Material	TSL (% per day)	TSL (% per degree day)
All sites	Brand A	6.69 ± 3.19	0.5 ± 0.23
	Brand B	3.12 ± 1.93	0.26 ± 0.17
	Control	2.22 ± 1.00	0.19 ± 0.09
Ely	Brand A	6.46 ± 1.23	0.50 ± 0.10
	Brand B	4.23 ± 2.11	0.33 ± 0.17
	Control	2.08 ± 0.93	0.17 ± 0.08
Upper Fawr	Brand A	7.22 ± 2.45	0.61 ± 0.20
	Brand B	2.97 ± 1.65	0.25 ± 0.14
	Control	2.48 ± 0.44	0.22 ± 0.04
Lower Fawr	Brand A	4.73 ± 1.71	0.42 ± 0.14
	Brand B	2.29 ± 0.73	0.21 ± 0.06
	Control	1.12 ± 0.73	0.10 ± 0.07
Upper Glandulais	Brand A	8.38 ± 3.75	0.67 ± 0.29
	Brand B	1.83 ± 1.06	0.15 ± 0.08
	Control	2.49 ± 1.16	0.21 ± 0.10
Lower Glandulais	Brand A	4.51 ± 2.32	0.36 ± 0.18
	Brand B	1.86 ± 0.90	0.16 ± 0.08
	Control	1.50 ± 1.18	0.12 ± 0.10
Upper Rhiwbina	Brand A	8.52 ± 2.92	NA
	Brand B	3.27 ± 1.21	NA
	Control	2.12 ± 0.90	NA
Lower Rhiwbina	Brand A	11.01 ± 0.28	0.94 ± 0.02
	Brand B	4.02 ± 1.69	0.35 ± 0.15
	Control	3.19 ± 0.39	0.28 ± 0.03
Upper Taff	Brand A	11.28 ± 2.91	NA
	Brand B	3.71 ± 2.94	NA
	Control	2.58 ± NA	NA
Middle Taff	Brand A	11.10 ± 0.64	NA
	Brand B	3.14 ± 2.18	NA
	Control	1.73 ± NA	NA
Lower Taff	Brand A	NA	NA
	Brand B	6.57 ± 2.30	0.55 ± 0.19
	Control	3.10 ± 0.50	0.27 ± 0.03

5.3.3 Environmental and temporal drivers of degradation

Initial Spearman's rank correlations across all materials and sites showed that raw TSL rates slightly increased with temperature ($r = 0.29$, $p < 0.05$) but declined with exposure time for both raw and temperature-adjusted metrics (d %: $r = -0.32$, $p < 0.05$; dd %: $r = -0.29$, $p < 0.05$). These trends were primarily driven by Brand A wipes, which showed strong positive correlations with temperature (% d: $r = 0.68$, $p < 0.05$; % dd: $r = 0.65$, $p < 0.05$) and strong negative correlations with exposure time (% d: $r = -0.88$, $p < 0.05$; % dd: $r = -0.89$, $p < 0.05$), but also strong positive correlations with river level (both $r = 0.71$, $p < 0.05$). No significant temporal or environmental relationships were observed for Brand B or the cotton strips.

Environmental variables were also interrelated in the combined correlation model. pH increased with biofilm biomass ($r = 0.64$) and TDS ($r = 0.56$), while biofilm biomass decreased with light intensity ($r = -0.23$), and temperature ($r = -0.28$), all statistically significant at $p < 0.05$. Temperature also declined strongly over time ($r = -0.92$, $p < 0.05$).

To assess environmental influences on TSL across sites, the effects of temperature, light, TDS, river level, flow rate, and AFDM (a proxy for biofilm biomass) were modelled on raw and temperature-adjusted TSL for each material type. Exposure time (Day) was included in separate models as a covariate to control for temporal variation. Mean pH was excluded due to multicollinearity.

When environmental variables were analysed without controlling for time (Fig. 5.8), clear material-specific degradation patterns emerged. Brand A TSL increased with temperature (exponential model; $R^2 = 0.14$, $p < 0.05$), and AFDM ($R^2 = 0.29$, $p < 0.05$), and decreased with TDS (quadratic model; $R^2 = 0.31$, $p < 0.001$). Temperature-adjusted TSL also showed a strong quadratic relationship with AFDM ($R^2 = 0.56$, $p < 0.01$). Brand B showed only a weak association between adjusted TSL and TDS (exponential model; $R^2 = 0.11$, $p < 0.05$). The cotton control had strong quadratic relationships between both TSL metrics and AFDM ($R^2 = 0.72 - 0.75$, $p < 0.05$) and raw TSL had a somewhat unimodal relationship with TDS (quadratic model; $R^2 = 0.19$, $p < 0.05$), with degradation peaking around 170 mg/L.

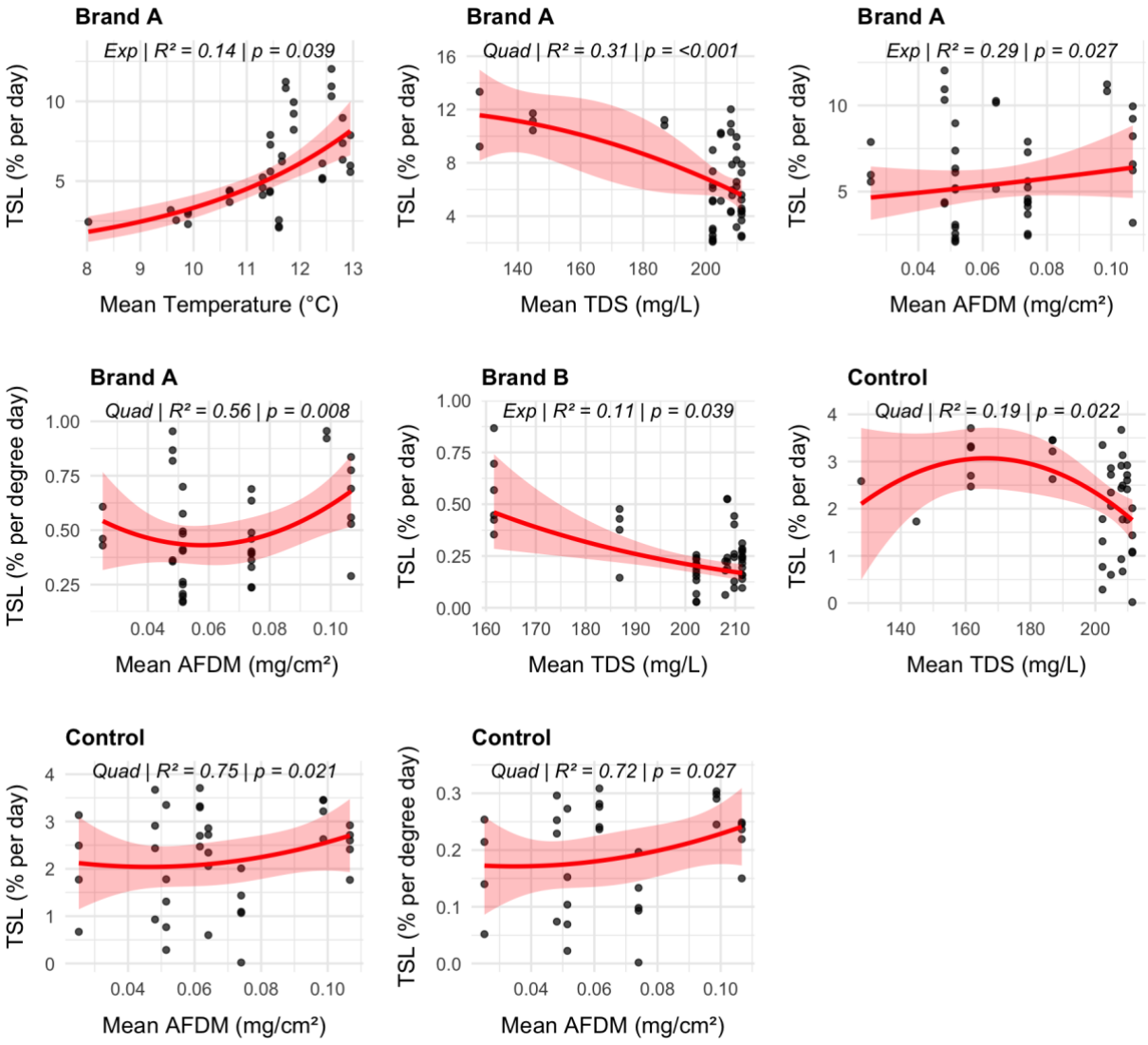

Fig. 5.8. Relationships between statistically significant environmental predictors and TSL metrics for each material type. Each plot shows the best-fit model from the modelling workflow and 95% confidence interval, without exposure duration (Day) held constant. Predictor values are presented unscaled for interpretability. Model forms include exponential (exp) and quadratic (quad) functions, and each model plot shows its associated R^2 and Benjamini-Hochberg adjusted p-values.

When controlling for exposure time (Fig. 5.9), Brand A degradation remained closely linked to environmental factors, with stronger model fits than in time-excluded models. Both TSL metrics increased with AFDM (exponential and quadratic models; $R^2 = 0.9$, $p < 0.01$), and river level (exponential and linear models; $R^2 = 0.42$ - 0.56, $p < 0.01$) but decreased with temperature (quadratic models; $R^2 = 0.75$ - 0.76, $p < 0.01$). Adjusted TSL also declined with TDS (quadratic model; $R^2 = 0.72$, $p < 0.05$). Notably, fitted temperature curves appeared inverse to the raw trend in data points, implying that exposure time masked some of the underlying temperature

effects. For Brand B, no significant environmental predictors were observed. Cotton controls again showed slightly positive AFDM associations (quadratic models; $R^2 = 0.76 - 0.77$, $p < 0.05$) and negative TDS effects (quadratic models; $R^2 = 0.35 - 0.39$, $p < 0.05$), consistent with models that excluded time. This suggests that time was a less influential driver of degradation for these cotton materials compared to Brand A wipes.

