Reefs of rubbish: the ecological implications of anthropogenic litter in streams

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Abstract

Anthropogenic litter (solid manufactured waste) is a significant and increasing problem worldwide. However, despite emerging evidence of the prevalence of litter in rivers, most research has focused on the marine environment. Anthropogenic litter has been shown to have a variety of environmental impacts in aquatic systems, but its consequences for rivers are poorly understood. This thesis aimed to address this by investigating the characteristics and ecological impacts of litter in rivers.

I undertook field surveys and in situ experiments to determine how macroinvertebrates and fish are affected by riverine litter. First, in a survey of small and heavily managed UK rivers, I found that anthropogenic litter density across a range of small and heavily managed UK rivers ranged from 0 to 8.7 items m^{-2}. This density is comparable to that reported in other aquatic systems, confirming that rivers contain considerable quantities of litter and demonstrating the need for increased research into litter in rivers. Plastic was the most common litter material, but its dominance was less than has been found in marine systems or on river banks. Thus, excluding non-plastic materials, like glass and metal, from riverine litter research risks omitting a significant proportion of anthropogenic litter from investigation. This study also found a positive correlation between litter density and macroinvertebrate diversity across rivers. This surprising result suggested that anthropogenic litter could increase the diversity of available habitat, especially in managed rivers that are otherwise scarce in habitat diversity. This finding was supported by the results of a second investigation comparing the macroinvertebrate communities inhabiting litter and natural mineral substrates (rocks); the dominant natural substrate in the sampled rivers. It found that communities on litter were consistently more diverse than rock equivalents, reflecting the greater surface complexity of the litter. Results also suggested that small-scale differences in the physical properties of litter types and rocks cause the different substrates to support distinct macroinvertebrate communities. In particular, flexible plastic and fabric items were inhabited by macroinvertebrate taxa that would typically live on macrophytes, suggesting that these types of litter items can mimic the structure of plants.

Anthropogenic litter, especially large litter items, may affect more than just the organisms which inhabit its surface. By altering local habitat conditions it could impact biota in the surrounding river. This was investigated through experimentally installing car tyres into two rivers (one sand-bed and one gravel-bed river) and monitoring their effects on macroinvertebrates and fish. The macroinvertebrate communities inhabiting tyre surfaces in the sand-bed river were significantly more diverse and included more sensitive taxa than the surrounding river bed, whereas tyre surface communities were relatively impoverished in the gravel-bed river. In both rivers, tyres significantly affected macroinvertebrate communities in the surrounding river bed, which could be attributed to the influence of the tyres on local flow and sediment conditions, similar to the effects of natural structures like large wood and boulders. Some small fish (<15 cm long roach, chub, and dace) were also affected by tyres. They spent more time and fed more downstream of tyres than they did upstream, sheltering in the zone of slower flow velocity which may allow them to preserve energy. Other fish species and sizes were less frequently observed and did not seem to respond to the presence of tyres.

This research is the first to show that anthropogenic litter can provide and create habitat in rivers. It suggests that, whilst litter should not be deliberately added to rivers, removing it could have the side effect of reducing local biodiversity, both for macroinvertebrates and fish, unless the habitat it provides is replaced by alternative materials.

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Chapter 1: Introduction

Humans have fundamentally changed river ecosystems through physical modification, habitat fragmentation, pollution, introduction of non-native species, and by the alteration of catchment climate, hydrology, soils and biogeochemistry (Kowarik, 2011; United Nations World Water Assessment Programme [WWAP], 2015; Reid *et al.*, 2019). As a result, approximately 60% of European surface waters are failing to achieve good ecological status according to the Water Framework Directive (EU Directive: 2000/60/EC; Kristensen *et al.*, 2018). The combined effects of these stressors has led to freshwaters having some of the highest rates of extinction of any environment (Strayer and Dudgeon, 2010; Burkhead, 2012; Grooten and Almond, 2018); rates that are expected to increase due to continued urbanisation and climate change (Dudgeon *et al.*, 2006; Pimm *et al.*, 2014). Healthy rivers contribute vital habitat, supporting a considerable proportion of global biodiversity (Reid *et al.*, 2019). They also provide important ecosystem services for humans such as water supply and purification, benefits to health and wellbeing, recreation opportunities, and carbon storage (Postel and Carpenter, 1997; Dodds *et al.*, 2013). In the UK, freshwater ecosystem services have an estimated value of £39.5 billion per year (Office for National Statistics [ONS], 2017). Therefore, understanding the effects of anthropogenic activities on river environments is of critical importance to improve management practices for both people and wildlife.

One so far under-researched aspect of human influence on rivers is that of anthropogenic litter. Anthropogenic litter is defined as any solid manufactured waste item that ends up in the environment through improper disposal (McCormick and Hoellein, 2016). This includes items which are deliberately discarded into the environment, as well as those that are unintentionally lost. I exclude microplastics (i.e. plastic pieces of less than 5 mm; Bellasi *et al.*, 2020; Li *et al.*, 2020) and other small artificial particles from this definition, because their small size means that their sources, dynamics and impacts are distinct from anthropogenic litter (Blettler *et al.*, 2018; Windsor *et al.*, 2019).

Anthropogenic litter is a significant and increasing problem worldwide (Bergmann *et al.*, 2015; Häder *et al.*, 2020). For example, of the 200 million tonnes of plastic produced globally per annum, it is estimated that 10% ends up in aquatic habitats (Vannela, 2012). Volumes of waste generation (and therefore quantities of litter) are increasing; an estimated 2.0 billion tons of waste was generated globally in 2016, but this is predicted to increase to approximately 3.4 billion tons in 2050 (Kaza *et al.*, 2018). A recent YouGov survey of UK adults conducted by the RSPB *et al.* (2021) found that 35% of survey respondents thought that 'litter and plastic pollution' was the biggest threat facing the freshwater environment. However, the impacts of anthropogenic litter on river environments are poorly understood. According to a 2018 study, only 13% of all scientific publications on plastic pollution in aquatic environments studied freshwaters, and of these only 19% included macroplastics (as opposed to microplastics) (Blettler *et al.*, 2018). Even less is known about anthropogenic litter made from other materials, such as metal, glass, and fabric, as these materials are often excluded from surveys (Vriend *et al.*, 2020).

1.1. Thesis aims

The aim of this thesis is to investigate the characteristics and ecological impacts of anthropogenic litter in rivers. It will address this overarching topic through a number of individual, but connected, research projects with the following aims:

1) To improve current understanding of the amount and types of litter present in UK rivers, and to investigate whether there is a relationship between litter density and macroinvertebrate communities (Chapter 4).
2) To assess whether there are differences in the macroinvertebrate communities inhabiting anthropogenic litter and natural mineral substrates (i.e. rocks) (Chapter 5).
3) To measure the impacts of large anthropogenic litter items on local habitats, macroinvertebrates and fish (Chapters 6 and 7).

1.2. Justification of aims

Although high concentrations of anthropogenic litter have been reported in several rivers (e.g. McCormick and Hoellein, 2016; Bruge *et al.*, 2018; Kiessling *et al.*, 2019), most surveys of litter composition and density have been limited to marine environments. Of those that have studied rivers, many only considered litter on the banks of the river, rather than on the river bed where aquatic organisms are more likely to be exposed to it. Moreover, several studies only sample plastic litter, despite evidence that considerable amounts of other litter materials are present in rivers (Hoellein *et al.*, 2014; McCormick and Hoellein, 2016). As such, our understanding of the characteristics of anthropogenic litter within rivers is limited. In Chapter 4, I surveyed anthropogenic litter densities and characteristics in a number of different UK rivers to address this research gap (Aim 1).

Chapter 4 also considered whether there are correlations between the quantity of anthropogenic litter in the river bed and the macroinvertebrate communities that live there (Aim 1). There have been a number of published papers on the effects of anthropogenic litter on marine organisms, including the hazards of entanglement with, or ingestion of, litter, changes to habitat conditions, and its effects on the colonising community (Gregory, 2009; Bergmann *et al.*, 2015). However, very few papers have looked at the ecological impacts of litter in rivers.

Macroinvertebrates are known to colonise non-toxic, solid substrates in aquatic systems, including anthropogenic litter. Because of the distinct and unusual physical structure of litter, and since the distribution of macroinvertebrates is strongly linked to the characteristics of the substrate they colonise (Death, 2000), communities inhabiting anthropogenic litter may be different to those on natural substrates. Chapter 5 tested this hypothesis by comparing macroinvertebrate communities inhabiting anthropogenic litter with those on natural mineral substrates in three rivers with high litter concentrations (Aim 2).

Large anthropogenic litter (i.e. that which is considerably larger than the typical sediment size and therefore protrudes above the river bed) has the potential to drive changes in the surrounding river bed habitat by interacting with the flow of water and the distribution of sediment and organic matter. Chapters 6 and 7 investigated the effects of experimentally introduced car tyres on macroinvertebrates and fish respectively (Aim 3). Tyres were installed into two rivers (one sand-bed and one gravel-bed) and Chapter 6 measured the response in macroinvertebrate communities which inhabit the surrounding river bed over a twelve-month period. Chapter 7 looks at the effects of tyres on fish. It explored whether tyres influence fish distribution and behaviour, as is the case for natural structures.

1.3. Thesis structure

This thesis is made up of eight chapters. Chapter 1 provides an introduction to the topic and details the thesis aims. The literature on anthropogenic litter is reviewed in Chapters 2 and 3; Chapter 2 focuses on the sources and dynamics of litter in rivers, whereas Chapter 3 details research into the effects of litter on people and aquatic ecology. The research conducted for this thesis is presented in Chapters 4 to 7. Chapter

8 includes an overall discussion of the results from these chapters and explores the fulfilment of the aims.

Chapter 2: The sources and dynamics of anthropogenic litter in rivers

Most anthropogenic litter research so far has been conducted in marine environments. Rivers have traditionally been seen as conduits of litter, rather than sinks themselves (Hoellein and Rochman, 2021). However, for plastics at least, this is beginning to change. Several recent reviews on the dynamics of plastics in rivers have now been published (Schwarz *et al.*, 2019; van Emmerik and Schwarz, 2019; Windsor *et al.,* 2019; Liro *et al.*, 2020; Al-Zawaidah *et al.*, 2021; Hoellein and Rochman, 2021). This information is summarised in the following paragraphs alongside consideration of other anthropogenic litter materials.

2.1. The sources of anthropogenic litter

Anthropogenic litter can enter river environments in a number of different ways. At the end of its useful life, solid waste is typically managed via systems which collect and transport waste to a management facility for disposal (Hoellein and Rochman 2021). This waste is most often stored as landfill or incinerated (Williams, 2005), but some is shipped to lower income countries to prevent it needing to be disposed of in the country of origin (Brooks *et al.*, 2018). A large quantity of the waste that has been created therefore still exists; for example, of all the plastic waste generated worldwide (an estimated 6,300 million tonnes up to 2015), 79% of this is currently stored in landfills or exists as litter in the environment (Geyer *et al.*, 2017). Litter may be lost from the system during the process of disposal (Ryberg *et al.*, 2019), and in some places, systems to manage waste are either absent or rarely used (Guerrero *et al.*, 2013). Losses from managed landfills in high income countries like the UK are, however, generally small, although historic landfills or those vulnerable to flooding may slowly release litter into rivers and coasts through erosion (Brand *et al.*, 2018).

A more significant source of riverine litter is deliberate dumping, which is both insidious and difficult to trace as individuals tend to dump anthropogenic litter secretly in order to evade detection and possible fines (McCormick and Hoellein, 2016). The illegal disposal of large amounts of solid waste is known as fly-tipping. This is a significant problem in the UK with 1,002,000 instances recorded in England in 2016/17, costing councils £57.7 m to clean up (Department for Environment Food and

Rural Affairs [Defra], 2017). Rivers are common fly-tipping sites; Williams and Simmons (1999) found that 50% of the 50 sites they surveyed along the length of the River Taff and its tributaries in Wales were used for fly-tipping. Littering is the inappropriate disposal of smaller amounts of waste, either deliberately or accidentally (Schultz *et al.*, 2011). Unlike fly-tipped waste, this litter typically comes from multiple individual disposal occurrences which build up over time. Litter may be directly dumped into the river, or it could be carried into rivers via wind, floods, or surface water runoff (Mihai, 2018; van Emmerik and Schwarz, 2019).

Another significant source of riverine anthropogenic litter is through wastewater management systems. Surface water runoff transfers litter from streets into stormwater drains, which may drain directly into rivers (Armitage, 2007). In combined wastewater systems, as are typical in the UK, these drains are combined with sewage systems, which also receive litter (especially sanitary waste) that is flushed down drains (Williams and Simmons 1999). Wastewater treatment works collect litter via screens or settling tanks (Carr *et al.*, 2016). However, not all wastewater gets treated, as systems can become overwhelmed by high volumes of water introduced during heavy rainfall. On these occasions, untreated sewage and associated litter is released directly into rivers (Williams and Simmons 1999; Morritt *et al.* 2014; McConville *et al.*, 2020). Additionally, misconnected wastewater pipes may lead to sewage waste being directly discharged at any time (Ellis and Butler, 2015). Sewage related litter can make up a significant amount of the anthropogenic litter assemblage; more than 20% of litter was sewage related in the Rivers Taff (Wales) and Thames (London, UK) by Williams and Simmons (1999) and Morritt *et al.* (2014) respectively.

Spatial variation in these sources means anthropogenic litter distribution can be linked to nearby land use and human activity. For instance, Williams and Deakin (2007) found higher litter densities near public infrastructure like bridges, footpaths, schools and shopping centres. Areas used for recreation (beaches or parks) have been found to have high amounts of smoking and food related litter (Hoellein *et al.*, 2014). Fly-tipping, however, is strongly linked to road access; Williams and Simmons (1999) found that 60% of surveyed sites with vehicle access experienced fly-tipping, compared to only 28% of sites without vehicle access. McCormick and Hoellein (2016) also found that the number of parking spaces, distance to footpaths/roads, and number of people observed during the study was positively correlated with the amount of litter at a site. Also relevant is the fact that people are more likely to drop litter in sites that are already littered because these sites are seen as less valuable (Bator *et al.*, 2011).

2.2. Processes affecting anthropogenic litter in rivers

Once anthropogenic litter reaches a river, it is subject to a number of process that determine how it moves and whether it is retained or exported from the system.

2.2.1. Litter transport

In a similar manner to sediment transport, the movement of litter is controlled by hydrological factors (e.g. flow velocity and discharge) and, in the case of floating litter, by the wind (Browne *et al.*, 2010). Field investigations of litter have shown a positive correlation between flood events and litter movements (Williams and Simmons, 1997). High flows have greater ability to mobilise litter, and so high volumes of transported litter have been reported during periods of increased discharge (Castro-Jiménez *et al.*, 2019; van Emmerik *et al.*, 2019a). Floods can lead to the deposition of litter outside of the channel itself as flood water recedes (Williams and Simmons, 1997), as well as causing the recruitment of additional litter from banksides and through surface runoff (Moore *et al.*, 2011; Mihai, 2018; Schirinzi *et al.*, 2020; Roebroek *et al.*, 2021). However, contrasting evidence indicates that the relationship between litter presence and hydrology is complex; for example, McCormick and Hoellein (2016) found no relationship between discharge and litter accumulation rates in the North Branch of the Chicago River (Chicago, USA). Rivers may transport litter far downstream of their original source, which can make it difficult to identify where a specific item originated.

The physical properties of anthropogenic litter are also important (van Emmerik and Schwarz, 2019), but their significance in controlling litter transport is less well understood. Most of the research on this topic has focused on microplastics. Some researchers have argued that microplastic particles move in a similar manner to natural particles of the same size, density and shape, such as organic matter or mineral sediment (Nizzetto *et al.*, 2016; Kooi *et al.*, 2018; Hoellein *et al.*, 2019). These principles have been used to design transport models for microplastics (e.g. Besseling *et al.*, 2017; Hoellein *et al.*, 2019). However, the utility of applying existing models of natural particle transport to microplastics is disputed because artificial particles have such different physical properties to natural particles (e.g. plastic has much lower density) (Windsor *et al.*, 2019). The same issue applies to the movement of anthropogenic litter. One of the significant challenges is the huge range in size and shape of litter items (Kooi *et al.*, 2018). Because size and shape are important determinants of a particles' mobility (Church, 2006), differences in these properties will alter the behaviour of litter relative to natural particles (Windsor *et al.*, 2019). For instance, although litter is larger than typical grains in the suspended load (generally sand grains or finer; Walling and Moorehead, 1989), some items (e.g. plastic bottles) have very low density, especially if they contain air, and can therefore float and be transported downstream with relative ease (Ryan, 2015; Rech *et al.*, 2014). The issue is further complicated by the huge range in litter types and characteristics, making it difficult to fully quantify its effects.

There is, therefore, large variability in the mobility of different types of anthropogenic litter, which is strongly related to their material type and consequent density. These differences not only determine whether or not a piece of litter will move, but also how it will move, and where in the water column it will be carried (Al-Zawaidah *et al.*, 2021). For instance, Ryan *et al.* (2009) argued that the proportion of litter made up of plastic increases with distance from source due to the relative ease of transporting plastic, which is low in density, compared to glass or metal. However, although plastic bags and packaging are easily mobilised, their flexibility and shape means that they have a tendency to become caught in vegetation (Williams and Simmons, 1997). Their lower density also means they are more likely to be transported in suspension, and therefore have a greater chance of being deposited on the banks. As such, Hoellein *et al.*, (2014) found that plastic was significantly more abundant in the riparian zone of the Chicago River because it is easily stranded, whereas glass was significantly more common in river sediments due to its higher density. In fact, Weideman *et al.* (2020) found that plastic litter in the Vaal River (South Africa) often only travelled short distances from its original source before being buried, deposited on river banks, or being caught on vegetation. This supports the work of McCormick and Hoellein (2016) who measured the residence times of common anthropogenic litter types in the Chicago River (USA). They found that plastic food wrappers had the longest residence time (368 days), closely followed by glass bottles (330 days), whilst aluminium cans had the shortest residence time (197 days).

Large anthropogenic litter items such as shopping trolleys or car tyres, which are comparable in size to boulders, are likely to be much less mobile than cobbles, gravel or sand. Immobile obstacles like these may be important controls on the movement of other materials. For instance, Williams and Deakin (2007) found that shopping trolleys in the River Tawd (Skelmersdale, UK) acted like nets, thereby accumulating other litter, organic matter and sediment.

Anthropogenic litter transport has been investigated through quantification of floating or suspended debris using drones or visual observations (e.g. van Emmerik *et al.*, 2018; Geraeds *et al.*, 2019), as well as stationary samplers like nets or booms (e.g. Carson *et al.*, 2013; Morritt *et al.*, 2014; Haikonen *et al.*, 2018). Alternatively, repeat clearance surveys have been used to quantify litter accumulation rates either on banks or the river bed (e.g. Williams and Simmons, 1997; McCormick and Hoellein, 2016). All of these methods have disadvantages, meaning the measurement of litter transport in situ is challenging. Focusing on floating debris ignores the fact that significant volumes of submerged litter are in motion (Morritt *et al.*, 2014), and therefore probably biases assemblages towards plastic and other buoyant materials. Visual observations are labour intensive and rely on the operator's ability to detect litter items (Al-Zawaidah *et al.*, 2021). Net samplers are limited by the size of the net and its mesh size, are restricted to use under a small range of river flows, and require regular checks to ensure aquatic organisms do not become trapped (Al-Zawaidah *et al.*, 2021). Repeat clearance surveys are time-consuming, and can also bias accumulation rates themselves, especially as littering rates are affected by amount of litter already present (Bator *et al.* 2011), and as litter presence could itself encourage the deposition of other litter items from the water column (Williams and Deakin, 2007; McConville *et al.*, 2020). Additionally, several studies have warned that the frequency of resampling significantly affects the estimated accumulation rate (Smith and Markic, 2013; Ryan *et al.*, 2014; McCormick and Hoellein, 2016).

2.2.2. Storage of litter in rivers

Anthropogenic litter can be temporarily or permanently stored in rivers. Common temporary retention spots include vegetation, river margins, and infrastructure (e.g. bridges, dams or sluices) as these are sites of high flow resistance and decreased flow velocity (Hoellein and Rochman, 2021). River channels characterised by slow flows, low slope, and high densities of vegetation or urban infrastructure are more likely to store litter (Bruge *et al.*, 2018), whereas litter transport is less likely to be impeded in channelised rivers, which have an absence of natural structures such as vegetation or islands (Liro *et al.*, 2020). In the River Thames (London, UK) McConville *et al.* (2020) found that litter was stored in two distinct types of sites: floating sites (where floating litter accumulates on slipways or beaches) and sinking sites (where heavier items like wet wipes and sanitary pads are deposited out of suspension in slow moving stretches of river). The litter composition stored at the two types of site was found to be significantly different (Bernardini *et al.*, 2020), indicating that the properties of litter, as well as channel shape, are important in determining litter storage. Litter storage is also possible within the floodplain because high flows can deposit items here (Williams and Simmons, 1997). Temporarily stored anthropogenic litter may be subsequently remobilised and transported when flow conditions change. The presence and characteristics of retention sites are therefore important controls on litter mobility that regulate when and where litter is transported or stored (Williams and Simmons, 1997; Windsor *et al.*, 2019). Notably, litter is often stored alongside accumulations of organic material, like wood and leaves (Al-Zawaidah *et al.*, 2021).

Aquatic vegetation is particularly important in controlling the storage of anthropogenic litter. Williams and Simmons (1997) released plastic sheeting tracers into the Rivers Cynon and Taff in Wales and monitored the time taken for these tracers to reach the end of the study reach approximately 1 km away. In the low energy River Cynon under low flows (discharge 0.6 m^3s^{-1}) all tracers were quickly trapped, most often at the first obstacle, but even under high flows in the River Taff (discharge 74.3 m^3s^{-1}) 56% of tracers were stranded (Williams and Simmons, 1997). Similarly, van Emmerik *et al.* (2019b) found that plastic transport was directly related to the seasonal presence of water hyacinths which caused extensive stranding of floating litter during spring and summer months. Riparian vegetation, and in particular overhanging branches, can become draped with anthropogenic litter. This is termed the 'Christmas tree effect' (Williams and Simmons, 1999).

Anthropogenic litter can also be stored more permanently in the river bed or banks through burial with sediment, decreasing an item's potential for remobilisation (Liro *et al.*, 2020). In this way, long term storage over years or decades is possible. For instance, a sediment core from a lake in London (UK) showed that plastic particles have been preserved in bed sediments since the late 1950s when mass production of plastic was in its infancy (Turner *et al.*, 2019). The chance of a litter item being buried is affected by its characteristics; for example, more dense litter is more likely to be deposited and buried. Items with an interior, such as bottles, cans and bags, can accumulate sediment, promoting sinking and reducing item mobility (McCormick and Hoellein, 2016). The properties of the river channel are also important. Certain channel shapes have been found to encourage high rates of litter burial. For instance, the inside of meanders on the River Thames (London, UK) have been found to contain extremely high densities of wet wipes within the sediment; up to a maximum of 210 wet wipes in 1 m^2 (McConville *et al.*, 2020). This could be considered as loss from the system, because buried litter is hidden under the river bed. However, it could still be encountered by burrowing organisms or it could be later remobilised via floods or erosion. Most litter surveys only monitor visible litter on the surface of river beds or banks (e.g. McCormick and Hoellein, 2016; Bruge *et al.*, 2018), so the extent and quantity of buried litter is unknown.

2.2.3. Transformation of litter

Many of the qualities desired of manufactured items (tensile strength, inertia, etc.) mean that litter items have extremely long lifetimes and so are highly persistent in the environment. Plastic, for instance, is estimated to take hundreds or thousands of years to degrade (Barnes *et al.*, 2009). However, litter is vulnerable to transformation through degradation processes, weathering and mechanical abrasion that break it down into smaller fragments (Andrady, 2011). Chemical oxidation and biological activity can also cause physical and chemical changes to litter. Ultimately, this could lead to the loss of litter from the environment through total decomposition. Litter breakdown rates are affected by abiotic factors like heat, moisture, and UV light (Gewert *et al.*, 2015), however, there have been very few field measurements of degradation in a river environment so our understanding of litter degradation under natural conditions is limited.

There is only one known study that investigated degradation times in a river environment, and this only in the riparian zone. Williams and Simmons (1996) conducted exposure trials on polyethylene backing strips for sanitary pads on river banks. Degradation was tested by measuring the tensile strength of materials after exposure. Test subjects tethered to the bank degraded rapidly during the first month, losing 20% tensile strength, but afterwards changed little for the remaining three months of investigation. Buried samples degraded even less, only losing 10% tensile strength (Williams and Simmons, 1996). Degradation rates are slower if litter becomes submerged or buried as ambient temperature is reduced and it is no longer exposed to UV light or physical abrasion (Williams and Simmons, 1996; Andrady, 2003). Therefore litter in rivers can be extremely long lasting.

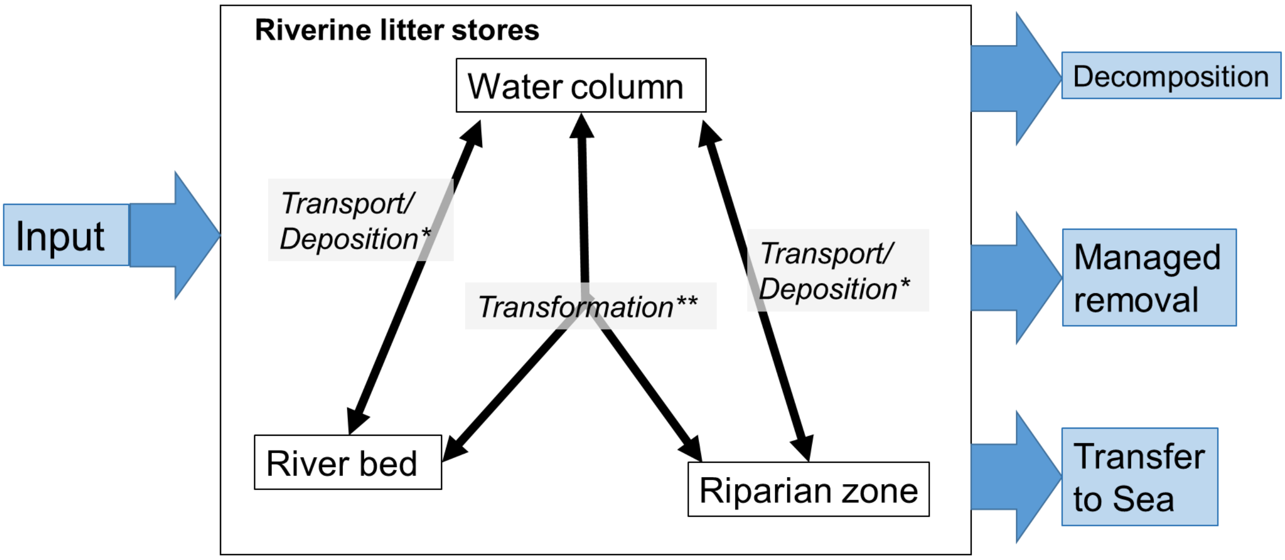
2.3. The loss of anthropogenic litter from rivers

Litter clearance and disposal schemes remove anthropogenic litter from rivers and transfer it to waste management systems for proper disposal (The Benioff Ocean Initiative, 2019). There are many devices which have been created to capture and remove floating plastic, but none are yet able to remove litter from the bed or banks (Helinski *et al.*, 2021), thus the majority of benthic riverine litter clearance is done manually by government agencies, volunteers, and informal waste collectors (Guerrero *et al.*, 2013; Hoellein *et al.*, 2015; McConville *et al.*, 2020; McDermott *et al.*, 2021). Surveys have shown that regular clearance of anthropogenic litter can significantly reduce litter densities. Vincent *et al.* (2017) used citizen science data on beaches around the Great Lakes USA to show that urban beaches had the lowest litter abundance during summer due to seasonal municipal beach cleaning, whereas other beaches with no litter clearance had no seasonal pattern in litter. McConville *et al.* (2020) reported that regular volunteer clean ups around the River Thames in London have successfully reduced the amounts of large immobile litter (e.g. tyres, bicycles) which have a slow return period. However, the clearance of regularly littered items (e.g. single use plastics) at high input sites is sometimes unable to keep pace with the quantity entering the river (McConville *et al.*, 2020). Carpenter and Wolverton (2017) argued that anthropogenic litter clearance would be ineffective at sites with high littering rates, but sites with poor public access and lower litter density could be more effectively cleared.

The other way in which litter could be lost from rivers is by export to the sea or to a lake. Whether litter is exported or remains stored in the river, depends on the balance between litter retention and transport, and so it is strongly linked to channel geomorphology, artificial structures, vegetation, and the river hydrology (Liro *et al.*, 2020). Several papers have modelled plastic emission from rivers (e.g. Lebreton *et al.*, 2017; Schmidt *et al.*, 2017; Tramoy *et al.*, 2019), basing their models on river discharge, population density, and the amount of waste which is mismanaged. For example, Lebreton *et al.* (2017) estimate that globally 1.2 to 2.4 million tonnes of plastic is exported from rivers per year, whilst Schmidt *et al.* (2017) predicted global plastic export rates of 0.4 to 4.0 million tons per year.

2.4. Summary

Based on this information a lifecycle model for litter can be proposed to describe the major processes controlling the entry, removal and transformation of litter whilst in the river environment (Figure 2.1).



*Deposition processes also include burial.

**Transformation includes fragmentation and physical or chemical changes to litter, but is not full decomposition as litter remains in the river.

Figure 2.1: Conceptual diagram showing the inputs, stores and outputs of riverine litter (rectangular boxes). The arrows show processes affecting where litter is stored in the river (black arrows) and how it is imported or exported from the system (wide blue arrows).

Riverine anthropogenic litter is thus affected by many factors. Its input is related to the surrounding land use, social factors, intensity of human activity, and the extent of waste production and management. What happens to this litter once it reaches the river is governed by the channel geomorphology, vegetation distribution, climate, hydrology and drainage patterns, as well as the properties if the litter itself. These factors determine the magnitude and nature of transport processes, as well as the likelihood of temporary or permanent storage, and potential for litter transformation or decomposition. This literature therefore demonstrates the potential for significant spatial and temporal variation in the sources and dynamics of litter, restricting the application of findings from previous research outside of the river or local area surveyed (Earll *et al.*, 2000). There is limited understanding of many of these factors and processes which affect litter in rivers, especially for materials other than plastic.