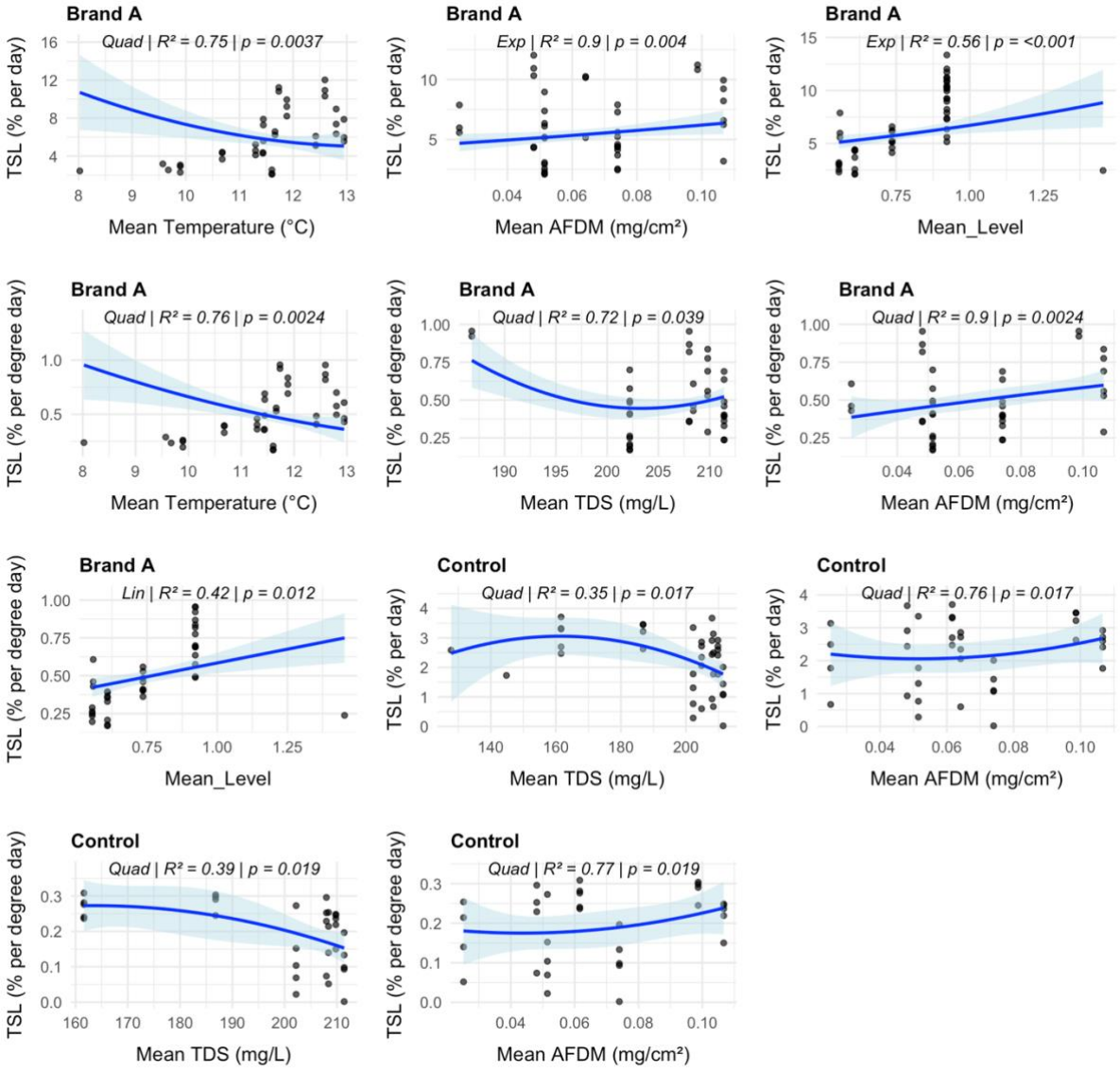

Fig. 5.9. Relationships between statistically significant environmental predictors and TSL metrics for each material type. Each plot shows the best-fit model from the modelling workflow and 95% confidence interval, with exposure duration (Day) held constant. Predictor values are presented unscaled for interpretability. Model forms include linear (lin), exponential (exp), and quadratic (quad) functions, and each model plot shows its associated R^2 and Benjamini-Hochberg adjusted p-values.

Although many relationships were best captured by quadratic models, interpretations at predictor extremes should be cautious due to sparse data. Site-level variance was negligible in ad-hoc mixed-effect models, so degradation patterns were primarily explained by local environmental conditions, discharge events (via mean water level), and exposure time, not site identity.

Overall, degradation sensitivity varied by material type. Brand A and the cotton controls responded consistently to organic and ionic conditions, with Brand A also showing sensitivity to hydrodynamic conditions (i.e. river level). In contrast, Brand B showed limited responsiveness to all measured environmental factors, suggesting slower degradation rates or potential influence from unmeasured or interacting stressors.

To further examine temporal trends, exposure time (Day) was modelled against each material's TSL rates using linear regressions (Fig. 5.10a). Brand A showed strong temporal sensitivity, with both raw and temperature-adjusted TSL declining over time ($R^2 = 0.64 - 0.65$, $p < 0.001$). No significant trends were found for Brand B or the cotton controls. Given non-linear trends observed in a prior mesocosm analysis (Allison et al., In prep/ Chapter 4), generalised additive mixed models (GAMMs) were fitted with Site as a random effect (Fig. 5.10b). For Brand A, GAMMs confirmed non-linear TSL declines over time (edf = 2.4 - 2.6, $R^2 = 0.71 - 0.73$, $p < 0.001$), suggesting rapid early degradation followed by plateauing. No significant temporal patterns were found for Brand B or the cotton control, reinforcing that degradation in these materials was either slower, or more influenced by non-temporal factors.

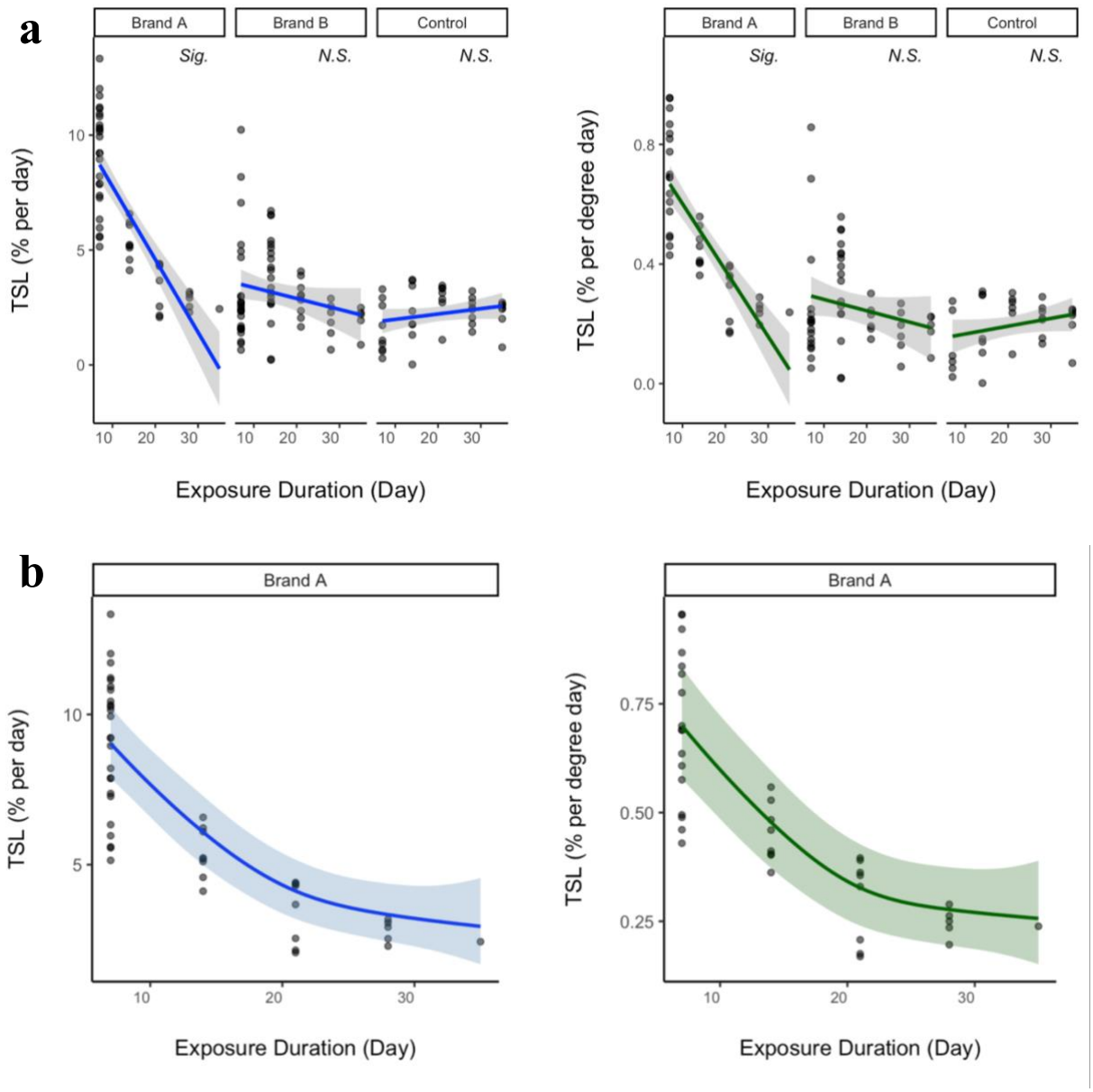

Fig. 5.10. a) Linear relationships between exposure duration (Day) and TSL metrics for each material type. Sig. indicates significant regression models and N.S. = non-significant models. b) Non-linear GAMM predictions for exposure duration (Day) vs. TSL metrics for statistically significant Brand A models. Both sets of models show 95% confidence intervals.

5.3.4 Photographic and SEM analysis of degradation

Photographic evidence revealed clear differences in degradation patterns between the two full-sized wet wipe brands across the urban sites (Fig. 5.11). Brand A degraded more rapidly and extensively than Brand B. By Week 5, Brand A wipes had visibly fragmented or deteriorated at several sites, including Ely, Upper and Lower Fawr, and Upper Rhiwbina. In contrast, Brand

B wipes retained more structural integrity, particularly at Lower Glandulais, Lower Fawr, and Upper Rhiwbina, where wipes remained largely intact.