Chapter 3: The effects of anthropogenic litter on rivers

Anthropogenic litter research is in its infancy, especially in rivers. Therefore, although this review of the effects of anthropogenic litter is focused on rivers, literature has been drawn from all aquatic environments, particularly marine systems, where there has been greater focus on litter and there is relevance to river systems. Primarily this review is about the ecological consequences of anthropogenic litter; however, I also consider its impacts on people and society because of the important human context.

3.1. Ecological effects

3.1.1. Entanglement, ingestion and the leaching of toxic chemicals

Recent research, primarily from the marine environment and focused on plastic litter, has documented many occurrences of harm to organisms through ingestion of, or entanglement with, anthropogenic litter (Beaumont *et al.*, 2019). Ingestion or entanglement can cause a range of lethal or sublethal effects including: drowning, suffocation, laceration, starvation or damage to the digestive system, reduced fitness, changes to animal behaviour, and affected reproduction or ability to catch food or avoid predators (see reviews: Derraik, 2002; Gregory, 2009; Gall and Thompson, 2015). According to recent estimates, entanglement with anthropogenic litter has been linked to severe injuries or death in 395 marine species, and harm from ingestion has been reported for 208 marine species (Gall and Thompson, 2015).

Entanglement has been more commonly reported than ingestion of litter, probably because its effects are more visible (Gall and Thompson, 2015). In marine environments, many of the occurrences of entanglement are caused by abandoned fishing gear which continues to trap organisms, known as ‘ghost fishing’ (Ayaz *et al.*, 2006). However, there are many different types of litter that can entangle organisms and even small organisms can become entangled. For instance, beetles crawl into discarded beverage bottles and cans, encouraged by any remaining liquid, but are subsequently unable to escape (Romiti *et al.*, 2021).

There are lots of documented reports of aquatic organisms consuming anthropogenic litter, but it is unclear why they do so. It could be that they mistake litter for food because of how it looks or smells (Schuyler *et al.*, 2014; Savoca *et al.*, 2017), or litter could be ingested accidently in the process of consuming their intended food items (Scherer *et al.*, 2017). Unlike biotic materials, there is no nutritional benefit to consuming anthropogenic litter, but organisms that ingest litter could be exposed to toxic chemicals associated with the litter (Gunaalan *et al.*, 2020). Small particles may be egested (Scherer *et al.*, 2017), but larger items can cause gastrointestinal blockage (Gall and Thompson, 2015), therefore both ingestion and its consequences are affected by the size of the organism relative to the litter item (Windsor *et al.*, 2019).

There has been considerably less research into these possible effects of litter in rivers as opposed to in the marine environment (Blettler *et al.*, 2018; Blettler and Wantzen, 2019). Only a few studies have documented anthropogenic litter entanglement for organisms in rivers (but see: Edwards and Cryer, 1987; Serena and Williams, 2010; da Silva *et al.*, 2018). So far there are no known published papers documenting the ingestion of litter larger than a couple of cm long in freshwater environments (Blettler *et al.*, 2018), but macroinvertebrates, fish and birds have all been found to ingest microplastics in lakes and rivers (e.g. Possatto *et al.*, 2011; Faure *et al.*, 2015; Holland *et al.*, 2016; Scherer *et al.*, 2017; Andrade *et al.,* 2019; Windsor *et al.*, 2019; Blettler *et al.,* 2019a). Possatto *et al.* (2011) reported that water birds also consumed small metal and glass particles. There has also been limited investigation of the consequences of ingesting litter for freshwater organisms, but in a recent laboratory study Silva *et al.* (2021) exposed invertebrates to different concentrations of microplastics and found that the abundance of deposit feeders and grazers significantly reduced in response to microplastics, however there were no changes to leaf decomposition rates or primary productivity.

Related to the risks of entanglement and ingestion, there is a danger that chemicals from anthropogenic litter could leach into the surrounding environment and be taken in by organisms. Toxic compounds associated with plastic are especially of concern (Rochman *et al.*, 2013). These include many additives added during its manufacture, as well as other chemicals that adsorb to the surface of plastic (Teuton *et al.*, 2009; Gunaalan *et al.*, 2020). Negative effects to organisms from exposure to plastic leachate have been found in several studies (see reviews: Oehlmann *et al.*, 2009; Ziccardi *et al.*, 2016; Hermabessiere *et al.*, 2017; Gunaalan *et al.*, 2020), but studies on freshwater organisms are limited to a small number of taxa. Additionally, most research is based on laboratory exposure studies of the dose-effect relationship, which are hampered by the use of unrealistic environmental conditions or concentrations and characteristics of litter and litter-associated toxins (Phuong *et al.*, 2016; Koelmans *et al.*, 2017). Other litter materials can also release toxic compounds, for instance tyres (Degaffe and Turner, 2011; Redondo-Hasselerharm *et al.*, 2018) or batteries (Nrior and Gboto, 2017), but there has been limited research into the toxic effects of litter materials other than plastic.

3.1.2. Anthropogenic litter as a form of habitat

Anthropogenic litter may also have ecological consequences through providing new surfaces for colonisation and changing habitat structure. Extensive colonisation of artificial structures has been reported in many aquatic environments, including river walls (Attrill *et al.* 1999; Hoggart *et al.*, 2011), coastal infrastructure (Morris *et al.*, 2017; Evans *et al.*, 2021), artificial reefs (Chapman and Clynick, 2006; Scott *et al.*, 2015; Folpp *et al.*, 2020), and anthropogenic litter (Katsanevakis *et al.,* 2007; Watters *et al.*, 2010; García-Vazquez *et al.,* 2018; Crocetta *et al.,* 2020; Song*, et al.,* 2021). Litter has also been found to provide shelter for mobile organisms like fish and crustaceans (Chapman and Clynick, 2006; Katsanevakis *et al.*, 2007). For example, the octopus *Octopus rubescens* uses beer bottles as dens (Anderson *et al.,* 1999), and a range of marine vertebrates and fish have been found to aggregate around floating debris (Aliani and Molcard, 2003). Hermit crabs have also been documented using litter as mobile homes instead of shells (Lavers and Bond, 2017). Similarly, case building caddisfly have been found to incorporate microplastics and other artificial particles into their cases (Ehlers *et al.*, 2019), which could affect the physical properties of their cases (Ehlers *et al.*, 2020).

Anthropogenic litter has very different physical properties, such as colour, density, surface texture, size and shape, to natural substrates; all of which could have consequences for colonising organisms. If litter provides a different type of habitat to that otherwise present, it could affect local communities by creating conditions that favour different species. In muddy and sandy shallow seas where litter provides the only available hard substrate, it has been found to increase the overall local biodiversity both by providing habitat for sessile species that inhabit hard-substrata, and shelter for mobile species (Katsanevakis *et al.*, 2007; Pace *et al.,* 2007). Similarly, researchers found that the communities of macroinvertebrates on individual pieces of anthropogenic litter in reservoirs in Poland were more diverse and significantly different to those inhabiting natural substrates (Jatulewicz, 2007; Czarnecka *et al.*, 2009). The authors suggested that these differences between the communities colonising litter and those on natural substrates were related to the novel physical structure of the anthropogenic litter. For example, bottles could protect organisms in their interiors (Katsanevakis *et al.*, 2007; Czarnecka *et al.*, 2009), and the smooth surface texture of plastic and glass materials might affect organism settlement (Pace *et al.*, 2007). Adamiak-Brud *et al.* (2015) noticed that leeches preferred hard litter substrates because they were easier to move around on, but that transparent materials (like glass or some plastics) were unsuitable, because leeches were exposed to solar radiation. Some authors have also noticed that suspension feeding invertebrates are often associated with litter (Mordecai *et al.*, 2011; Bergmann and Klages, 2012; Kiessling *et al.*, 2015), perhaps because colonising the litter enables then to elevate themselves into the water column and thereby gain better access to food particles suspended in the water.

Colonisation of floating anthropogenic litter could enable rafting organisms to travel long distances, especially because litter tends to have a longer lifespan than natural raft materials (Kiessling *et al.*, 2015). Thus, litter could play a role in the spread of species. In the marine environment, 387 taxa have been documented to raft on floating litter, this includes pro- and eukaryotic microorganisms, seaweeds, and a range of invertebrates (Kiessling *et al.*, 2015). There is concern that invasive species could be transported across oceans, facilitating their spread (Barnes, 2002; Gregory, 2009; Rech *et al.*, 2018). Although all of this research has been based in marine environments, floating litter is also present in rivers and can travel long distances downstream, therefore this could have relevance for river environments too.

Anthropogenic litter has also been found to provide reproductive habitat for a number of species. For example, Katsanevakis *et al.* (2007) found that a marine gastropod (*Hexaplex trunculus*) attached egg masses to litter items in shallow seas, and molluscan egg masses were common on litter trawled from the Tyrrhenian Sea (Crocetta *et al.*, 2020). Goldstein *et al.* (2012) and Majer *et al.* (2012) found that high floating microplastic concentrations provided abundant oviposition sites for pelagic skaters (*Halobates* spp). Similarly, a project that provided half-cylindrical ceramic tiles, with the aim of increasing the availability of spawning substrates for a freshwater relict darter fish (*Etheostoma chienense*), found that egg-clutches on artificial substrates were twice the size of those on natural substrates (Piller and Burr, 1999). As of yet there has been limited research into these interactions, for instance there is no known research on whether the eggs are affected by toxic chemicals leaching from the litter. However, these findings suggest that the presence of anthropogenic litter could have implications for the reproduction of egg laying species.

3.1.3. Changes to habitat caused by anthropogenic litter

Anthropogenic litter could also affect organisms indirectly through inducing habitat changes in the surrounding river bed. Plastic sheeting and bags have been shown to smother bottom sediments on the seafloor and estuaries, leading to anoxia and hypoxia by inhibiting gas exchange, and causing changes to local communities (Goldberg, 1997; Green *et al.*, 2015). Green *et al.* (2015) found that the anoxic conditions that developed under plastic bags resting on the bed of brackish mudflats, reduced primary productivity and organic matter, which changed the assemblage and reduced the abundance of invertebrates. However, in a similar experiment in the intertidal zone of an estuary in Brazil, Clemente *et al.* (2018) found no significant differences for organic matter, redox potential, and primary productivity between samples taken from beneath plastic bags and controls, although there were still differences in the macroinvertebrate community. In a similar project comparing beach sediment smothered by plastic litter to areas without litter, Uneputty and Evans (1997) found lower densities of diatoms, but higher abundance of meiofauna underneath litter. They suggested that the plastic prevented light reaching the sediment, worsening conditions for diatoms, but that the plastic benefited meiofauna by encouraging organic matter deposition (Uneputty and Evans, 1997). These differences between studies probably relate to the length of time plastic remained covering the same area of sediment. Bags were fixed in place for nine weeks in Green *et al.*'s (2015) study, but bags in Clemente *et al.*'s (2018) study were regularly remobilised by the flow, suggesting that regular movement of litter could ameliorate this impact (Clemente *et al.*, 2018). Uneputty and Evans (1997) measured litter as it was found on the beach so could not record how long items had been present for.

Evidence that anthropogenic litter could change the form of river habitats is also emerging, but is so far poorly studied (though see Chapters 6 and 7). For instance, large litter could encourage deposition; Williams and Deakin (2007) found that shopping trolleys accumulated organic matter, sediment and additional litter items in the River Tawd in Skelmersdale (UK). Similarly, Thames 21, a river conservation charity based in London (UK) found considerable deposition of wet wipes (50 to 200 wet wipes m^{-2} in a 2019 survey) on the inside of riverbends in the River Thames where slower currents caused the sinking waste to collect in 'mounds' (McConville *et al.*, 2020). These wet wipe mounds encourage further deposition of additional litter and fine sediments by increasing local channel roughness and binding together sediments. Bathymetric surveys have shown that the mounds are growing in height and area; the largest grew by 70cm in the eight months between Sept 2018 and May 2019 (McConville *et al.*, 2020).

Riverine anthropogenic litter could prompt changes in habitat by increasing channel roughness and thereby changing the pattern of flow velocity and distribution of sediment, as has been shown for natural habitat structures like wood, boulders and macrophytes (Shamloo *et al.*, 2001; Gurnell, 2002; Green, 2005; Afzalimehr *et al.*, 2019). These structures obstruct the flow, reducing downstream flow velocities and altering hydraulic patterns around itself, and accelerating flow around and through the structure (Abbe and Montgomery, 1996). At the reach scale, this increases flow complexity and decreases mean channel velocity (Linstead, 2001), but locally, areas of concentrated flow expose coarse bed material (Mutz, 2000; Montgomery *et al.,* 2003), whereas patches with reduced flow velocities deposit fine sediment and organic matter (Keller and Swanson, 1979; Daniels, 2006). This introduces notable habitat diversity through maintaining coarse gravel patches even where fine sediment is prevalent (Osei *et al.*, 2015a), and creating diversity in hydraulic conditions (Pilotto *et al.*, 2016). Given that river organisms are strongly related to habitat characteristics, changes to habitats caused by litter could influence their distribution, diversity, and community composition.

3.2. Impacts on people and society

Although this thesis focuses on the ecological implications of anthropogenic litter, litter also has consequences for human society. High concentrations of anthropogenic litter affect the aesthetics of an area, and therefore crucially, people's opinion of a place. Several studies have investigated the consequences of litter for beach visitors and coastal tourism. Almost 70% of visitors to coastal beaches said in interviews that they would stop visiting a beach with high amounts of litter (Brouwer *et al.*, 2017). Similarly, the wellbeing benefit from outdoor recreation is lower at coastlines with litter (Wyles *et al.*, 2016). This influences the tourist value of an area. For example, after heavy rainfall caused an unusually high amount of litter to collect on the beaches of Goeje Island, South Korea, there were 500,000 fewer visitors that year compared to the previous (a reduction of 63%), costing €23 to €29 million in lost revenue (Jang *et al.*, 2014). Significant expenditure is therefore required to clear beach litter for visitors. In the UK the municipal cost of clearing beach litter is estimated to be around €18 million annually (Mouat *et al.*, 2010). Anthropogenic litter has also been linked to rates of crime and antisocial behaviour (Keizer *et al.*, 2008; Brown *et al.*, 2010), and it decreases local property value (Schultz *et al.*, 2011).

Blockage of drainage and sewage systems by anthropogenic litter can also affect flood risk. Litter can block culverts and trash screens (Honingh *et al.*, 2020) and exacerbate local flood extent (Tjia, 2020). Several studies have sought to identify factors causing culvert blockage to improve design and estimate risk at specific sites (Blanc, 2013; Wallerstein and Arthur, 2013). Sewer blockage caused by litter which is erroneously flushed down toilets is also a significant and costly problem to clear up. Wet wipes in particular are the main cause of sewer blockages; 78% by weight of samples collected from blocked sewers, pumps and wastewater treatment works inlets were baby wipes (Drinkwater and Moy, 2017).

Human health can be negatively affected by anthropogenic litter. Pieces of broken glass or metal, syringes, or other sharp objects can cause injury to people that encounter these types of litter. Evidence of how often this occurs is limited, due to a lack of reporting (Werner *et al.*, 2016). However, Campbell *et al.* (2016) reported that 22% of beach users reported injuries from litter, even though the site they investigated had relatively low litter density. There are also risks from disease associated with litter. Anthropogenic litter could also be a vector for potentially harmful microorganisms, particularly a concern in the marine environment, where floating litter could enable the spread of diseases over long distances (Keswani *et al.*, 2016). Certain types of litter (e.g. wet wipes, sanitary products) are associated with sewage, which could mean they harbour faecal indicator organisms and pathogens (McCormick *et al.*, 2014). Litter also poses indirect health hazards through encouraging pests like rats and mosquitos (Schultz *et al.*, 2011). For example, Banerjee *et al.* (2013) found that household waste in Kolkata, India, supported populations of the mosquito species which is a vector of dengue fever. However, at present there is limited information to assess these potential risks to human health (Keswani *et al.*, 2016).