Rapid early degradation at the Ely site (Weeks 2 and 3) appeared to be driven by biological activity, specifically the burrowing behaviour and accumulation of *Gammarus pulex* into the fine mesh bags containing full-sized wipes – an interaction not seen with wipe strips. It is likely that these amphipods were juveniles; small enough to enter through the 100 µm mesh early in the exposure period and subsequently grew inside the bags. Wipe samples from the Upper and Middle Taff sites were unavailable after Week 1, however, complete wipe degradation observed downstream at Lower Taff by Week 4 suggests similarly strong upstream conditions.

Interestingly, at Upper Fawr and Upper Glandulais in Week 4, Brand B wipes unexpectedly showed more fragmentation than Brand A. These variations underscore the complex nature of wet wipe degradation, which depends not only on material composition but also on local environmental and biological factors.

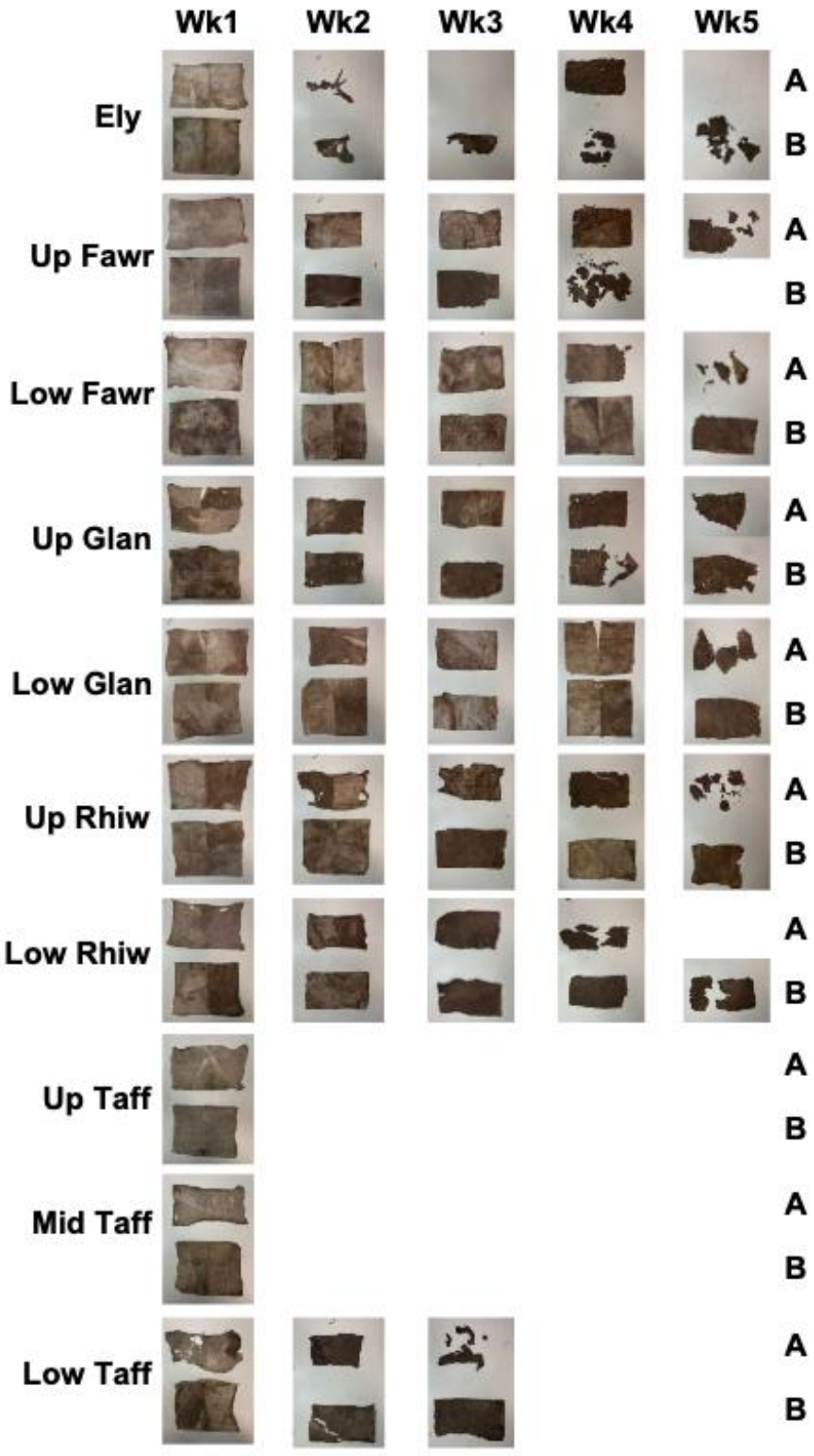

Fig. 5.11. Visual assessment of full-sized wet wipe (Brand A and B) degradation in each flowing urban site over the study period. In order from top to bottom, site abbreviations represent: Upper Fawr, Lower Fawr, Upper Glandulais, Lower Glandulais, Upper Rhiwbina, Lower Rhiwbina, Upper Taff, Middle Taff, Lower Taff. Upper and Middle Taff samples were lost after Week 1. Missing images in all other instances reflect high or complete degradation.

Scanning electron microscopy (SEM) revealed distinct fibre morphologies between the two cellulosic wet wipe brands, offering insight into their specific material compositions and potential degradation behaviour. In non-incubated wipes (Fig. 5.12), Brand A was predominantly composed of wide, flattened ribbon-shaped fibres, characteristic of natural cellulosic materials such as cotton and wood pulp (Harter et al., 2022; Ziklo et al., 2024). At broader magnifications, a smaller proportion of cylindrical, smooth-surfaced fibres – typical of regenerated cellulose such as viscose – were also observed (Salama et al., 2021), suggesting a blended composition but favouring natural fibres. Brand B showed similar fibre morphologies to Brand A but in a reverse pattern: more frequent smooth, rounded fibres and fewer flattened structures, indicating a higher proportion of regenerated cellulose than natural.

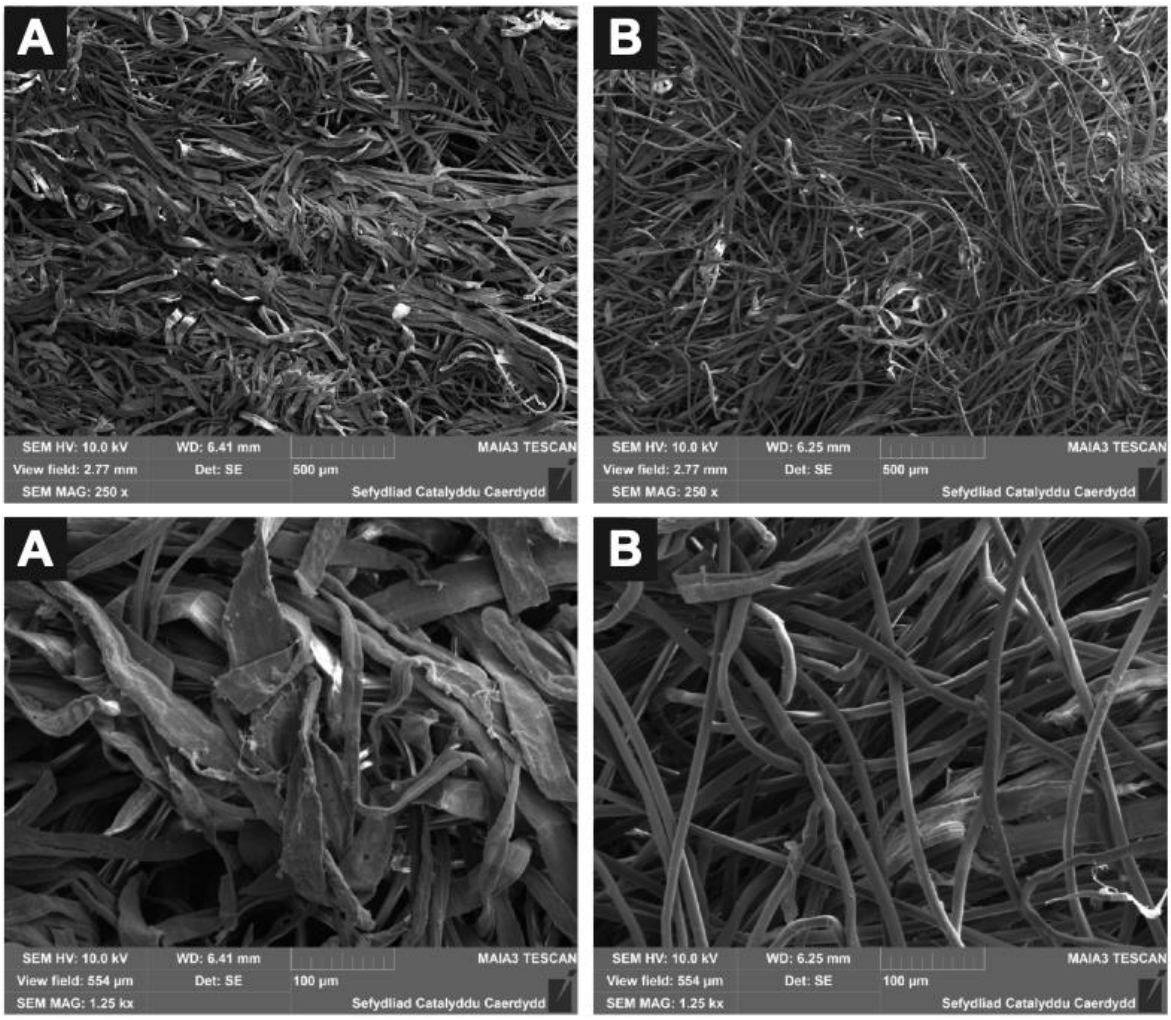

Fig. 5.12. SEM images of non-incubated cellulosic wet wipe brands (A and B) at different magnifications.

Elemental analysis using energy dispersive spectroscopy (EDS) further supported these findings. Both brands were dominated by carbon and oxygen, as expected for cellulosic materials. Titanium was detected in non-incubated Brand B (~1.67% by weight), possibly derived from titanium dioxide – a common additive used for textile whiteness or UV protection (Fig. 5.13). Gold (Au) and palladium (Pd) traces in the spectrum reflect the sputter coating applied during SEM sample preparation.