3.3. Summary

Litter is an extremely heterogenous pollutant that comes in a variety of types with diverse characteristics (e.g. shape, material, colour, size, surface complexity, chemical properties). Consequently, there are many ways that organisms could be affected by litter. For example, the shape and size of litter determines its surface area, which affects mobility, scope for colonisation, and potential to leach chemicals (Windsor *et al.*, 2019). This diversity makes understanding the impacts of litter challenging, especially as most research focuses on plastic litter rather than other materials.

Most previous research has been focused on the marine environment, however, rivers are very different environments, not only in terms of the processes that affect litter, but also in terms of the characteristics of the environment and the organisms which live there. This means that the sources, dynamics, and impacts of litter are likely to be different, so directly applying findings from marine studies to rivers is likely to be inappropriate. Yet, very little research has so far addressed the effects of litter on river organisms (Blettler *et al.*, 2018). It is important to understand the ecological impacts of anthropogenic litter specific to rivers in order to manage its negative impacts, and to legislate for sustainable and appropriate disposal of waste.

Chapter 4: Anthropogenic litter density is positively associated with macroinvertebrate diversity

4.1. Introduction

4.1.1. Anthropogenic litter density and composition

High concentrations of anthropogenic litter have been found in a range of aquatic environments (Oigman-Pszczol and Creed, 2007; Mordecai *et al.*, 2011; McCormick and Hoellein, 2016). It has been found even in isolated and uninhabited areas such as remote islands (Duhec *et al.*, 2015; Lavers and Bond, 2017), the Arctic seafloor (Tekman *et al.*, 2017) and ocean trenches (Shimanaga and Yanagi, 2016). The vast majority of surveys have been conducted in marine environments (Blettler *et al.*, 2018), despite rivers being considered a major conduit of litter to oceans (Lebreton *et al.*, 2017; Castro-Jiménez *et al.*, 2019), and increasing evidence that rivers themselves are important sinks for litter (Schwarz *et al.*, 2019; Winton *et al.*, 2020). In rivers, most surveys focus on floating (e.g. González-Fernández and Hanke, 2017; van Emmerik *et al.*, 2018) or riparian (e.g. Rech *et al.*, 2015; Kiessling *et al.*, 2019) anthropogenic litter. Benthic litter is less accessible and more difficult to quantify (Vriend *et al.*, 2020), but ignoring this fraction of riverine litter risks excluding an important aspect of the litter problem.

Anthropogenic litter may be deliberately or accidently discarded directly into rivers, or could be transported into the river through natural processes, such as wind or surface runoff (van Emmerik and Schwarz, 2019). Known sources of anthropogenic litter to rivers include fly-tipping, whereby large amounts of waste are illegally dumped (Williams and Simmons, 1999; Rech *et al.*, 2015). Alternatively, litter may build up over time through individual people dropping smaller amounts of litter. This litter is often concentrated near recreational areas and commercial centres with high human activity (Williams and Deakin, 2007; McCormick and Hoellein, 2016; Priestley, 2017), and is typically associated with convenience goods, which are consumed shortly after purchase and the litter (e.g. fast food wrappers, smoking litter, or beverage containers) discarded directly into or near to the river (Carpenter and Wolverton, 2017). Another common source is wastewater management systems. The majority of UK wastewater treatment is combined sewage systems, so at times of high water volume, wastewater is released into rivers via Combined Sewage Overflows (CSOs), which deliver sewage related litter directly to rivers (Williams and Simmons, 1999; Morritt *et al.*, 2014; McConville *et al.*, 2020). Once anthropogenic litter has entered the river it is subject to a number of processes that determine its distribution, including: transport downstream, stranding in vegetation, burial by sediments, degradation, or removal by clean-up efforts (see Chapter 2 and reviews by: Schwarz *et al.*, 2019; van Emmerik and Schwarz, 2019; Liro *et al.*, 2020).

The small number of studies to date that have investigated the amounts and types of anthropogenic litter in rivers have reported high densities, comparable to those found in marine environments (McCormick and Hoellein, 2016), although many papers only surveyed plastic pollution. Researchers have tended to focus on plastic because of its high abundance (Derraik, 2002; Ryan *et al.*, 2009) and extremely slow rates of degradation (Anrady, 2003), but common anthropogenic litter materials also include glass, metal, fabric, paper, rubber, ceramics, and manufactured wood (Rech *et al.*, 2015; McCormick and Hoellein, 2016). Excluding these materials could mean that the litter quantity is underestimated (Vriend *et al.*, 2020). This is especially likely for rivers, given that previous comparisons of litter composition in marine environments and rivers suggest that riverine litter may not be as dominated by plastic as litter found on coasts (Hoellein *et al.*, 2014; McCormick and Hoellein, 2016). Other key differences in anthropogenic litter composition between the different ecosystems have also been reported. For example, fishing related litter is common in marine benthic surveys, but is rarely found on beaches or in rivers (McCormick and Hoellein, 2016; Bruge *et al.*, 2018). Conversely, Winton *et al.* (2020) found that sewage related litter was much more common in rivers or lakes than in marine studies. This suggests that applying what is known about litter characteristics in the marine environment is unlikely to be appropriate to rivers, especially given the high spatial and temporal variability in litter characteristics (Earll *et al.*, 2000).

Assessing the density and composition of anthropogenic litter can provide insight into the possible sources and consequences of litter (McCormick and Hoellein, 2016; Bruge *et al.*, 2018), important for informing management practices. Building understanding of these dynamics is critical because of the pervasive and persistent nature of litter worldwide. This chapter extends our knowledge of the nature of the litter problem in the UK by sampling benthic litter in several small rivers across a range of litter densities.

4.1.2. The ecological impacts of anthropogenic litter

A variety of different ecological impacts have been hypothesised for anthropogenic litter (see Chapter 3 for more details). This includes the damaging effects of entanglement with, or ingestion of litter (Gall and Thompson, 2015) and the risk of encountering toxic pollutants which leach from litter (Rochman *et al.,* 2013). Anthropogenic litter may also affect organisms through providing alternative habitats, for example by providing otherwise rare hard substate (Katsanevakis *et al*., 2007) or substrate which is more complex than that which is naturally available (Czarnecka *et al*., 2009). This could provide opportunity for species that are able to colonise litter but would otherwise be unable to find suitable habitat. Other proposed ecology-litter interactions include affecting egg-laying behaviour (Piller and Burr, 1999; Crocetta *et al*., 2020), providing shelter (Chapman and Clynick, 2006; Katsanevakis *et al*., 2007) and facilitating organism dispersal via the colonisation of floating litter (Barnes, 2002; Gregory, 2009).

In this chapter, I focus on investigating the effects of anthropogenic litter on macroinvertebrates. Macroinvertebrates are ideal study organisms as they are abundant, widespread, and their distribution is strongly linked to local environmental conditions (Li *et al*., 2010).

Not all of the potential impacts of anthropogenic litter detailed in Chapter 3 are relevant to macroinvertebrates. For instance, because of the small size of macroinvertebrates relative to litter, entanglement and ingestion impacts are less likely than they are for larger organisms, however, litter could provide a novel substrate for colonisation (Windsor *et al*., 2019). At the scale of individual items of litter, several published papers have found that litter items supported higher macroinvertebrate diversity and significantly different communities compared to natural substrates (Czarnecka *et al*., 2009; García-Vazquez *et al*., 2018; Song *et al*., 2021), but none of these studies have been conducted in rivers (although see Chapter 5). Macroinvertebrates are highly sensitive to substrate characteristics (Death, 2000), so litter could provide or create conditions that favour certain species over others. The overall effect of litter might therefore be an increase in habitat diversity, meaning that rivers with high litter densities could support greater diversity of macroinvertebrates or specific species that would naturally be absent. This is potentially counter-intuitive given that litter is generally perceived to be harmful, but has important implications for how litter should be managed if such an effect exists.

4.1.3. Research approach

My objectives for this study were to: (1) quantify and compare anthropogenic litter density and characteristics between a number of small UK rivers, (2) assess whether there are differences between the results and other aquatic systems worldwide, and (3) to investigate whether there are any associations between the quantity of litter at sampling sites, and the macroinvertebrate communities therein. It was hypothesised that differences in the litter found in the rivers sampled here and those reported from other aquatic environments relate to differences in the sources and transport of litter in the different habitats. It was also predicted that litter density is positively correlated with macroinvertebrate diversity due to corresponding increases in substrate (and therefore habitat) diversity caused by the presence of anthropogenic litter. This chapter also provides context for rest of the thesis by providing detail on the characteristics of anthropogenic litter found in small UK rivers, similar to the sites sampled in the rest of this thesis.

4.2. Methods

4.2.1. Study sites

Fieldwork was conducted on four small UK rivers within Nottinghamshire and Leicestershire (Figure 4.1), with anthropogenic litter present in all four. These were Alfreton Brook (AB), Black Brook (BB), River Leen (RL) and Saffron Brook (SB). Rivers were chosen that were reasonably similar in terms of channel size, slope, discharge, geology, and planform (Figure 4.2; Table 4.1). All of the rivers are strongly affected by anthropogenic influences, in that they have been channelised, cleared of natural habitats, and most have been reinforced with bank and or bed protection. At each river, two or three reaches were chosen for sampling with the aim of selecting sites across a range of litter densities. The selected reaches were, as far as possible, straight channels with homogeneous morphology, in order to avoid comparing samples from sections with very different habitat characteristics. All sites were classified as good or moderate for invertebrates according to the Water Framework Directive (WFD: 2000/60/EC) in 2019, indicating that invertebrate communities are not restricted to just the most pollution tolerant species. Sampling was conducted between May and July 2018.

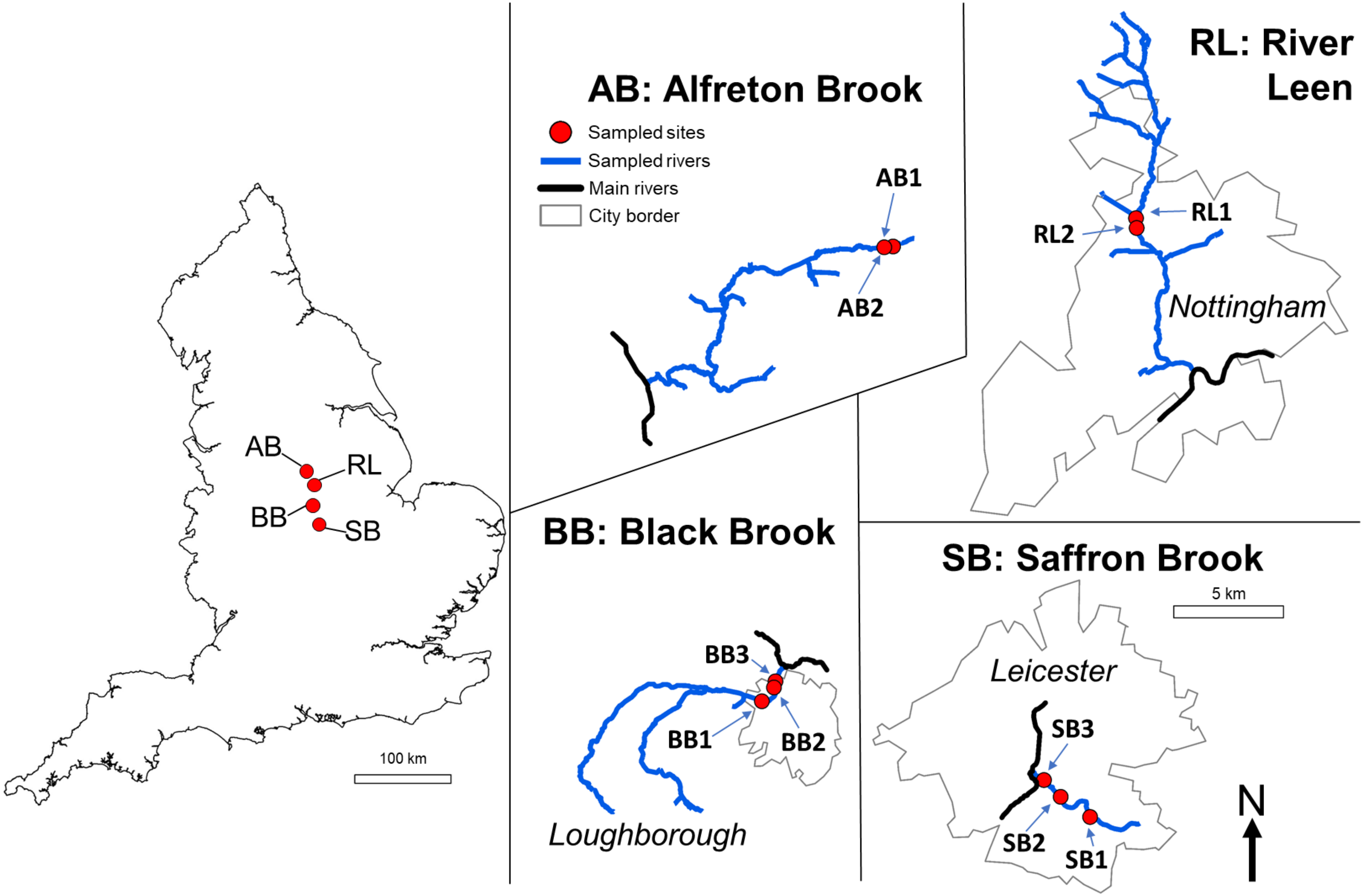


Figure 4.1: Map of sampled sites showing their location within England, and the specific sites within each river relative to the urban area and main river they flow into. Sites are labelled with the abbreviated river name and numbered proceeding downstream.

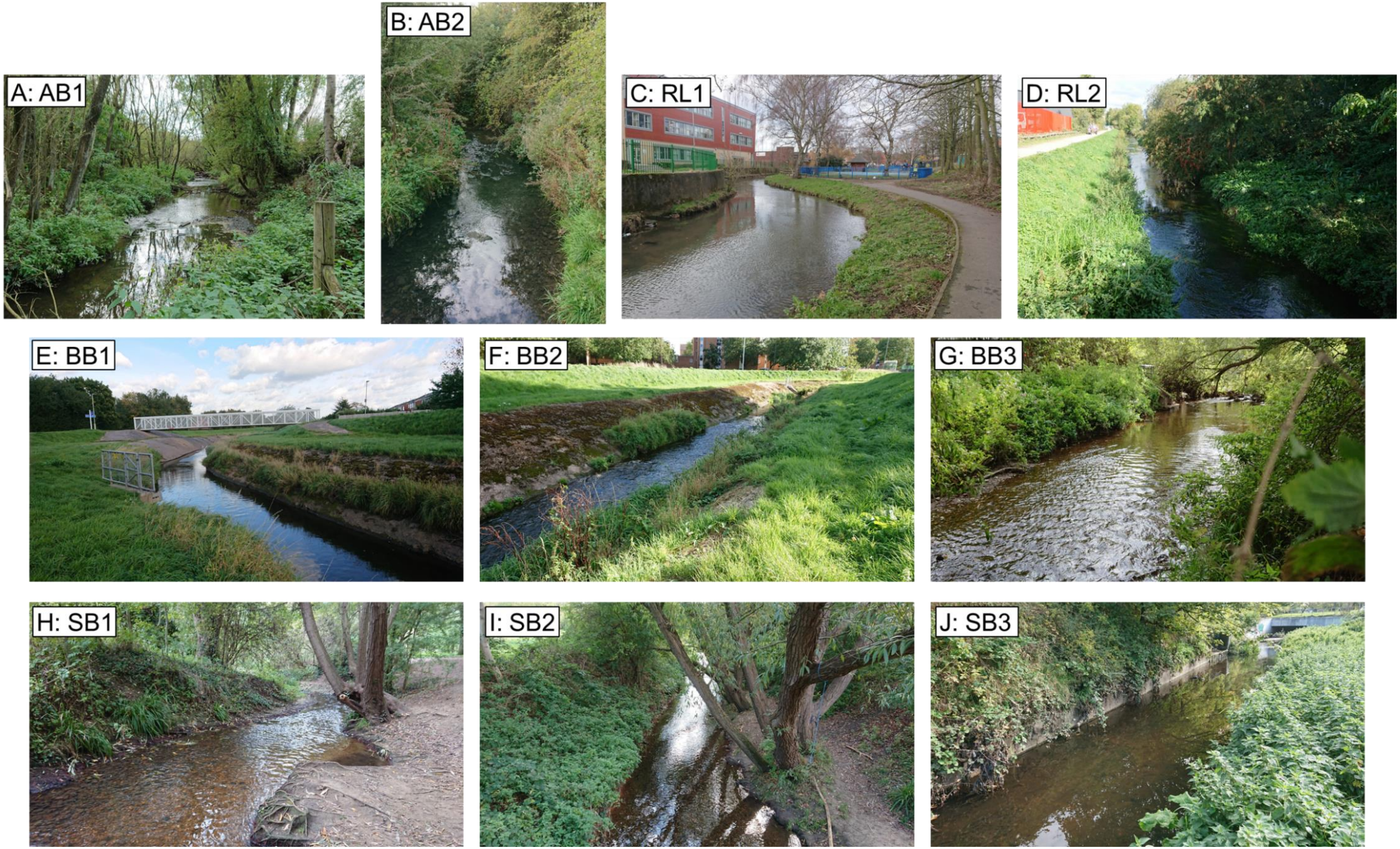


Figure 4.2: Photograph of each of the sampled reaches.

Table 4.1: Details of the sampled reaches. The river width (m) and depth (m) were measured at a representative cross-section during sampling, with the mean depth calculated from measurements taken at five equidistant points along the channel. Sampled sections were 2.5 m long, therefore the area of river bed sampled was 2.5 times the river width (see Section 4.2.2 for details). The 2019 Water Framework Directive (WFD) classifications were taken from the Environment Agency (EA)'s Catchment Data Explorer (EA, 2021a,b,c,d).

River	Reach	River width (m)	Mean river depth (m)	River area sampled (m^2)	Invertebrates WFD status	Physico-chemistry WFD status
Alfreton Brook	AB1	2.8	0.1	7.0	Moderate	Moderate
	AB2	3.0	0.1	7.5		
Black Brook	BB1	2.6	0.4	6.5	Good	Moderate
	BB2	3.4	0.3	8.5		
	BB3	5.9	0.2	14.8		
River Leen	RL1	6.0	0.3	15.0	Good	Good
	RL2	5.5	0.2	13.8		
Saffron Brook	SB1	3.1	0.1	7.8	Moderate	Moderate
	SB2	4.3	0.1	10.8		
	SB3	3.9	0.2	9.8		

4.2.2. Field methods

At each site, a characteristic 50 m long reach was identified and divided into twenty 2.5 m sections along the length, marked using stakes. Five of these sections, one every 10 m along the reach, were randomly selected for sampling of macroinvertebrates and anthropogenic litter. This was so that sampled sections would be spread out along the reach, whilst also preventing sample selection bias. Because the rivers differed in width, the area of river bed sampled was not consistent between reaches (see Table 4.1), this was addressed in the treatment of litter and macroinvertebrate data before analysis.

4.2.2.1. *Macroinvertebrates*

Benthic macroinvertebrate samples were taken first to ensure that macroinvertebrate communities would not be disturbed by anthropogenic litter collection. Samples were collected using a 1 mm mesh kick net and following an adapted kick sampling methodology. For a period of two minutes, the operator disturbed the river bed upstream of the net using their foot, thereby dislodging macroinvertebrates which were carried into the net by the flow. The operator systematically moved across and upstream through the section in order to proportionally sample all of the different types of habitat present (e.g. marginal areas, areas with different flow velocity, depth or substrate type, macrophytes, or organic matter) according to their relative area. This duration of kick sampling is less than typical for the EA standard (Murray-Bligh, 1999), because the area of river bed being sampled was considerably smaller in this study. This method of kick sampling gave a semi-quantitative measure of the macroinvertebrate populations that is spatially comparable to the litter survey. The method is standardised by sampling effort (i.e. sampling is conducted for the same length of time and is spread proportionally across habitat types) rather than by area (Everall *et al.*, 2017), therefore differences in the river width (and thus sampled area) at each reach would not affect macroinvertebrate samples. It is theoretically possible that sampling larger areas of river would increase the chance of incorporating more types of habitat (and therefore greater habitat diversity). However, this is unlikely because habitats did not differ much within individual cross sections in these rivers, apart from marginal areas which were always sampled (regardless of width), and because sampling effort was distributed proportional to the relative size of different habitat types. Samples were preserved in the field and then transported to the laboratory for processing and identification.

4.2.2.2. *Anthropogenic litter*

After collection of the macroinvertebrate samples, the five sections were re-surveyed for anthropogenic litter by collecting all litter with b-axis greater than 1 cm. Litter smaller than this was difficult to reliably spot so was excluded (following McCormick and Hoellein, 2016). Only litter which was floating or visible on the bed of the river was sampled (although items may have been partially buried).

Several studies have explained the need for harmonisation in litter categorisation and the difficulties of comparing between surveys which use different metrics (Owens and Kamil, 2020; Vriend *et al.*, 2020). With this in mind, all litter items were classified according to item type, material composition and intended function. Item type classification was based on the protocol from Wenneker and Oosterbaan (2010), which is a standard for beach litter monitoring, adding additional categories for litter where necessary (Table 4.S 1). This gave 86 distinct litter types, which are comparable to other studies. There were nine material categories assigned based on the dominant material type: building materials (including asbestos, bricks, roof tiles, and concrete), fabric, glass, metal, mixed (where there was no one dominant material type), paper (including cardboard), plastic, rubber, wood (only manufactured wood, including sawn timber and processed wood), or other. Intended function was split into eleven categories: clothing and jewellery, commercial (including shopping trolleys/baskets), construction, electricals, food and drink (including cigarettes), household, packaging, recreation (including fishing equipment), sewage (associated with sewage waste), transport (including car/bike parts or tyres), and unknown. Where items could have been assigned to food and drink or packaging (e.g. food wrappers) they were classed as food and drink litter. Dividing litter into intended functions can be used to give an indication of the source of the litter (Santos *et al.*, 2009; McCormick and Hoellein, 2016).

4.2.3. Laboratory methods

Macroinvertebrate kick samples were processed by first washing through a 500 µm mesh sieve and then manually separating macroinvertebrates from remaining bed sediments. Taxa were identified to mixed taxonomic levels, keeping levels consistent between samples and sites. The majority of taxa were identified to species or genus, except Oligochaeta which were identified to subclass and most Diptera which were identified to family (apart from Chironomidae which were identified to tribe, with Chironomidae pupae considered as a separate taxa as they are thought to exhibit habitat preferences distinct from larval stages: Armitage *et al.*, 1995). Identification followed Cham (2012), Dobson *et al.* (2012), Edington and Hildrew (1995), Elliott (2009), Elliott and Dobson (2015), Elliott and Humpesch (2010), Friday (1988), Holland (1972), Macan (1977), Reynoldson and Young (2000), Savage (1989) and Wallace *et al.* (1990).

4.2.4. Data analysis

All data analyses were performed using R statistical software (version 4.0.4: R Core Team, 2021).

Because the rivers differed in width, the area of river bed sampled was not consistent between reaches. To account for these differences the abundance of anthropogenic litter was converted to litter density. This also facilitates comparison between the densities found in this study and those recorded elsewhere. Litter densities and the proportional abundance of different materials and intended functions were then examined visually.

To test whether enough of the river bed was sampled to get an accurate representation of litter density over the whole reach, I surveyed litter density along the whole reach at SB3 and BB2 and compared this to the mean density across the five sampled sections in each reach, respectively. I found that the five sections suitably represented the total litter population in both rivers, with differences between their mean and the total litter density being within the standard error of the five sub-samples.

Macroinvertebrate data analysis was performed with the full dataset of mixed taxonomic resolution. Macroinvertebrate abundance was the count of individuals within each sample. Macroinvertebrate diversity was determined by calculating Hill's numbers (^{0}D, ^{1}D and ^{2}D) using *vegan* (Oksanen *et al.*, 2020). Hill's numbers are defined to the order of q (^{q}D) which conveys the relative weighting of rare and common taxa (Hill, 1973). ^{0}D is equivalent to estimated taxon richness, which is weighted towards rare species due to its insensitivity to relative frequencies. ^{1}D is equal to the exponential of Shannon's Diversity index, weighted towards common species, and ^{2}D to the inverse of Simpson's Diversity index, weighted towards highly abundant species (Tuomisto, 2010). Taken together, these indexes provide complementary information on the diversity (taxon richness and evenness) of samples.

To look for a possible relationship between macroinvertebrates and litter density I used Linear Mixed Effects (LME) models using the *lme4* package (Bates *et al*, 2015). Because samples taken from the same reach of river, and those taken from within the same river, were not independent of one another, these factors were accounted for by using random effects with reaches explicitly nested within river. Litter density was included as a fixed factor, and its significance tested via likelihood ratio tests (distributed as X^2) of the full model against a simpler model without the litter density factor.

4.3. Results

4.3.1. Anthropogenic litter

In total, there were 1203 pieces (142.5 kg wet mass) of anthropogenic litter sampled over 506.3 m^2 of river bed, with a mean density (± SE) of 2.0 ± 0.3 litter items m^{-2} (Figure 4.3). Maximum litter density was in RL where there were up to 8.7 items m^{-2}.

Only five sections across all rivers had no litter and four of these were in BB. Glass, metal, and plastic made up the majority of litter collected (these materials accounted for 76% of all litter found over all rivers). The most common litter functions that could be identified were food and drink (30%), sewage (16%) and packaging (11%). The intended function of anthropogenic litter items could not be identified for 20% of cases, most often because items were broken and degraded having been in the river for a long time.

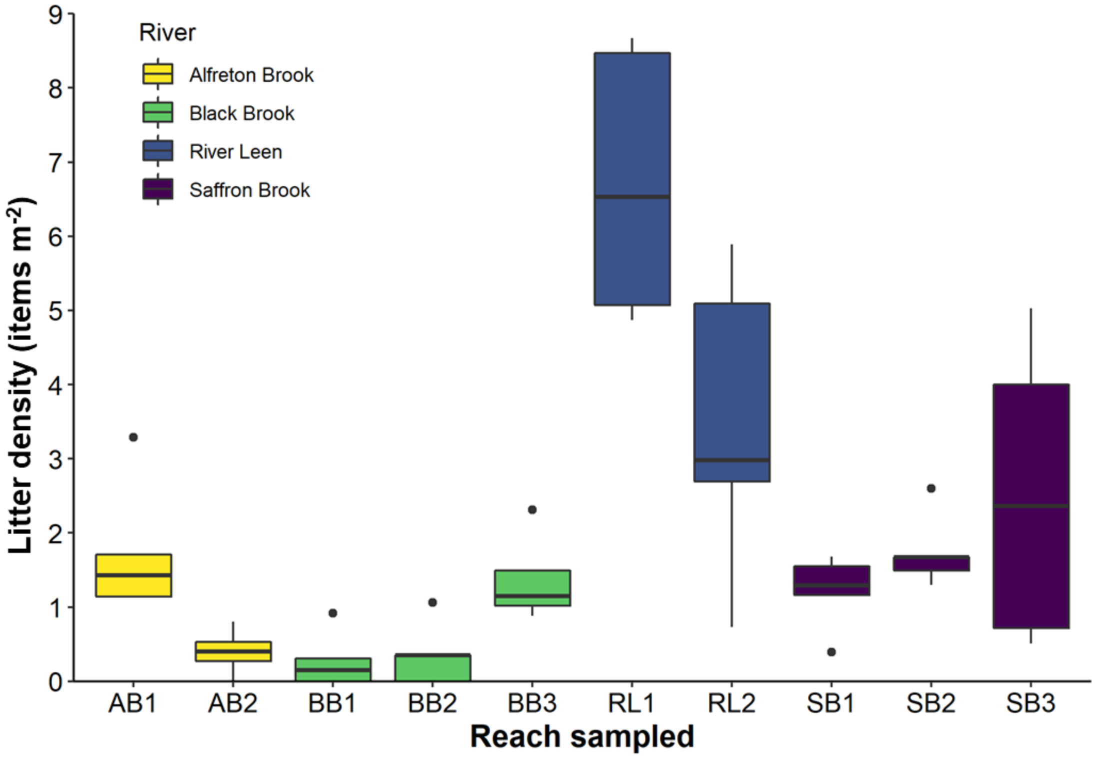
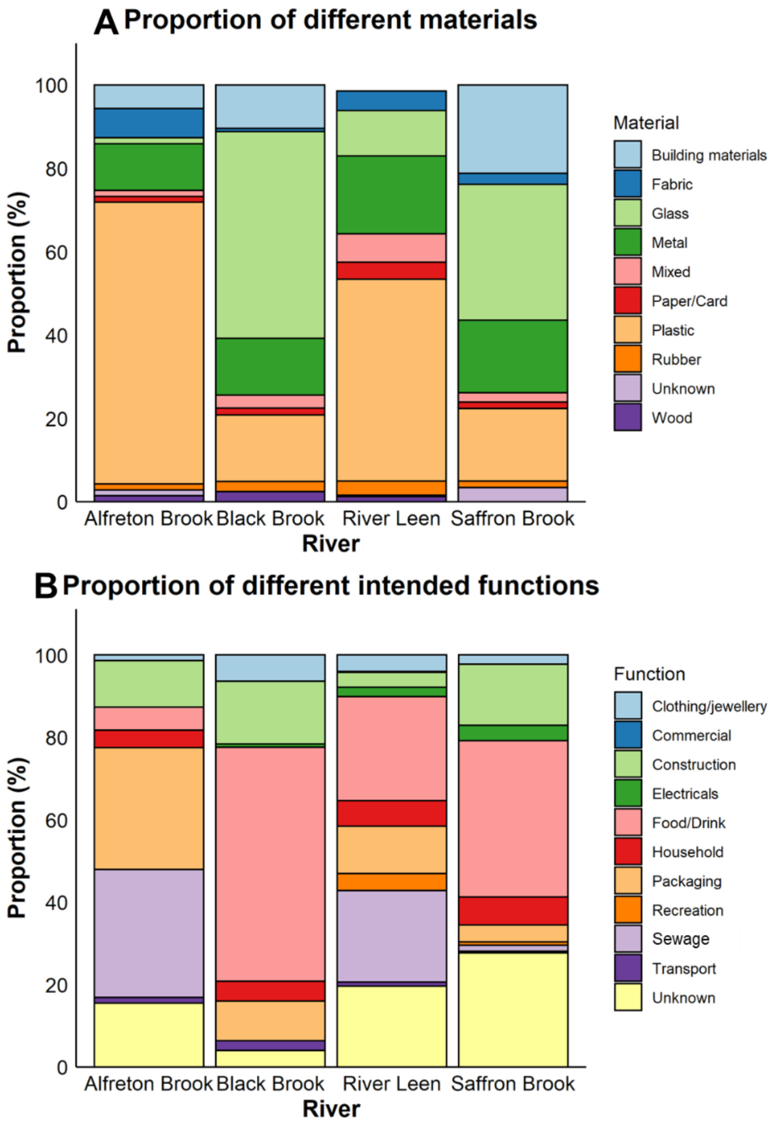


Figure 4.3: The litter density as litter items m^{-2} found in each of the sampled sections sorted by reach. Density was calculated via summation of litter found in each section divided by the total area of river bed sampled. Boxes are coloured according to river.