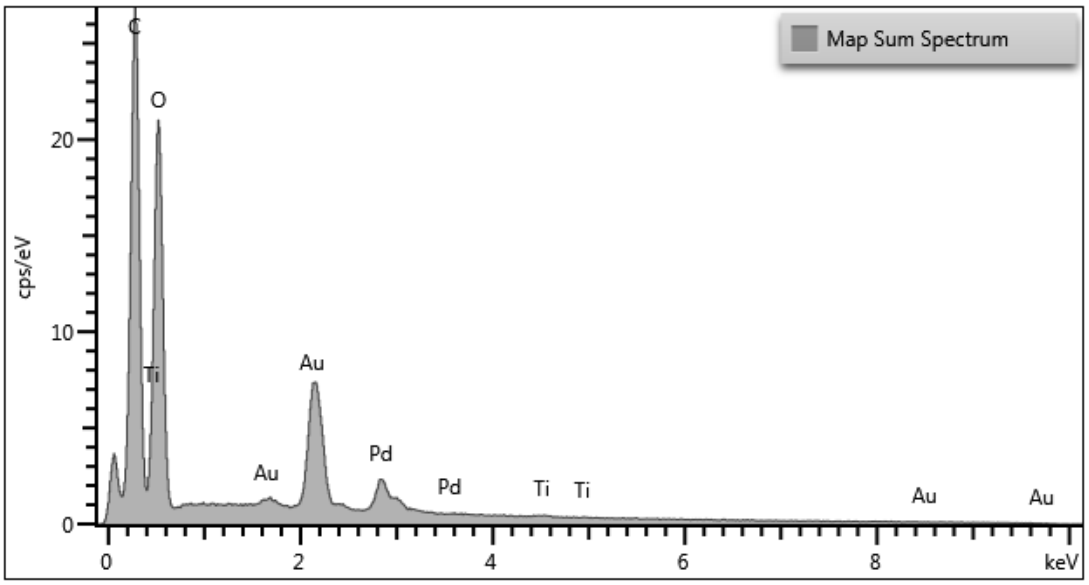

Fig. 5.13. Elemental analysis spectrum for non-incubated Brand B cellulosic wipes using energy dispersive spectroscopy (EDS).

SEM imaging of incubated wet wipes retrieved from the Lower Nant and Upper Glandulais sites helped to explain degradation patterns over time (Fig. 5.14). Brand A wipes exhibited more pronounced signs of degradation. By Week 3, ribbon-shaped fibres at both sites showed surface roughening, fraying, and partial detachment. At Lower Nant, these fibres were additionally coated with a thin, web-like matrix suggestive of early biofilm formation, intermixed with entrained fine sediment and organic debris which became more obstructive by Week 5 across both sites. While fibre outlines remained partially visible beneath this debris, signs of further erosion and embrittlement were evident at Lower Nant, particularly in areas of exposed fibres. At Upper Glandulais, degradation was more subtle, with fibre architecture still discernible but less visibly damaged. Mild pitting, surface cracking, and occasional tearing

were observed, though surface structural decay was less advanced than at Lower Nant from what could be seen.

Brand B wipes remained structurally intact for longer. At Week 3, cylindrical fibres displayed relatively smooth surfaces at both sites, though some abrasion and fine pitting were evident. At Lower Nant, thin, filamentous matrices – possibly early biofilm – were visible in some regions, entangled with particulate debris similar to Brand A. By Week 5, fibres in both sites were also densely embedded within thick layers of amorphous surface matter. No strong evidence of fibre fraying or fragmentation was found, though occasional faint scarring and coating adhesion were present. Some fine filaments observed on Brand B surfaces by Week 5 likely originated from environmental sources and were not degradation products.

Compared to non-incubated controls (e.g., Fig. 5.13), EDS spectra of incubated wipes showed elevated signals for aluminium, silicon, iron, magnesium, potassium, and calcium - elements typical of mineral sediments - supporting SEM observations of entrapped riverine debris on the wipes.

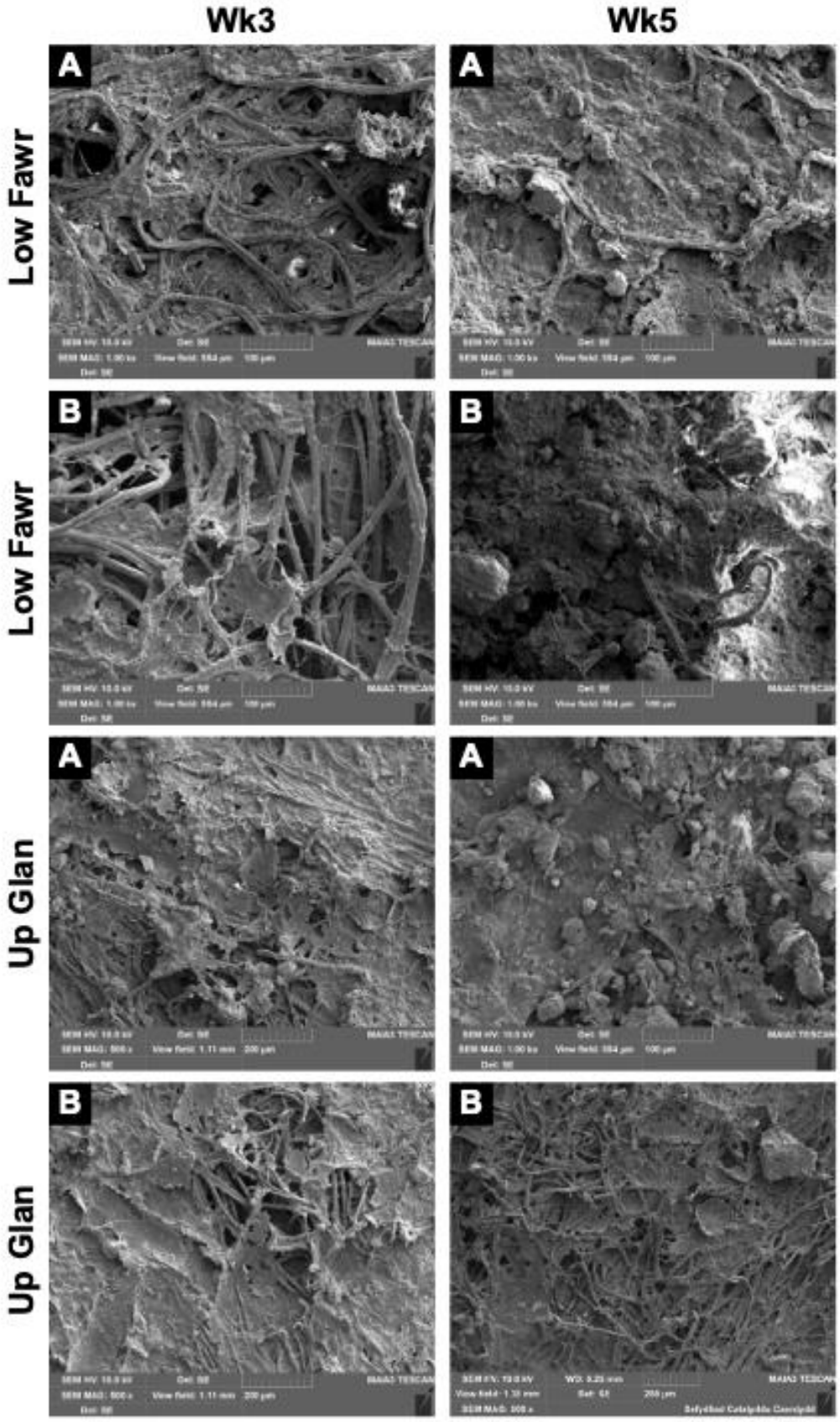

Fig. 5.14. SEM images of Brand A and B cellulosic wet wipes over time for the Lower Nant Fawr and Upper Glandulais sites.

5.4 Discussion

Growing concern over plastic pollution and increasing regulatory pressures, such as recent UK legislation (DEFRA, 2024a), have accelerated the adoption of plastic-free, cellulose-based wet wipes. These products are marketed as biodegradable and environmentally friendly, yet their degradation in freshwater environments – particularly urban rivers and streams where they commonly accumulate – remains poorly understood. This chapter provides the first multi-site, in-situ assessment of biodegradable wet wipe degradation in flowing urban waters using tensile strength loss (TSL) as a degradation proxy and cotton strip bioassays as ecological baselines.

5.4.1 Degradation behaviour in urban rivers and streams

Cellulose-based wipes displayed clear material- and site-specific degradation patterns, with Brand A consistently degrading more than twice as fast as Brand B, and both outperforming cotton controls. These findings reinforce patterns observed in controlled mesocosm experiments (Allison et al., in prep), highlighting that differences in degradation behaviour are strongly linked to intrinsic compositional differences.

SEM analyses confirmed these compositional differences. Brand A wipes contained predominantly wide, ribbon-shaped fibres typical of natural celluloses (e.g. wood pulp), with minor presence of cylindrical fibres suggestive of regenerated cellulose (e.g. viscose or lyocell) (Salama et al., 2021; Harter et al., 2022; Ziklo et al., 2024). Conversely, Brand B followed the opposite pattern, greater amounts of regenerated fibres than natural. Typically, in blends, the longer regenerated fibres provide the physical strength of the wipe structure by tangling with other fibres, while shorter length natural fibres such as wood pulp encourage water or chemical absorption and degradation (Tipper, 2016; Harter et al., 2022). The larger surface area and rougher texture of natural wipe fibres can also enhance microbial colonisation and enzymatic activity (Ziklo et al., 2024). Accordingly, the material-specific degradation patterns and SEM observations align with broader evidence that natural cellulose textiles (including wipes) physically and chemically degrade more readily in aquatic conditions than regenerated forms (Durukan and Karadagli, 2019; Zambrano et al., 2020b; Kwon et al., 2022; Kwon et al., 2023; Smith et al., 2024).