The types of materials and intended functions of anthropogenic litter were highly variable between rivers (Figure 4.4). Only one piece of glass was found at AB, but glass made up half of the litter items at BB and 33% of items in SB. Plastic items were common across sites, especially in RL (48%) and AB (68%), which also had high proportions of sewage waste (22% in RL and 31% in AB). Sewage related litter was much rarer in BB and SB. Food and drink related litter was the most common intended function of litter in BB (57%), SB (38%) and RL (25%), but was rare in AB where the majority of litter was packaging or sewage related. Metal items made up modest proportions at all sites (11 to 18%), whereas paper and wood were always rare. Building materials only made up high proportions in BB and SB (10% and 21% respectively). This was primarily bricks, but notably asbestos was found in RL and SB. These materials, and some metal items, made up the majority of construction waste which made up 11 to 15% of litter items at BB, SB, and AB. Only 12 items (1% of the total) were made of unknown materials.



		Alfreton Brook	Black Brook	River Leen	Saffron Brook
Material	Building materials	5.6	10.4	1.5	21.2
	Fabric	7.0	0.8	4.6	2.7
	Glass	1.4	49.6	10.9	32.6
	Metal	11.3	13.6	18.7	17.4
	Mixed	1.4	3.2	68.0	2.3
	Paper/Card	1.4	1.6	4.1	1.5
	Plastic	67.6	16.0	48.4	17.4
	Rubber	1.4	2.4	3.4	1.5
	Wood	1.4	2.4	1.2	0.0
	Other-Unknown	1.4	0.0	0.3	3.4
Intended usage	Clothing/jewellery	1.4	6.4	4.0	2.3
	Commercial	0.0	0.0	0.1	0.0
	Construction	11.3	15.2	3.6	14.8
	Electricals	0.0	0.8	2.3	3.8
	Food-Drink	5.6	56.8	25.3	37.9
	Household	4.2	4.8	6.2	6.8
	Packaging	29.6	9.6	11.4	4.2
	Recreation	0.0	0.0	4.2	0.8
	Sewage	31.0	0.0	22.2	1.5
	Transport	1.4	2.4	0.9	0.4
	Unknown	15.5	4.0	19.7	27.7

Figure 4.4: The proportions of total litter in each river that was made of each A) material and B) intended function category.

The most common items of identifiable anthropogenic litter are shown in Figure 4.5. Glass bottles (or fragments of bottles) were the most common litter items in BB and SB and were second in RL; making up 17% of all litter found overall. Metal drinks cans were also common in these rivers, suggesting that litter from beverage consumption was prevalent. Wet wipes were the next most frequently sampled item after glass bottles, but these were only found in AB, SB and RL. AB also had high numbers of sanitary pads (the second most common litter item in this river) suggesting that sewage is a significant source of litter here (31% of litter in AB was sewage related). Other single use packaging items like plastic bags, plastic packaging, plastic food packaging and metal foil wrappers were also in the top ten most common litter types. The high abundance of asbestos and ceramic tiles and bricks suggests that fly-tipping of construction waste also makes up a notable proportion of anthropogenic litter. Mixed fishing equipment were only found at RL and were almost all children's rock-pooling nets, probably sourced from the public park just upstream.

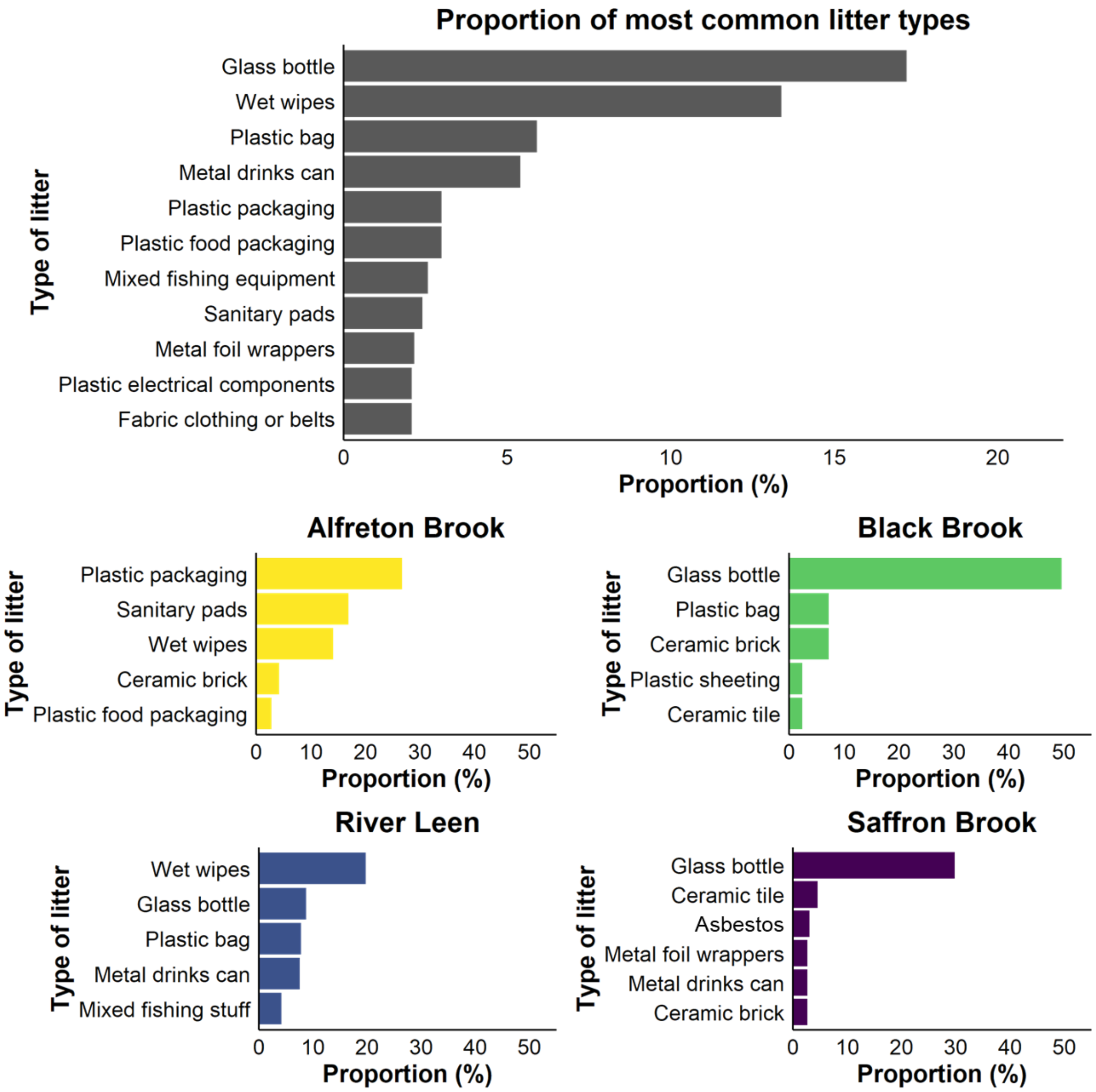


Figure 4.5: The most common anthropogenic litter types across all rivers, and the top five items for each of the four rivers (six types are shown for Saffron Brook as two types were joint for fifth). Items which could not be identified (e.g. plastic other) have been excluded from this figure.

4.3.2. Macroinvertebrates

Across all samples, 68 taxa from 46 families were found (Table 4.2). There were three non-native taxa found: *Crangonyx pseudogracilis* (found in SB and RL), *Potamopyrgus antipodarum* (found in all rivers), and *Planaria torva* (only found in SB). The full taxa list is shown in Table 4.S 2.

Table 4.2: The characteristics of macroinvertebrate communities found in each of the sampled rivers.

River	Number of taxa represented	Mean abundance per sample (±SE)
Alfreton Brook	23	1916 (± 246)
Black Brook	49	440 (± 63)
River Leen	55	1624 (± 168)
Saffron Brook	39	1016 (± 174)

The dominant taxa were common across rivers. These were Oligochaeta, *Gammarus pulex/fossarum* agg., and Chironomidae (mostly Chironomini, Orthocladiinae/Diamesinae, and Tanytarsini); all of which made up more than 10% of total individuals found in all rivers, excepting *G. pulex/fossarum* agg. which was slightly less common in BB and RL (< 7%). Simuliidae were also very common in RL (10% of individuals collected). There was a low abundance of gastropods, apart from *P. antipodarum* which was very common in RL and AB (23% and 31% respectively). Ephemeroptera, Plecoptera and Trichoptera taxa (EPT taxa) were uncommon, although *Baetis* sp. made up 5% or more of individuals sampled in all rivers, and *Serratella ignita* individuals made up 13% of the total abundance in BB. There were 13 Trichoptera (caddisfly) taxa found overall, mostly in BB (10 taxa found) and RL (11 taxa). However, their abundances were low, and only two caddisfly individuals were found in Alfreton Brook (both *Hydroptila* sp.).

Figure 4.6 shows that there was no relationship between the area of river bed sampled and the macroinvertebrate abundance, ${}^{1}D$ and ${}^{2}D$. Area sampled and ${}^{0}D$ were positively associated, as is often the case in ecology. However, as none of the other metrics were correlated with area, and because the trend in ${}^{0}D$ is mainly caused by the largest areas being in RL where taxon richness was highest, the kick sampling method was considered to be an appropriate sampling method that adequately represents the macroinvertebrate community regardless of the area of river bed sampled.

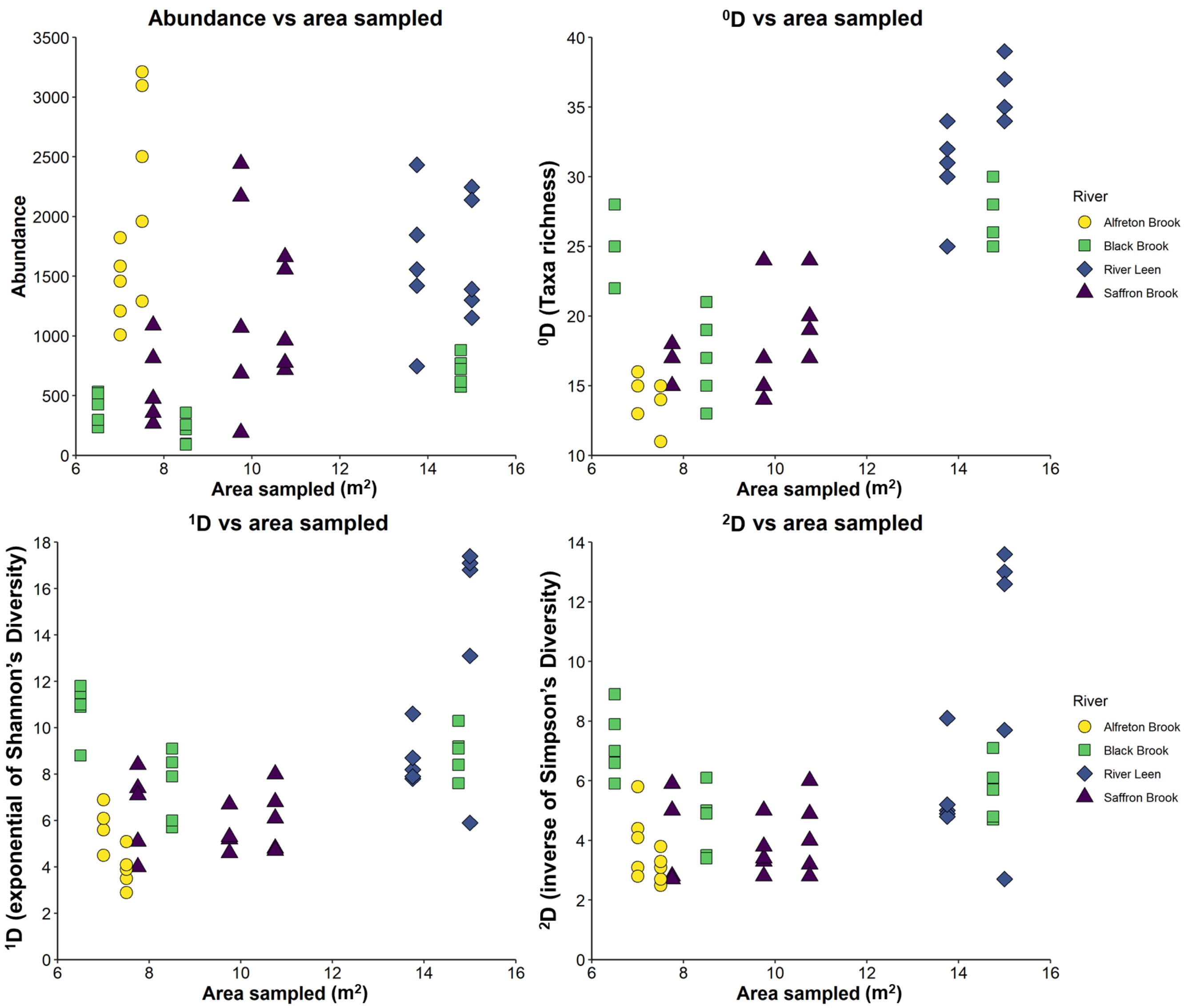


Figure 4.6: Scatterplots of the area of river bed sampled and the macroinvertebrate abundance and diversity found in each section. Different colours/symbols are used to display the data from each river.

LME models found no relationship between litter density and either macroinvertebrate abundance or ^{0}D (Figure 4.7). However, litter density was significantly and positively associated with ^{1}D (X^2 (1) = 5.71, $p = 0.02$) and ^{2}D (X^2 (1) = 6.30, $p = 0.01$). For every 1 litter item m^{-2} increase in density, these models predict a 0.57 ± 0.22 increase in ^{1}D and 0.52 ± 0.18 increase in ^{2}D. This indicates that the community was more diverse in sections where anthropogenic litter density was higher. It represents an increase in evenness, as taxon richness did not change with litter density.

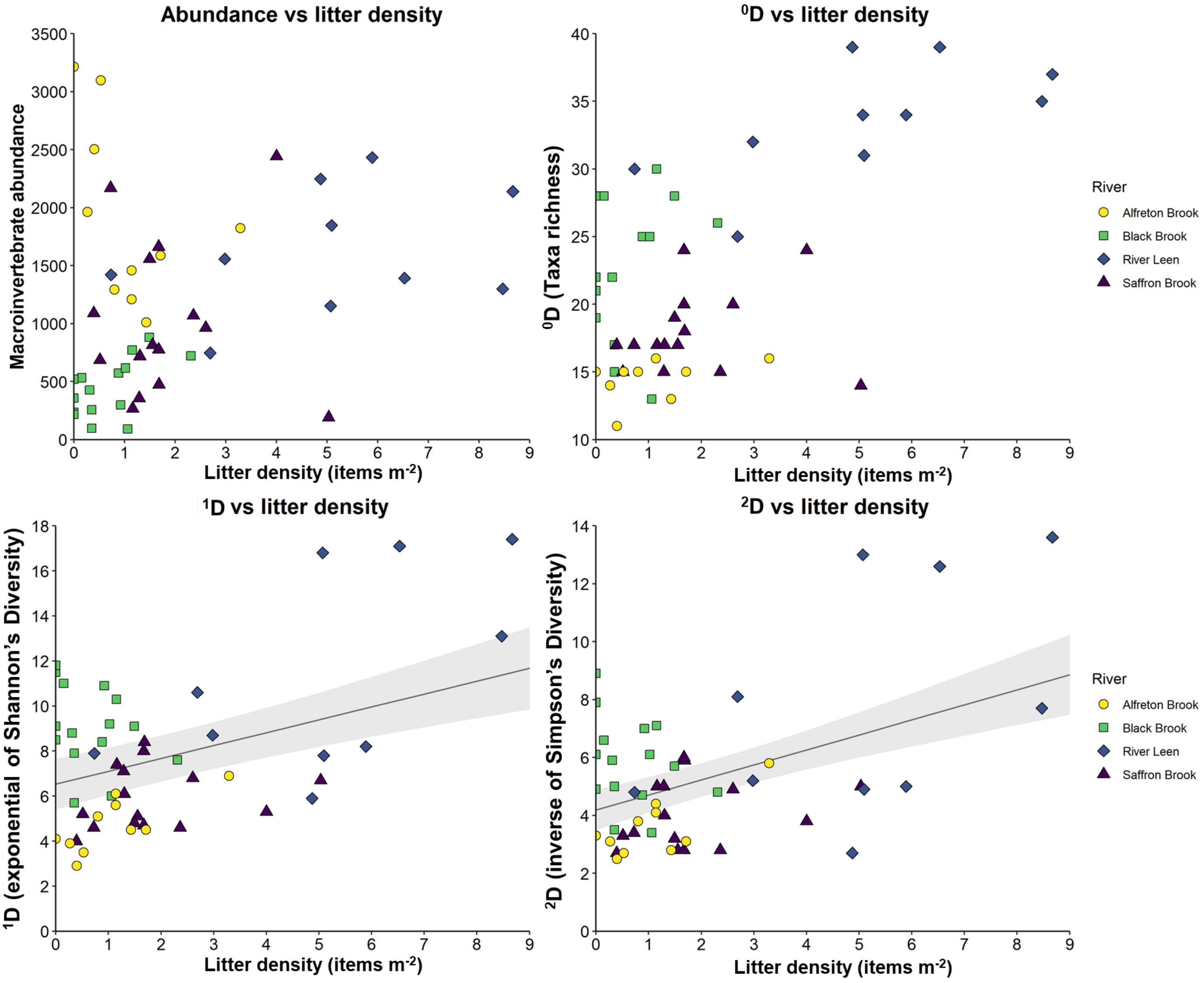


Figure 4.7: Scatterplots showing the relationship between litter density (number of items m^{-2}) with macroinvertebrate A) abundance, B) ^{0}D (taxon richness), C) ^{1}D (exponential of Shannon's Diversity), and D) ^{2}D (inverse of Simpson's Diversity). The trendline is that created by the relevant Linear Mixed Effect model with a shaded error band shown according to the standard error of the predicted values. Different colours/symbols are used to display the data from each river.

4.4. Discussion

4.4.1. Anthropogenic litter in sampled rivers

The density of anthropogenic litter found in this study varied between rivers and reaches. In particular, RL sites had far greater litter density (mean = 5.1 ± 0.8 items m^{-2}) than other rivers. The sites surveyed in this chapter were specifically chosen to cover a range of litter densities, but examining the differences between sites can provide information about the sources of litter and why densities might differ between sites. For instance, it is likely that the higher density of litter in the RL relates to its surrounding land use and high level of accessibility. Past researchers have found positive relationships between the amount of anthropogenic litter in a river and various metrics of human activity in the surrounding area, such as the distance to roads or footpaths, number of parking spaces, amount of pedestrian traffic, and intensity of urban development (Williams and Deakin, 2007; McCormick and Hoellein, 2016; Carpenter and Wolverton, 2017; Cowger *et al.*, 2019). RL sites were situated close to Bulwell town centre, which is a densely built-up area with a high local population, and easy public access to the river via footpaths on both banks and a park with children's play area. Highly littered sites like this are often seen as less valuable, encouraging a positive feedback that means people are more likely to litter at a site that already has litter (Williams and Simmons, 1999; Bator *et al.*, 2011). In contrast to the RL, AB sites were much less accessible. Although there is a footpath with public access along the river, the sites were distant from any large population centres and only a few people were seen during sampling. Dense trees on both banks also inhibit riverside access. This probably explains why there was much less food and drink related litter in this river compared to the others. Food and drink anthropogenic litter predominates in areas with high numbers of pedestrians and nearby commercial services (Williams and Deakin, 2007).

Another difference between the surveyed rivers is that sewage related anthropogenic litter was never found in BB and was rare in SB, but made up 31% of litter in AB and 22% in RL. Therefore, around a quarter of the anthropogenic litter in these rivers has likely come from sewage, probably through CSOs or misconnected sewage pipes. Other studies have found similarly high amounts of sewage related litter in rivers. Williams and Simmons (1999) found that 23% of litter in the River Taff (South Wales) was sewage related, which they linked to the presence of CSOs. More than 20% of litter caught in fyke nets in the River Thames (London, UK) was sewage related litter (Morritt *et al.*, 2014), and extremely high densities of wet wipes (50 to 200 wet wipes m^{-2}) have also been found at several sites on the foreshore of the Thames in London

UK (McConville *et al.*, 2020). This is concerning as it implies that raw sewage has been released into these rivers, posing a risk to people as well as river organisms (Seager and Abrahams, 1990; Mulliss *et al.*, 1997). The presence of sewage related litter in rivers seems to be one of the main differences between marine and riverine litter (Winton *et al.*, 2020). However, not all rivers have sewage litter; McCormick and Hoellein (2016) found that less than 1% of litter was sewage related in their survey of rivers in Chicago, USA.

Anthropogenic litter density was also highly variable within reaches. For example, the litter density in RL2 ranged from 0.7 to 5.9 items m^{-2}; a more than 700% increase between two sections that were only 25 m apart. Litter clustering could be a result of local spatial heterogeneity in anthropogenic litter sources. For example, fly-tipping can create small patches of extremely high litter density (Rech *et al.*, 2015). Alternatively, this could be a result of patterns in the movement and retention of litter within the river. Rivers transport litter in the flow until either an obstacle, or changes in flow velocity, cause litter to be deposited and stored. Litter accumulation rates are known to be high around vegetation (Williams and Simmons, 1997; van Emmerik *et al.*, 2019b), infrastructure like bridges or dams (Hoellein and Rochman, 2021; Kiessling *et al.*, 2021), or slow flowing areas like meander bends or river margins (McConville *et al.*, 2020). If sediment is deposited with the litter, this could lead to partial or complete burial of litter and therefore longer-term retention of litter within the river (McCormick and Hoellein, 2016; Liro *et al.*, 2020). River properties, such as flow conditions, channel shape, and vegetation, are therefore important in determining small-scale litter patterns.

It was also noticed that large, immovable items of litter could promote further accumulation of litter by acting as a key piece upon which other items of litter, as well as organic matter and fine sediment, could gather (Figure 4.8). For instance, at RL2, a metal box (45 x 41 x 11 cm) had collected 18 other pieces of litter. At BB2, 89% of the litter found in one section (8 pieces in total) were entangled around a metal radiator. The same effect was observed for shopping trolleys by Williams and Deakin (2007), although no study is known to have investigated this yet. The phenomenon is similar to the effect of large wood in rivers; a key piece of wood obstructs flow and reduces local flow velocity, encouraging the deposition of additional wood, sediment and organic matter (Gurnell *et al.*, 2002). The development of litter jams is particularly interesting because they could affect river geomorphology, and thus habitats, by virtue of their size.

Figure 4.8: Anthropogenic litter accumulations and interactions with wood, organic matter and sediment observed during the course of this fieldwork.

4.4.2. Comparison of litter in the surveyed rivers with records from other aquatic systems

The density of anthropogenic litter found in this study ranged from 0 to 8.7 items m^{-2}, with a mean density (± SE) of 2.0 ± 0.3 items m^{-2}. This is at the upper end of the values recorded in previous riverine studies (Table 4.3) and these results fall within the range found by studies from a range of aquatic environments. For comparison, marine beach litter has been much more widely studied and reported densities are hugely variable, from more than 600 items m^{-2} (Lavers and Bond, 2017), to more commonly reported values of between 1 to 5 items m^{-2} (e.g. Abu-Hilal and Al-Najjar, 2004; Rosvelt *et al.*, 2013; Aytan *et al.*, 2019; Ertas, 2021;) similar to those found in the rivers surveyed here. Litter density on the sea floor tends to be lower. Typical benthic litter densities found by marine studies are well below 0.1 items m^{-2} (Galgani *et al.*, 2000; Mordecai *et al.*, 2011; Pham *et al.*, 2014; Maes *et al.*, 2018). This provides further evidence that rivers are affected by significant amounts of litter and so a broadening of research focus to include rivers, as well as marine systems, is needed.

It is worth noting, however, that it is challenging to compare between studies given the huge variety in sampling methods and units of measurement. In particular, many surveys measured litter by transect-based abundance (items m^{-1}), only report percentage abundance of litter types, or only consider certain types of litter.

Most previous studies have found that plastic was the most dominant litter material (Table 4.3), as was true for AB and RL in this study (67 and 48% of litter was plastic in each river respectively). Plastic also dominates in marine environments (Galgani *et al.*, 2000; Pham *et al.*, 2014; Aytan *et al.*, 2019), and this is often the justification for focusing anthropogenic litter research exclusively on plastic. However, the most common material found in BB and SB was glass (50% and 33% respectively). Metal and building materials were commonly found in the sampled rivers, as well as smaller amounts of fabric, paper, rubber, and wood. Excluding these materials is likely to lead to underestimations of the quantity of anthropogenic litter in rivers (Vriend *et al.*, 2020), especially given that the longevity of many of these materials is similar to that of plastic. It is possible, however, that the high relative abundance of glass items could be an artefact of the fragility of this material. 85% of all the glass bottles recorded in this study were fragments rather than whole items, therefore individual bottles could have been recorded multiple times. This is a problem with quantifying anthropogenic litter by number (García-Rivera *et al.*, 2017).

Table 4.3: Published values from studies which quantified the density (items m^{-2}) of anthropogenic litter (or just plastic) in rivers. N refers to the number of samples each statistic is based on. Location is the river(s) and country in which the study was based. Habitat shows whether the survey was based on the river banks (riparian) or the river bed (benthic), and the size limit details the minimum size (cm) at which litter was collected in each study. For studies that collected all anthropogenic litter, % plastic items is the percentage of all items which were plastic (shown as a range when studies reported values for multiple sites separately).

	Reference	Statistic	Litter density (items m^{-2})	N	Location	Habitat	Survey method	Size limit (cm)	% plastic items
All anthropogenic litter	This study	Mean (±SE)	2.00 (± 0.30)	50	Rivers in Nottinghamshire and Leicestershire (UK)	Benthic	Manual collection	1	16 to 68%
	Hoellein *et al.* (2014)	Mean (±SE)	0.10 (± 0.02)	3	North Branch of Chicago River (Chicago, USA)	Riparian	Manual collection	1	53%
		Mean (±SE)	0.08 (± 0.02)	3		Benthic	Manual collection	1	16%
	Rech *et al.* (2015)	Range in median per river	0.14 to 3.42	20	Rivers in Chile	Riparian	Manual collection	1.5	not recorded
	McCormick and Hoellein (2016)	Mean (±SE)	0.29 (± 0.08)	15	Rivers near Chicago (USA)	Riparian	Manual collection	1	48 to 65%
		Mean (±SE)	0.12 (± 0.02)	15		Benthic	Manual collection	1	21 to 46%
	Bruge *et al.* (2018)	Range in mean per river	0.01 to 4.00	240	Adour River (France)	Riparian	Manual collection	0.5	94%
	Kiessling *et al.* (2019)	Mean (±SD)	0.54 (± 1.20)	1564	Rivers in Germany	Riparian	Manual collection	not recorded	31%
	Owens and Kamil (2020)	Standing stock	0.58	1	Tukad Badung River (Bali)	Riparian	Manual collection	not recorded	88%
		Standing stock	3.26	1	Karamana River (Kerala, India)	Riparian	Manual collection	not recorded	81%
	Schöneich-Argent *et al.* (2020)	Range in mean per river	0.0014 to 0.0055	558	Estuaries of Rivers Elms, Weser and Elbe (Germany)	Benthic	Trawling	0.5	65 to 100%
Just plastic	Battulga *et al.* (2019)	Mean	0.15	12	Selenga River (Mongolia)	Riparian	Manual collection	2	
	Blettler *et al.* (2019)	Range	0.35 to 5.05	6	Paraná River (Argentina)	Riparian	Manual collection	2.5	
	Bernardini *et al.*, 2020	Mean (±SD)	27.70 (± 41.79)	1056	River Thames (London, UK)	Riparian	Manual collection	not recorded	
	Mitchell *et al.* (2021)	Mean (±SD)	0.61 (± 0.43)	6	Paraná River (Argentina)	Riparian	Manual collection	2.5	

Some studies have addressed this issue of quantifying litter by measuring anthropogenic litter density by mass (e.g. McCormick and Hoellein, 2016; Bruge *et al.*, 2018; Cowger *et al.*, 2019). This is useful as it also gives an indication of the size of the litter, which could conceivably be related to the impacts it has on river organisms (Windsor *et al.*, 2019). However, to get an accurate measure, litter needs to be cleaned and fully dried to compensate for the varying amounts of water absorbed by different materials and sediment stuck to its surface (e.g. McCormick and Hoellein, 2016). This was not possible in this study due to limits on time and laboratory space. Additionally, 38 anthropogenic litter items could not be removed from the river bed because they were too embedded or heavy to move, and excluding these large items from mass calculations would significantly bias data. This included several scaffolding poles, an oil drum, part of a brick wall, a piece of corrugated iron, and several car tyres. Similarly, asbestos could not be handled as specialised equipment would be needed to investigate this litter material. Measuring item surface area is a possible alternative that, unlike measuring mass, has the benefit of not being biased by item density, which varies substantially between anthropogenic litter materials. It is, however, time consuming to measure, so would be difficult to apply in practice. Litter counts remain the most common method and could be objectively applied to all sites in this study, thus it was deemed an acceptable approach to meet the aims of this study. However, future research should ideally seek a unified approach (McCormick and Hoellein, 2016; Owens and Kamil, 2020), measuring both abundance and mass or surface area by unit area.