Differences in chemical additives also likely contributed to the observed degradation responses. For instance, both brands contained a range of antimicrobial preservatives (e.g., benzoic acid, phenoxyethanol, potassium sorbate, sodium benzoate), as listed on their packaging, which may have delayed microbial colonisation until leached out in water (Windler et al., 2013; Malis et al., 2019; Sait et al., 2021). However, the rate and extent of chemical leaching for these materials are unknown and may vary depending on water chemistry, antimicrobial chemical composition, wipe structure and exposure duration. Although this hypothesis was not directly tested in the present study, future work could investigate additive effects more explicitly – for example, by comparing degradation rates in pre-washed wipes (to remove surface preservatives) versus untreated controls under experimental microbial exposure conditions.

Mean degradation rates in this study were substantially higher than those recorded in the mesocosm study. Brand A degraded over three times faster in urban waters, while Brand B and cotton strips both degraded at roughly double their mesocosm rates. These trends were consistent for both raw and temperature-adjusted TSL metrics, suggesting that natural urban streams may offer more dynamic or favourable conditions for degradation than upland rural upland streams (See Section 5.4.2 for further discussion).

Comparative findings from Metcalf et al. (2024) further contextualise these results. When applied to Equation 1 for TSL % per day, their beach mesocosm study found daily TSL rates of 2.06 ± 1.36 % and 3.04 ± 1.72 % for home and commercially compostable wipes, respectively. These rates for compostable wipes, which, like biodegradable wipes, are ultimately designed to biologically degrade though typically under more controlled conditions (Nizamuddin and Chen, 2024), were lower than the cellulosic wet wipes rates observed in this urban stream study. This contrast highlights how varying conditions in environments where wipes often accumulate, can significantly influence degradation trajectories for wipes.

Despite signs of early-stage degradation, persistent wipe fragments were found across all degradation studies – after 5 weeks in both the stream mesocosm and current urban water studies, and up to 15 weeks for home-compostable wipes in the beach mesocosm study (Metcalf et al., 2024). This persistence raises questions about the real-world biodegradability of these materials, especially given that industry standard often relies on laboratory or industrial composting tests (Zambrano et al., 2020b; Liao and Chen, 2021). Moreover, high TSL values

may partly reflect mechanical fibre loss via hydrodynamics or sediment abrasion, rather than complete microbial breakdown (Kwon et al., 2022; Kargar and Joksimovic, 2024). This highlights the potential for cellulose-based wipes to act as a source of vast, albeit short-term, microfibre pollution in urban freshwater systems (Allison et al., 2025), reinforcing the need for more robust and environmentally representative biodegradability assessments.

5.4.2 Environmental, temporal, and hydrodynamic drivers of degradation

Degradation of cellulose-based wipes in urban rivers was also shaped by a combination of environmental conditions, hydrodynamics, and exposure duration. AFDM, serving as a proxy for microbial biomass, showed the strongest and most consistent positive association with degradation, especially for Brand A and controls. These findings align with well-established ecological evidence that microbial abundance and diversity are key to successful cellulose decomposition in freshwater systems (Burdon et al., 2020; Polman et al., 2021; Hayer et al., 2022). Unlike the earlier mesocosm study, in which low nutrient levels likely constrained microbial growth and where AFDM estimates were confounded by greater algal interference (Winterbourn et al., 1992; Ledger and Hildrew, 2008), the urban sites here showed higher AFDM levels and stronger TSL correlations. This reinforces the value of field-based testing and supports TSL as a reliable proxy for in-situ freshwater biodegradation (Tiegs et al., 2013; Colas et al., 2019; Hill et al., 2022). However, it should be noted that AFDM provides a general estimate of organic biomass and does not differentiate between microbial groups (e.g. bacteria, algal, fungi). As such, the observed associations with degradation reflect an indirect relationship – capturing total biofilm presence rather than the specific microbial agents responsible for cellulose breakdown.

TDS was also an important predictor, though its effects were non-linear and negatively curved. Degradation decreased at high TDS levels, particularly for Brand A and cotton controls, which contrasts with previous findings for cotton strips (Colas et al., 2019). This indicates possibly inhibitory thresholds for microbial growth and biodegradation under nutrient rich and poor conditions. While moderate TDS may support microbial activity by providing dissolved nutrients for energy and stable ionic conditions, high TDS values – typical of heavily urbanised and polluted waters – may reflect environmental stressors that inhibit microbial growth,

community structure, or disrupt enzymatic activity (Woodward et al., 2012; Griffiths and Tiegs, 2016).

Temperature emerged as a significant positive driver of Brand A's raw TSL in Spearman's correlation and regression models excluding time. This was consistent with findings from the earlier mesocosm experiment (Allison et al., in prep; Chapter 4) and broader literature showing that elevated temperatures enhance microbial metabolism and cellulolytic enzyme activity, thereby accelerating cellulose breakdown (Griffiths and Tiegs, 2016; Zambrano et al., 2019; Zambrano et al., 2020b; Nagamine et al., 2022). However, when exposure time was included as a covariate, Brand A temperature effects reversed – showing negative associations with both TSL metrics. This apparent contradiction suggests a masking effect, whereby the strong negative correlation between temperature and time ($r = -0.92$) suggests that temporal degradation trends – such as early physical breakdown – absorbed explanatory power that would otherwise be attributed to temperature. Linear temporal regressions confirmed that cumulative exposure duration alone was a strong predictor of Brand A degradation (see Fig. 5.10), highlighting that time-dependent processes played a more dominant role in driving wet wipe breakdown than temperature variation alone.

Hydrodynamic conditions were also investigated as potential drivers of degradation. While site-level mean flow rates varied by over three orders of magnitude – from small residential streams (< 0.2 m^3/s) to high-volume river channels (up to ~22.5 m^3/s in the Taff) – no significant associations were observed between flow rate and degradation in the regression models. This suggests that static, site-level flow metrics may be less predictive of degradation than dynamic hydrological fluctuations. By contrast, river level – used as a regional proxy for daily discharge variation – showed a strong positive relationship with Brand A degradation, even after accounting for exposure time. This implies that temporal shifts in discharge, such as those triggered by rainfall events, may facilitate degradation by increasing mechanical abrasion, turbulence, or hydrodynamic stress – processes known to physically weaken cellulosic materials or increase microfibre release (Kwon et al., 2022; Kargar and Joksimovic, 2024).

It should also be emphasised that flow rates were derived from scaled, static means per site and did not reflect temporal variability or peak flow events. This simplification may have limited the ability of models to detect hydrological influences on degradation across sites or over time.

In contrast, river level was derived from a continuous time series and better captured short-term discharge dynamics. These findings suggest that episodic increases in flow (e.g. during storm events) may be more important for driving wet wipe degradation in urban rivers than long-term average flows. This is unlikely to result from wet-dry cycling, as samples remained fully submerged throughout the study, but more plausibly reflects enhanced sheer stress during discharge peaks.

Temporal patterns of degradation differed by material type and may have reflected their underlying composition and structural properties. For Brand A wipes, TSL rates declined rapidly within the first three weeks before plateauing - a trajectory mirrored in the earlier mesocosm experiment for raw TSL in both wipes (Allison et al., in prep/Chapter 4). This two-phase pattern reflects an initial phase of physical disintegration followed by slower biological breakdown. SEM imagery supported this: by Week 3, Brand A fibres at both Lower Nant and Upper Glandulais exhibited surface erosion, fraying, and partial fibre detachment. These structural changes likely reflect hydrodynamic abrasion and fibre shedding in the early stages of aquatic incubation, particularly of natural cellulose components (Kwon et al., 2022; Kargar and Joksimovic, 2024). In parallel, the presence of thin filamentous matrices interwoven with fibres and debris at both sites could suggest early-stage biofilm colonisation. By Week 5, Brand A wipes were heavily embedded within mineral-rich organic matter, which may have both obscured visible fibre decay and impeded further microbial access – possibly explaining the observed TSL plateau.

In contrast, Brand B exhibited slower, more linear TSL loss throughout the study period, with minimal change in degradation rate over time. SEM images revealed smoother, cylindrical fibres with more limited signs of erosion or fraying, consistent with its higher proportion of more resistant regenerated cellulose (Tipper, 2016). While faint pitting and scarring was observed at Week 3, typically indicative of microbial activity (Nagamine et al., 2022), the fibres remained structurally intact through Week 5. Web-like coatings – possibly indicative of early microbial colonisation – were also visible at Lower Nant in Week 3 samples, but it did not appear to progress in tandem with the temporal degradation patterns. By Week 5, extensive sediment accumulation was observed on all Brand B samples, particularly at Lower Nant, with fibres tightly encrusted in mineral and organic debris. This coating may have further hindered microbial access or obscured any subtle surface-level degradation and could help explain Brand B's low sensitivity to both environmental and temporal predictors. Its resistance to degradation

likely reflects a combination of regenerated cellulose structure, debris entrapment, and possibly stronger or more persistent additive barriers.

Cotton controls, by comparison, exhibited a more gradual but steady increase in TSL across the study period. Their woven structure and higher physical integrity meant that debris entrapment was limited, and was likely buffered against early physical disintegration, allowing microbial and environmental degradation processes to act more gradually. This is consistent with their known performance in standardised bioassays (Tiegs et al., 2013; Colas et al., 2019; Hill et al., 2022) and supports the role of cotton as a valid ecological comparator in TSL-based biodegradation studies.

5.4.3 Limitations

While this study provides important baseline insights into the degradation of cellulose-based wipes under real-world urban river conditions, several limitations must be acknowledged.

TSL served as a practical and ecologically relevant proxy for studying cellulose degradation (Colas et al., 2019; Jabiol et al., 2020; Hill et al., 2022) but it cannot distinguish between physical disintegration and chemical (including microbial) breakdown. While SEM and EDS analyses helped identify surface changes and organic and inorganic attachments, they did not clearly reveal microbial or enzymatic activity patterns over time. More targeted assessments – such as microbial respiration assays or biological oxygen demand tests – could better isolate biodegradation specifically (Tiegs et al., 2013; Zambrano et al., 2020b; Nagamine et al., 2022), although these methods are often difficult to implement practicably and reliably in field settings (Mulholland et al., 2001; Jouanneau et al., 2014; Irving et al., 2024). Likewise, cellulose degrading microbial communities were not directly characterised. Although AFDM provided a coarse proxy for biofilm biomass over the study period, methods such as eDNA analysis or metagenomic sequencing would yield clearer insight into the composition and function of cellulolytic taxa in urban river settings.

Chemical additives and their leaching were also not measured. Antimicrobial preservatives in biodegradable wipes, such as benzoic acid, phenoxyethanol, and sodium benzoate, are commonly used to extend wipe shelf life, but may also inhibit microbial colonisation post-

disposal (Malis et al., 2019). Additionally, cellulosic wipes may also include chemical binders to improve the wet strength of cellulosic wipes (Tipper, 2016), but there is limited knowledge on the degradation performance of such binders in dynamic wastewater and freshwater systems. Therefore, the environmental persistence of such additives could have influenced degradation dynamics in this study.

Hydrodynamic influences were also subject to methodological constraints. Site-level flow rates were estimated as static mean values and did not capture temporal variability or discharge peaks. This simplification may have obscured potential relationships between flow dynamics and degradation. In contrast, river level data – available as continuous time series – were better suited to capturing short-term hydrological fluctuations but were only used as regional proxies and not directly measured at all sites.

Another key limitation was the heavy accumulation of organic and inorganic debris on wipe surfaces, despite the use of fine mesh bags. These encrustations may have hindered microbial or hydraulic access, limited degradation, particularly at later stages of exposure. Simultaneously, these findings also reflect the complexities of natural systems and may provide a key reason for the persistence of these cellulosic materials in flowing freshwater systems. Finally, although fine mesh bags were used to exclude macroinvertebrates, full-sized wipes, but not strips, at the River Ely site showed signs of interference from *Gammarus pulex*. While outside the scope of the study, this underscores the important role of shredders in cellulosic material degradation in freshwater ecosystems (Tiegs et al., 2007), and raises questions about the ingestion and ecotoxicology risks as well as dietary preferences of aquatic invertebrates towards these wipes (see Courtene-Jones et al., 2024).

5.4.4 Implications for freshwater environments

The persistence and fibre shedding of cellulose-based biodegradable wet wipes in urban rivers raise important environmental concerns. Despite being marketed as eco-friendly, these materials do not fully degrade under real-world aquatic conditions within short timeframes. Both field observations and SEM evidence confirmed that structural remnants of wet wipes remained after five weeks, with degradation strongly moderated by material type, environmental and hydrodynamic exposure, and surface fouling. These findings challenge

prevailing assumptions behind current biodegradability claims and reinforce calls for more ecologically representative testing standards (Liao and Chen, 2021; Allison et al., 2023).

A key implication of this study is the potential for these wipes to contribute to short-term microfibre pollution (see Chapter 3). The early-stage fraying fibre detachment observed in natural cellulose fibres suggests that hydrological variation – as reflected in the river level findings – may have facilitated rapid microfibre release, aligning with prior studies (Kwon et al., 2022; Kargar and Joksimovic, 2024; Allison et al., 2025). While these fibres may ultimately degrade, their temporary but vast presence in freshwater systems could pose ingestion and toxicity risks for aquatic organisms (Lusher et al., 2013; Courtene-Jones et al., 2024). The latter is especially true if fibres can also act as vectors for other pollutant and pathogen transport (Caruso, 2019; Metcalf et al., 2024), with additional potential exposure risks to human health. Additionally, the persistence of such materials retaining antimicrobial preservatives may have subtle but cumulative effects on aquatic microbial diversity, or may perhaps enhance antimicrobial resistances, particularly in systems already polluted by wastewater discharges (Reddy et al., 2022).

These findings hold direct relevance for wastewater management and regulatory policy. Flushed wipes – especially those that evade wastewater treatment via misconnections or sewer overflows – may persist in rivers longer than anticipated, undermining the environmental credibility of "biodegradable" claims. Product labelling, eco-label claims, and legislative definitions for these products must more accurately reflect the conditions in which wipes are likely to degrade. Life-cycle assessments of plastic alternatives should incorporate in-situ degradation evidence, not just composting or wastewater-based tests, to ensure more sustainable product transitions. Finally, considerations for possible breakdown products, such as microfibres, and their environmental fate should be accounted for in degradation standards.

5.5 Conclusion

Building on prior mesocosm-based research, this chapter presents the first in-situ assessment of biodegradable wet wipe degradation in urban rivers and streams. In these systems – where wipes are most likely to accumulate – degradation was strongly shaped by wipe composition, environmental conditions, and exposure time. Natural cellulose-rich wipes degraded more rapidly than regenerated cellulose-dominant wipes and cotton bioassays, likely because of

greater natural microfibre shedding and microbial biodegradation. While microbial biomass and total dissolved solids emerged as key environmental drivers, exposure duration explained the most variation in degradation across materials, reflecting physical degradation behaviour in response to hydrodynamic conditions. Despite this, substantial wipe fragments and sediment-laden fibres persisted after five weeks, raising concerns about the environmental relevance of current biodegradability claims.

These findings carry important implications for ecological risk, product regulation, and consumer disposal behaviour. They reinforce the need for biodegradability standards and labelling criteria that reflect real-world freshwater conditions, including the role of hydrodynamics, debris accumulation, and microbial community dynamics. Future research should prioritise longer-term studies, detailed microbial characterisation, and the environmental fate of released fibres and additives, to fully evaluate the ecological impact of these so-called biodegradable alternatives.

Chapter 6

Conclusions: Recommendations and Future Research

6 Conclusions: Recommendations and Future Research

6.1 Overview

The growing shift towards plastic-free alternatives, such as cellulosic wet wipes, has been driven by widespread concerns over plastic pollution and its impacts on aquatic ecosystems. However, significant uncertainties remain regarding the environmental behaviour, degradation, and ecological risks of these emerging wet wipe materials, particularly in freshwater environments where they often accumulate. Against this backdrop, the overarching aim of this thesis was to critically evaluate the full life cycle of commercially available cellulosic wet wipes – representative examples of plastic-free alternatives – to understand their environmental fate and assess the ecological risks they may pose to freshwater systems. Across the six research chapters, this thesis employed an interdisciplinary approach utilising life-cycle thinking, emissions-based mathematical modelling, and empirical mesocosm and in-situ degradation experiments in rivers. By integrating these different analyses, this research provides the first holistic assessment of cellulosic wet wipes as an emerging class of freshwater pollutants, offering vital insights into whether plastic-free alternatives genuinely represent environmentally safer choices.

Collectively, the research presented in this thesis produced several novel findings that advance understanding of plastic-free wet wipe alternatives within the context of freshwater pollution. It synthesised the current knowledge on cellulosic polymers, their usage in wet wipes and their flushed disposal, to evaluate the key physicochemical properties and environmental conditions most likely to influence their overall degradation and environmental fate in freshwaters (Chapter 2). It provided the first comprehensive quantification of macro- and microfibre emissions of flushed wet wipes to rivers, including the likely pathways such emissions are transported through (Chapter 3). The underestimated environmental persistence of cellulosic ‘biodegradable’ wet wipes was also empirically identified under experimental (Chapter 4) and realistic (Chapter 5) river conditions, alongside the key environmental drivers influencing their degradation rates. Importantly, the thesis challenges the assumption that bio-based alternatives inherently offer lower environmental risks, highlighting the need for a full life cycle and system-based approach when evaluating emerging materials intended to replace plastics.

Chapter 2 critically examined whether flushed biodegradable wet wipes can truly degrade under real-world environmental conditions, through a theoretical analysis of their full life cycle. It highlighted that, although cellulosic wet wipes are marketed with eco-labels such as biodegradable and flushable, they often do not completely degrade, and current testing standards do not tend to reflect realistic disposal conditions. Moreover, at the time of the research, such wipes still often contained a mixture of cellulose fibres and synthetic plastic fibres, alongside a range of property enhancing chemical additives including strengthening and antimicrobial agents that could consequently inhibit their degradation or leach out with ecotoxicological risks when disposed in real aquatic systems. Even for solely cellulose-based wipes, physical fragmentation was identified as the most likely breakdown mechanism in wastewater and freshwater environments, while true molecular degradation, via microbial colonisation and enzymatic activity, appeared slow and incomplete outside controlled conditions.

The chapter concluded that most flushed biodegradable wipes will likely not degrade fully in the environment within a reasonable time period, instead persisting as microfibre pollutants, in which they create risks to aquatic life synonymous with plastic counterparts. By adopting a life-cycle perspective from manufacturing to disposal, this chapter critically challenged assumptions of environmental safety around plastic-free alternatives and identified key knowledge gaps – particularly the need for a quantification of these pollutants flushed into these aquatic systems and their possible transport pathways, in-situ degradation studies, and investigation of the key environmental drivers influencing their physical and chemical breakdown under real aquatic conditions. The thesis then starts to address these in subsequent chapters.

Chapter 3 developed the first comprehensive emission-based model specifically quantifying wet wipe emissions into rivers, addressing a key gap in understanding the environmental fate of these emerging pollutants. By integrating flushed disposal rates, experimental microfibre shedding data in wastewaters from Kwon et al. (2022), likely wastewater system transport pathways from the surrounding microplastic literature, and scaling emissions from local to European scales, the model revealed that wastewater treatment plants (WWTPs), combined sewer overflows (CSOs), and agricultural runoff are critical routes for both solid wet wipe and microfibre pollution. Notably, while WWTPs removed much of the solid material, substantial microfibre emissions were still predicted to escape through effluents and land-applied sludge.

Agricultural runoff emerged as a dominant, yet under-recognised, diffuse source of microfibre emissions to rivers. Novel geo-spatial delineation of sewer overflow drainage areas enhanced the model's precision, offering a transferable framework for other emerging micropollutants. Critically, comparison against laundry-derived microfibre pollution highlighted that while laundry fibres dominate overall microfibre loads, wet wipes pose an underappreciated additional source, particularly for intact macro-sized pollution. Together, these findings provide new, actionable insights into the scale, pathways, and mitigation priorities for wet wipe-derived pollution across freshwater systems, directly addressing a major knowledge gap identified in earlier Chapters 1 and 2.

Chapter 4 provided the first analysis of the degradation behaviour of two commercially available cellulosic 'biodegradable' wet wipe brands in near-realistic mesocosm river environments, offering a major advance over previous laboratory-based or theoretical studies. By combining tensile strength loss (TSL) metrics and environmental monitoring, the study showed that although cellulosic wet wipes degraded faster than cotton bioassays used as ecologically relevant controls (Tiegs et al., 2013), both wipe brands persisted for several weeks under freshwater conditions, challenging assumptions about their rapid environmental breakdown. Notably, the study found that early-stage degradation was dominated by physical fibre fragmentation rather than microbial activity, particularly for natural cellulose-rich wipes, reinforcing risks of microfibre pollution. Degradation rates were strongly influenced by environmental factors such as pH, total dissolved solids (TDS), temperature, and exposure time. However, they were largely unaffected by microhabitat treatments (hyporheic, submerged, surface), suggesting broader environmental and temporal drivers outweighed local hydrological differences. This chapter importantly demonstrated that biodegradability claims based on controlled standards do not necessarily translate into real-world freshwater environments, providing critical new empirical evidence to inform regulatory testing frameworks and product design improvements. It also validated temperature-adjusted TSL as a reliable field-based metric for tracking degradation across heterogeneous freshwater contexts.

Chapter 5 expanded the mesocosm-based findings in Chapter 4 by providing the first in-situ multi-site assessment of biodegradable wet wipe degradation within dynamic urban river environments. Again, using TSL as a proxy for material degradation and cotton strips as ecological controls, it was found that river degradation was strongly material-specific, with natural cellulose-rich wipes degrading significantly faster than regenerated cellulose-

dominated products. In contrast to mesocosm results, degradation rates were higher across urban riverine sites, highlighting the importance of natural environmental variability – including microbial activity, hydrodynamics, and sediment debris interactions – in accelerating wipe breakdown. Environmental factors such as microbial biomass (measured as ash-free dry mass) and total dissolved solids (TDS) emerged as important drivers of degradation, while exposure duration exerted a dominant cumulative effect. SEM imaging reinforced the findings in this study by revealing greater surface degradation features – such as surface erosion, pitting and fibre detachment – in the predominantly natural cellulose wipes than regenerated cellulose samples. Microbial-induced degradation was also inferred by the presence of web-like biofilm structures on both wipe brands. Although some material breakdown occurred, wipe fragments remained present after five weeks at most sites. These results underscore that even biodegradable-labelled wipes can persist in freshwater environments, particularly those made with regenerated cellulose, and that real-world degradation is shaped by complex, site-specific factors.

6.2 Research limitations and caveats

While this thesis provides a novel, comprehensive assessment of cellulosic wet wipes as underestimated freshwater pollutants, several overarching limitations must be acknowledged.

Firstly, and as identified in the critical analysis undertaken in Chapter 2, existing literature on the different life stages of wet wipes (plastic and non-plastic) are limited. This meant that analyses and discussion throughout this thesis relied on fundamental theory, limited data sources and conservative assumptions. This was particularly the case for the emissions modelling in Chapter 3, which relied strongly on assumptions regarding disposal practices, sewer system configurations, fibre generation rates, and treatment plant filtration efficiencies, drawing on heterogenous secondary data sources. Although sensitivity analyses involving best-to-worst case scenarios and catchment-level applications helped constrain uncertainties, actual emission magnitudes and pathways may differ in systems with different demographic infrastructural, or climatic characteristics. Furthermore, direct empirical validation of wet wipe emissions into freshwater systems remains limited, reflecting a broader challenge in emerging pollutant modelling. Nonetheless, the model provided baseline flushed wet wipe emission

inputs to rivers, as well as a flexible and transferable framework that can be adapted and refined as better empirical data becomes available.

Secondly, the environmental nature of the experimental degradation studies, specifically the use of stream-fed mesocosm flumes (Chapter 4) and urban river field sites (Chapter 5), meant that findings were inevitably influenced by spatio-temporal variability characteristic of dynamic freshwater systems. In the mesocosm experiments, although upland stream water and natural sediment inputs were used to simulate realistic conditions, flow regimes were partially controlled and larger biota (e.g., macroinvertebrates, fish) were deliberately excluded using fine mesh bags. Similarly, the urban field experiments excluded larger aquatic organisms to help standardise degradation assessments across multiple sites, however, natural flow regimes were introduced this time to better capture flowing water interactions on the materials. Consequently, biophysical processes of degradation, derived from biotic mechanical fragmentation or ingestion, were not captured.

Although this design choice allowed for clearer attribution of degradation patterns to environmental factors such as temperature, biofilm biomass, and water chemistry, it also means that natural ecological interactions may have accelerated degradation in fully open freshwater systems. Additionally, natural organic and inorganic debris transport within freshwaters resulted in their attachment to wet wipe samples in both studies, potentially hindering their overall degradation and masking environmental and biological (e.g. microbial activity) effects of degradation. Therefore, degradation findings should be most appropriately interpreted as conservative estimates of degradation under typical environmental conditions, rather than maximum potential degradation rates.

While strong statistical associations were identified between environmental drivers and degradation rates in Chapters 4 and 5, experimental manipulation of individual drivers was beyond the study's scope. Consequently, definitive cause-effect relationships cannot be confirmed. However, the consistency of patterns across mesocosm and field contexts, combined with supporting mechanistic understanding from prior research (see Chapter 2), lends strength to the inferences drawn. Integrative studies of this type, linking multiple lines of evidence, remain rare for emerging plastic-free pollutants and are essential for informing future monitoring, policy, and management initiatives.

Finally, while this thesis adopted a life cycle assessment approach to help understand the environmental fate of cellulosic wet wipes, particularly their degradation and emission potential in freshwater environments, it did not assess broader life cycle sustainability indicators such as greenhouse gas emissions, eutrophication potential, or land and water use impacts during production. These environmental trade-offs are also critical to evaluating whether plastic-free alternatives are genuinely more sustainable than plastic-based counterparts. Recent work by Zhang et al. (2021), assessing multiple wet wipe types in China using full life cycle impact categories, demonstrates the value of integrating such metrics into comparative assessments. Incorporating these dimensions into future work would complement the fate-focused perspective taken in this thesis and help guide more balanced decisions about the sustainability of biodegradable product designs and associated regulations.

6.3 Recommendations

Flushed cellulosic wet wipes represent a growing and under-recognised pollutant in freshwater systems. Addressing this issue requires upstream interventions across the supply chain – from product design to policy and consumer behaviour (Fig. 6.1). The following solutions draw directly on findings from this thesis to identify and discuss key leverage points across the wet wipe lifecycle.

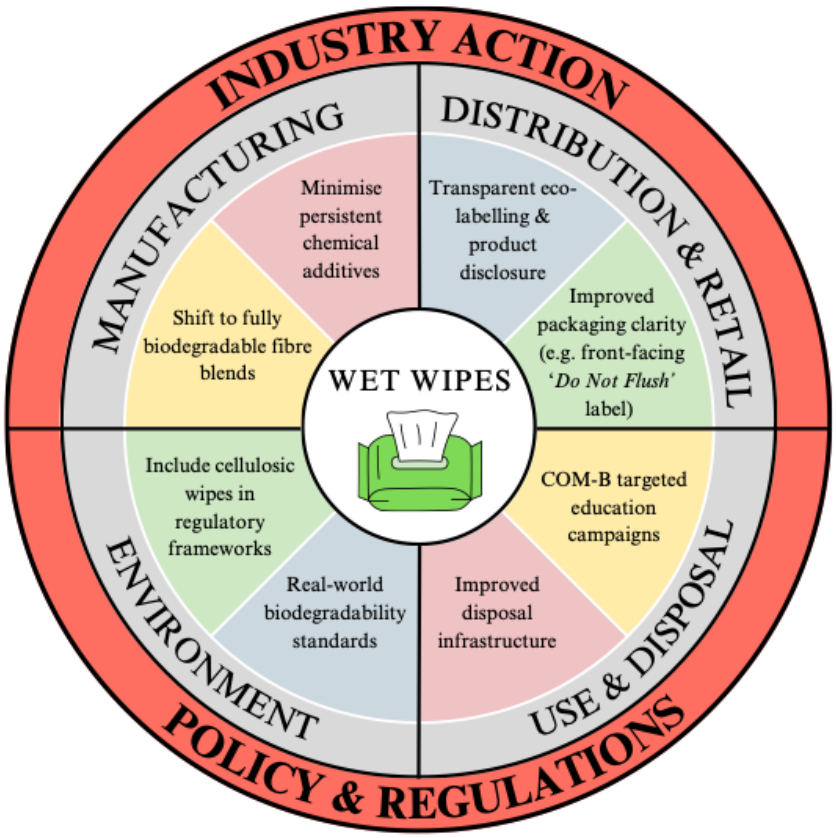

Fig. 6.1. Conceptual diagram illustrating key recommendations (Section 6.3) for targeted interventions across the life cycle of cellulosic wet wipes.

6.3.1 Manufacturing

Across this thesis - from theoretical, life-cycle-based analysis (Chapter 2) to emissions modelling (Chapter 3) and degradation studies (Chapters 4 and 5) – fibre composition emerged as a key determinant of environmental persistence. Wipes made primarily from natural cellulose (Brand A) degraded more quickly but shed more microfibres, whereas predominantly regenerated cellulose-based wipes (Brand B) were more resistant to breakdown. These findings reflect differences in underlying physicochemical properties, such as fibre crystallinity, surface area, size, and bonding techniques, all influencing the overall degradability performance of wipes in freshwater systems. Additionally, the presence of organic and inorganic debris in wastewaters and rivers can promote biofouling, further hindering degradation, as observed in Chapters 4 and 5.

To mitigate these impacts, manufacturers would need to move beyond vague 'biodegradable' claims and commit to full transparency in product composition, including fibre types and additives. Product formulations would need to prioritise truly biodegradable, low-toxicity materials while minimising the use of persistent additives such as wet strengthening agents, antimicrobial preservatives, and surface coatings, particularly those that can impair biodegradation after consumer disposal, and therefore contribute to blockages, and pose environmental risks through chemical leaching. Crucially, wipes and their by-products would need to be designed to fully disperse and degrade at the molecular level shortly after disposal.

6.3.2 Policymakers

The thesis highlights critical regulatory gaps in how biodegradable and flushable wipes, especially those made from cellulosic fibres, are classified, labelled, and governed. Although legislation such as the UK's recent 2023 Single-Use Plastics Ban and the EU's 2019 Single-Use Plastics Directive represent important steps toward addressing plastic wet wipe pollution, they currently exclude cellulosic wipes despite current evidence that such products can persist and contribute to microfibre pollution (Chapters 3 - 5). As a result, such products remain outside the scope of extended producer responsibility (EPR) schemes, bans, and performance-based labelling requirements, creating a policy loophole.

Labelling regulation remains fragmented and inconsistently enforced. For instance, the EU now mandates front-facing plastic content warnings on plastic-containing wipes, and California's 2021 AB 818 legislation requires "Do Not Flush" labels on all wipes in the state, alongside mandatory education campaigns by the manufacturers. These labels have also been introduced on a voluntary basis by some environmentally conscious non-woven manufacturers in Europe and the UK, following the now outdated labelling requirements of EDANA's 'flushability' guidelines and Code of Practice (Drinkwater and Moy, 2017). Such labelling on wet wipes has been shown to improve awareness but often fails to change disposal behaviour unless paired with meaningful public engagement (European Commission, 2020). Additionally, these labels are often not front-facing; they appear on the back of packaging among the small print and are overshadowed by the front-facing biodegradable or bio-based eco-labels, which may be incorrectly associated with safe toilet disposal. For cellulosic wipes, this suggests that clearer, universal, legal definitions of biodegradability and bio-based are urgently needed, and their

product packaging labels and explanations would need to be more clearly communicated to consumers to avoid continued incorrect flushing disposal.

Certification schemes would also need to evolve. Chapter 5 showed that certified biodegradable wipes persisted for weeks in real urban river environments, revealing a disconnect between lab-based degradation timeframes and real-world outcomes. Future biodegradability standards need to include performance-based testing under freshwater conditions, with defined quicker timeframes and residue limits. Prior voluntary schemes, such as Water UK's now-withdrawn 'Fine to Flush' certification, failed due to consumer confusion and continued sewer and river contamination (Joksimovic et al., 2020; Water Research Centre, 2024). To avoid repeating such mistakes, biodegradability frameworks need to include dynamic, real-world validation, and account for full life cycle impacts – including additives and micropollutant by-products such as fibres – and support clearer communication to consumers.

6.3.3 Consumer behaviour

Despite growing awareness of wipe pollution stemming from toilet flushing, this incorrect disposal practice remains commonplace due to convenience, habit, lack of clarity in labelling, and widespread misconceptions about the environmental safety of 'plastic-free' products. Chapter 5 confirmed that wipes persist in urban rivers, demonstrating how everyday disposal decisions have tangible and lasting environmental consequences.

Addressing this issue requires behaviour change strategies that go beyond generic awareness campaigns. Studies such as Allison et al. (2024b) emphasise the value of applying behavioural science frameworks such as COM-B model and Behaviour Change Wheel to understand the psychological, social, and situational drivers of disposal habits. They suggest that interventions that target moments of routine disruption, such as new parenthood, illness, or product switching may be particularly effective in forming new, more sustainable habits. Furthermore, disposal behaviour is also strongly shaped by socio-cultural norms and the material environment (Alda-Vidal et al., 2020). For instance, wet wipes stored next to toilets without adjacent bins can promote habitual flushing.

Confusion around the meaning of labels such as ‘biodegradable’, ‘bio-based’, and ‘flushable’ has been identified as a key barrier to proper disposal. Consumers often misinterpret these terms or overlook disposal guidance (European Commission, 2020), especially when environmentally friendly claims are more prominently displayed than disposal instructions. Even when ‘do not flush’ labels are included, their impact depends on visibility and public understanding. National education campaigns and coordinated public messaging therefore need to accompany any regulatory reforms to correct such misunderstandings and reinforce appropriate bin disposal practices – particularly for products marketed as environmentally friendly.

Empowering consumers with accurate, consistent, and accessible information, while embedding supportive infrastructure and behavioural cues, is essential to reducing all forms of wet wipe pollution at its source.

6.3.4 Industrially compostable wipes

One possible multi-faceted intervention could be transitioning towards industrially compostable wipes, whereby suitable wipes would be disposed of with food waste and taken to controlled composting facilities to biodegrade into compost, typically using anaerobic digestion (Purkiss et al., 2022). This disposal route could ensure predictable and efficient biological decomposition, minimising widespread environmental contamination. However, this assumes that manufacturing efforts are made to remove any harmful properties and byproducts, minimise environmental impacts associated with manufacturing (consistent with LCAs), and that wipes are 100 % bio-based with shown high and fast biodegradability for all constituents (including additives), particularly within food waste composting environments.

Due to past consumer confusion associated with biodegradable and flushable labels – particularly for wipes disposed via the toilet rather than solid waste routes - any transition would also require national public education campaigns to ensure labelling is correctly interpreted and disposal guidance is followed (Allison et al., 2024a). Furthermore, widespread consumer transition to these alternatives may reinforce the idea of routine disruption, which as mentioned earlier, may be more effective for long-term sustainable habits (Allison et al.,

2024b). This may require in depth marketing analyses to identify the predominant types of consumers contributing to the current biodegradable wipe market to ensure appropriate uptake.

Transitions to compostable wipes would also need to account for additional economic and resource costs associated with composting materials to make sure these do not create barriers and disincentives to such products, both for consumers and manufacturers. More importantly, this option is currently constrained by the inconsistent availability and accessibility of dedicated food waste collection systems and industrial composting infrastructure at scale, particularly in countries like the UK and the US, where few facilities are equipped to process compostable materials other than food waste (Ellen MacArthur Foundation, 2022; Purkiss et al., 2022; Razniewska, 2022). To support the viability of compostable alternatives, national policy would need to commit to scaling up composting infrastructure and expanding its capability beyond food waste alone, ensuring that emerging compostable products have a credible, low-impact end-of-life pathway.

6.4 Future research areas

Given the paucity of research on cellulosic wet wipes prior to this thesis, there remains considerable scope for further research.

First, there is a need for broader spatio-temporal assessments of cellulosic wet wipes across diverse hydrological and climatic contexts in which wet wipes typically end up. Both experimental studies in this thesis focused on upland and lowland sites in South and Mid Wales, UK, and while these provided valuable environmental realism, their generalisability to other geographical regions and seasonal conditions needs to be tested.

Secondly, the exclusion of macroinvertebrates and larger biota in the degradation experiments (Chapters 4 and 5) provided only part of the understanding of biotic interactions with these pollutants. While incidental observations of physical interactions between aquatic organisms and wet wipe materials were recorded in the field study (Chapter 5) – specifically the presence of *Gammarus pulex* in the mesh bags containing full-sized wipes alongside accelerated degradation - direct investigation of biotic interactions and ecotoxicological effects was beyond the scope of this thesis. As such, while the physical persistence and partial degradation of wipes

were well characterised, the biological consequences of wet wipe fibre ingestion or exposure, including potential sub-lethal or toxicological impacts, remain unknown. Given the emerging evidence linking microfibre ingestion from wipes to adverse effects in freshwater biota (McCoy et al., 2020), as well as broader cellulosic microfibre ecotoxicological impacts (Courtene-Jones et al., 2024), future research should prioritise experimental assessment of direct organismal responses to both intact wipes and their degradation products to fully characterise ecological risks.

Third, the chemical composition of commercial wipes - particularly the identity, function, and environmental fate of property-enhancing additives - remains poorly understood. Chapter 2 highlighted the wide variability and opaqueness of chemical additive formulations in wet wipes, yet their potential to impair degradation or contribute to ecotoxicity in freshwater systems remains poorly understood. This knowledge gap largely stems from limited manufacturer disclosure, which makes it difficult to identify and test for specific compounds post-disposal. One study assessed preservative content in plastic and non-plastic wipe products and estimated human exposure risks (Pack et al., 2021), but did not examine environmental leaching or aquatic effects. As such, analytical identification and untargeted leachate toxicity testing are essential to characterise real-world wipe additive behaviour following environmental disposal and to better assess their ecological risks.

Fourth, while this thesis adopted a LCA lens to investigate material properties, emissions, degradation, and likely freshwater risks, it did not evaluate other sustainability indicators such as greenhouse gas emissions, eutrophication, deforestation, or water use. Recent comparative LCA studies suggest that bio-based alternatives, including wet wipes may also carry trade-offs, especially during manufacturing, that require careful consideration when assessing plastic alternatives (Echeverria, 2021; Zhang et al., 2021b). Expanding environmental assessments to include these indicators is essential to ensure plastic-free alternatives are genuinely sustainable across their full life cycle.

Finally, while touched on in this chapter, future work should explore how socio-technical systems, including infrastructure, policy implementation, and consumer practices, mediate the environmental fate of alternative wipes. As highlighted in this thesis, disposal pathways and systems responses are shaped not just by product properties, but by behavioural, infrastructural

and regulatory contexts. Interdisciplinary research integrating environmental science, policy, and behavioural sciences will be critical for guiding sustainable plastic-free alternatives.

6.5 Conclusions

This thesis provides the first comprehensive, life cycle-based assessment of cellulosic wet wipes as emerging freshwater pollutants. In response to the rising demand for plastic-free alternatives, it interrogates the environmental trade-offs of cellulosic products that are widely marketed as biodegradable yet remain poorly understood in terms of their environmental fate and persistence.

Through critical review, emissions modelling, and experimental degradation studies, this thesis demonstrates that cellulosic wet wipes, particularly those composed of natural and regenerated cellulose, can persist in freshwater systems and release large amounts of microfibres, despite claims of biodegradability. Findings from river mesocosms and urban field environments reveal that degradation is time and context-dependent, modulated by fibre type, and hindered by chemical additives and environmental debris, while the emissions modelling highlights their significant contribution to freshwater microfibre loads via wastewater pathways.

These findings call into question current industry and regulatory standards for flushability and biodegradability, revealing that such claims are often based on unrealistic test conditions and poorly communicated to consumers. The work underscores the need for transparent material disclosure, performance-based biodegradability testing, improved consumer labelling, and coordinated policy reform to close existing loopholes. By combining a life cycle lens with field-relevant ecological realism, this thesis advances understanding of the environmental performance of cellulosic wet wipes, identifies key risks and system-level gaps, and provides a foundation for targeted, evidence-based interventions across the product lifecycle. In doing so, it offers essential insights for guiding the development of genuinely sustainable plastic-free alternatives.

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