This study is not the only one to have found that plastic litter is less dominant in the river bed compared to the riparian zone. Hoellein *et al.* (2014) and McCormick and Hoellein (2016) measured litter in benthic and riparian habitats of rivers in Chicago and found that plastic was much more common on banks, and glass or metal more common in the river channel. This pattern is probably because plastic is generally less dense and often has buoyant properties, meaning that plastic litter is more easily transported by the river, more likely to get stranded on riparian vegetation, and more likely to be deposited on banks during floods (McCormick and Hoellein, 2016; Kiessling *et al.*, 2019). Although litter in riparian and benthic zones is probably interrelated due to regular transfers between habitats, these differences in litter composition suggest that using riparian surveys to approximate litter within the river channel may bias towards plastic materials. This is a challenge for future studies, especially those in larger rivers, where manual collection of benthic or suspended litter is time consuming and often impossible. Notably, only 0.7% of anthropogenic litter recorded during this study was floating, and my observations suggest that these items are rapidly transported through the sampled rivers because their channels have been artificially straightened and cleared of obstacles to enable urban development. Floating litter looked like it had only recently been introduced to the river, as it had no biofilm coverage.

In terms of item function, the majority of anthropogenic litter across sites in this study was food and drink related (especially food packaging and beverage bottles or cans) or sewage related waste (mostly wet wipes and sanitary pads). This kind of single-use litter has been found to predominate along rivers and coasts by many other researchers. Common litter types across studies worldwide include: drinks bottles and cans, plastic bags, food wrappers, cigarette butts, cotton buds, and wet wipes (Lahens *et al.*, 2018; Blettler *et al.*, 2019b; McConville *et al.*, 2020; Schirinzi *et al.*, 2020; van Emmerik *et al.,* 2020). Although the relative amount of these types of litter varies between studies, they are consistently dominant (Winton *et al.*, 2020), and so there have been many calls to target single use litter, especially plastic, in recent years (McConville *et al.*, 2020; McDermott *et al.*, 2021). However, it is worth mentioning that anthropogenic litter surveys often find small amounts of incongruous litter which is unique to individual sites. For example, I found several children's rock-pooling nets in the RL because just upstream of the sampling sites is a shallow area used by children to paddle and fish. This underlines the fact that the signature litter found at a specific site is likely to be unique to that site.

4.4.3. The relationship between anthropogenic litter density and macroinvertebrates

This study found a positive correlation between macroinvertebrate diversity (^1D and ^2D) and the density of anthropogenic litter. This counter-intuitive result will be explored in more detail in subsequent chapters, but the most probable mechanism leading to this result is that the presence of litter increases habitat heterogeneity. Anthropogenic litter comes in a range of shapes, sizes, and physical properties, making litter a novel and diverse substrate for macroinvertebrates to colonise. For instance, bottles provide shelter within their interiors (Katsanevakis *et al.*, 2007; Czarnecka *et al.*, 2009), and solid materials provide hard surfaces which are preferred by many taxa (Czarnecka *et al.*, 2009; Adamiak-brud *et al.*, 2015). High densities of litter therefore, may be able to alter the structure of the river bed and increase habitat heterogeneity, thereby enabling the river to support more diverse communities. This could be particularly relevant in rivers like those sampled in this study which have been straightened and cleared of natural habitat structures such as large wood and marginal vegetation.

Unlike for larger organisms, entanglement with litter is less likely to pose a risk to invertebrates due to their small relative size (Windsor *et al.*, 2019). No known studies have investigated whether riverine litter can trap macroinvertebrates as happens on land (e.g. Romiti *et al.,* 2021), but this seems unlikely given that most aquatic macroinvertebrates are adept at moving around in water. Additionally, entanglement risks are thought to be lower in rivers compared to marine environments because of the smaller size of its fishing industry (van Emmerik and Schwarz, 2019), which is the main cause of entanglement in seas and oceans (Gall and Thompson, 2015). It is possible that macroinvertebrates which have colonised litter could be affected by the leaching of chemicals from the litter, but whether or not macroinvertebrates in direct contact with anthropogenic litter are at risk is not yet known.

Similarly, macroinvertebrates can only ingest tiny particles much smaller than their body size, like microplastics (e.g. Scherer *et al*., 2017; Windsor *et al*., 2019). If microplastic concentration is related to anthropogenic litter it is possible that the macroinvertebrates inhabiting sites with the highest litter densities were affected by microplastics. However, it is uncertain whether such a correlation exists, given the difficulties in comparing the amounts of these different pollutants due to the different sampling methods used to measure them. Anthropogenic litter is a source of microplastics though plastic degradation (Andrady, 2011), and microplastics have also been found in greater quantities around urban areas (Luo *et al*., 2019; Grbić *et al*., 2020; Weideman *et al*., 2020). However, microplastics are much more mobile as they require less energy to entrain and transport (Windsor *et al*., 2019; Weideman *et al*., 2020), and not all sources of microplastics are sources of litter (e.g. runoff from agricultural land which has had sewage sludge applied: Horton and Dixon, 2017). Even if there was a correlation, the effects of microplastics are uncertain given that ingestion studies are typically restricted to single organisms, and our understanding of a dose-effect relationship is limited by the use of unrealistic litter concentrations and environmental conditions (Phuong *et al*., 2016; Koelmans *et al*., 2017). Although still an emerging area of science, meaning risks are not yet fully understood, several reviews have found that evidence for negative effects of microplastics at current environmental concentrations is weak (Burns and Boxall, 2018; Everaert *et al*., 2018).

4.4.4. Conclusions and implications

Through this study I have explored some of the characteristics of litter found in a range of small urban rivers in the UK. There is a large range in litter density and composition between and within rivers, which can be linked to differences in human activity and patterns of litter retention. Many of these results are similar to those of other studies, but the lower proportion of plastic found in this project suggests that overly focusing research on plastic risks ignoring a significant portion of the litter problem. In particular, only sampling riparian or floating litter means large amounts of riverine litter will not be recorded and measured litter assemblages will be skewed away from denser materials such as metal, glass, ceramic and masonry.

A positive correlation between anthropogenic litter density and macroinvertebrate diversity was also found. Given the relatively small number of sites, and the large scatter in the relationship, this deserves more investigation, but it could reflect an increase in habitat diversity created by the presence of litter. As such, removal of litter could be damaging to macroinvertebrates and other organisms that live on and around litter (Backhurst and Cole, 2000).

4.5. Supplementary materials

Table 4.S 1: Classification of anthropogenic litter by item type, primary material, and intended function.

Code	Item type	Material	Intended function
1	Plastic bag	Plastic	Packaging
2	Plastic bottle	Plastic	Food/drink
3	Plastic food packaging	Plastic	Food/drink
4	Plastic car parts	Plastic	Transport
5	Plastic lighter	Plastic	Household
6	Plastic stationery	Plastic	Household
7	Plastic crisp packet	Plastic	Food/drink
8	Plastic sweet packet	Plastic	Food/drink
9	Plastic toy	Plastic	Household
10	Plastic cup	Plastic	Food/drink
11	Plastic cutlery	Plastic	Food/drink
12	Plastic mesh bag	Plastic	Packaging
13	Plastic string or webbing	Plastic	Unknown
14	Plastic sheeting	Plastic	Packaging
15	Plastic gun cartridge	Plastic	Other
16	Plastic shoe	Plastic	Clothing/Jewellery
17	Plastic DVD, cassette tape or CD	Plastic	Household
18	Plastic cards e.g. credit card	Plastic	Household
19	Plastic electrical components	Plastic	Electricals
20	Plastic coat hanger	Plastic	Household
21	Plastic packaging	Plastic	Packaging
22	Plastic other	Plastic	Several possible categories
23	Rubber balloon	Plastic	Household
24	Rubber tyre	Plastic	Transport
25	Rubber band	Plastic	Unknown or household
26	Rubber tennis ball	Plastic	Recreation
27	Rubber gloves	Plastic	Unknown
28	Rubber hair band	Plastic	Clothing/Jewellery
29	Rubber other	Plastic	Several possible categories
30	Fabric clothing or belts	Fabric	Clothing/jewellery
31	Fabric furnishing or carpet	Fabric	Household
32	Fabric sacking	Fabric	Construction
33	Fabric shoe or lace	Fabric	Clothing/jewellery
34	Fabric car part	Fabric	Transport
35	Fabric toy	Fabric	Household
36	Fabric other	Fabric	Several possible categories
37	Paper cardboard	Paper	Several possible categories
38	Paper smoking wrappers	Paper	Food/drink
39	Paper cigarette butts	Paper	Food/drink
40	Paper food packaging	Paper	Food/drink
41	Paper other	Paper	Several possible categories
42	Wood lollipop stick	Wood	Food/drink

43	Wood other	Wood	Several possible categories (mostly construction)
44	Metal bottle caps	Metal	Food/drink
45	Metal drinks can	Metal	Food/drink
46	Metal household appliances	Metal	Household
47	Metal foil wrappers	Metal	Food/drink
48	Metal food packaging	Metal	Food/drink
49	Metal oil drum	Metal	Unknown
50	Metal wire	Metal	Unknown
51	Metal jewellery	Metal	Clothing/jewellery
52	Metal battery	Metal	Electricals
53	Metal stationery	Metal	Household
54	Metal cutlery	Metal	Household
55	Metal tools	Metal	Construction or household
56	Metal nitrous oxide canister	Metal	Recreation
57	Metal coin	Metal	Household
58	Metal toy	Metal	Household
59	Metal shopping trolley/basket	Metal	Commercial
60	Metal broom/hoover part	Metal	Household
61	Metal sign	Metal	Construction
62	Metal key	Metal	Household
63	Metal cooking equipment	Metal	Household
64	Metal other	Metal	Several possible categories
65	Glass bottle	Glass	Food/drink
66	Glass cups	Glass	Household
67	Glass mirror	Glass	Household
68	Glass other	Glass	Several possible categories
69	Ceramic tile	Ceramic	Construction
70	Ceramic brick	Ceramic	Construction
71	Ceramic crockery	Ceramic	Household
72	Ceramic other	Ceramic	Several possible categories
73	Sanitary pads	Plastic	Sewage
74	Tampons	Plastic	Sewage
75	Wet wipes	Plastic	Sewage
76	Mixed umbrella	Mixed or plastic	Clothing/Jewellery
77	Mixed bike part	Mixed	Transport
78	Mixed phone part	Mixed	Electricals
79	Mixed fishing equipment	Mixed	Recreation
80	Chewing gum and blu tac	Plastic	Household or food/drink
81	Concrete	Building	Construction
82	Asbestos	Building	Construction
83	Unknown toy	Unknown	Unknown
84	Unknown rope	Unknown	Unknown
85	Unknown food packaging	Unknown	Unknown
86	Unknown	Unknown	Unknown

Table 4.S 2: List of the 68 taxa (indented) recorded during the study. Asterisks denote non-native species.

PLATYHELMINTHES
RHABDITOPHORA
Tricladida
Dugesia sp.
*Planaria torva**
Polycelis sp.

MOLLUSCA
GASTROPODA
Ectobranchia
Valvata piscinalis
Hygrophila
Physa fontinalis
Ancylus fluviatilis
Anisus vortex
Bathyomphalus contortus
Gyraulus albus
Gyraulus crista
Hippeutis complanatus
Littorinimorpha
Bithynia tentaculata
*Potamopyrgus antipodarum**
Stagnicola palustris
Radix balthica
BIVALVIA
Veneroida
Musculium sp.
Pisidium sp.
Sphaerium sp.

ANNELIDA
OLIGOCHAETA
CLITELLATA
Arhynchobdellida
Erpobdella octoculata
Rhynchobdellida
Glossiphonia complanata
Helobdella stagnalis

ARTHROPODA
MALACOSTRACA
Amphipoda
*Crangonyx pseudogracilis**
Gammarus pulex/fossarum agg.
Isopoda
Asellus aquaticus
INSECTA
Coleoptera
Nebrioporus elegans
Elmis aenae
Limnius volckmari
Oulimnius sp.
Haliplus sp.
Helophorus sp.
Diptera
Dasyhelea sp.
Ceratopogoninae
Chironomini
Orthocladiinae/Diamesinae
Prodiamesinae
Tanypodinae
Tanytarsini
Chironomidae pupae
Empididae
Ephydridae
Antocha sp.
Limoniidae
Limnophora sp.
Dicranota sp.
Psychodidae
Simuliidae
Stratiomyidae
Tipulidae
Ephemeroptera
Baetis sp.
Caenis luctuosa
Serratella ignita
Hemiptera
Sigara dorsalis
Megaloptera
Sialis lutaria
Odonata
Calopteryx splendens
Trichoptera
Brachycentrus subnubilus
Glossosoma boltoni
Agapetus sp.
Goera pilosa
Hydropsyche pellucidula
Hydroptila sp.
Lepidostoma hirtum
Athripsodes cinereus
Mystacides azurea
Polycentropus flavomaculatus
Psychomyia pusilla
Tinodes waeneri
Rhyacophila dorsalis

Chapter 5: Anthropogenic litter is a novel habitat for aquatic macroinvertebrates in urban rivers

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Author contributions to the article were as follows:

Hazel Wilson was responsible for study design, fieldwork, laboratory work, data analysis, and writing the article. Matthew Johnson, Markus Eichhorn and Colin Thorne (the supervisory team) advised on study design, data analysis, and commented on the article draft. Hazel was assisted in the identification of macroinvertebrates by Professor Paul Wood (Loughborough University), who also commented on the article draft.

5.1. Introduction

Growing public and political interest around problems of anthropogenic litter has encouraged a recent proliferation of studies into its occurrence and abundance in the environment, and its ecological impacts (e.g. Agamuthu *et al.*, 2019; Beaumont *et al.*, 2019). In marine ecosystems, anthropogenic litter has been shown to reduce organism fitness and cause mortality through entanglement and ingestion (Kühn *et al.*, 2015; Agamuthu *et al.*, 2019), expose organisms to harmful chemicals via leaching of pollutants (Rochman, 2015), modify physical habitat structure (Kiessling *et al.*, 2015), and aid the spread of invasive species (Tyrrell and Byers, 2007; Rech *et al.,* 2016). Despite urban rivers being some of the most anthropogenically modified landscapes on Earth (see review: Walsh *et al.*, 2005), and acknowledgement of the persistence and prevalence of anthropogenic litter in urban rivers (e.g. Rech *et al.,* 2015; McCormick and Hoellein, 2016), the effects of anthropogenic litter on these ecosystems have yet to be fully explored (Blettler *et al.,* 2018).

Urban rivers are typically limited in habitat diversity and quality due to historical channelization, dredging and bed/bank stabilisation works. Coupled with elevated concentrations of pollutants, and changed hydrological and sediment inputs, this has resulted in characteristic low diversity communities in many urban rivers (termed the 'Urban Stream Syndrome': Walsh *et al.,* 2005). Urban rivers also receive disproportionately large inputs of anthropogenic litter which, although undesirable, may provide different and more complex shapes and textures of substrate. Anthropogenic litter may also interact with flow patterns, increasing habitat heterogeneity in a similar manner to large rocks, wood and aquatic macrophytes. These natural habitat structures are largely absent in urban rivers due to regular removal practices and the high frequency of disturbance events (Blauch and Jefferson, 2019). Anthropogenic litter may therefore act as a proxy for habitats lost through urbanisation, supporting biodiversity which would otherwise be absent (Chapman and Clynick, 2006). For example, flexible anthropogenic litter may perform similar physical functions to aquatic macrophytes.

Macroinvertebrates are known to readily colonise artificial surfaces as long as they are non-toxic (e.g. the use of artificial substrate samplers; Beak *et al.*, 1973). As macroinvertebrate community structure is strongly related to habitat, especially the size, diversity, and arrangement of river bed substrates (Death, 2000; Jowett, 2003), this makes them useful model organisms to assess the effects of anthropogenic litter on urban rivers. The atypical physical structure of anthropogenic litter could provide a novel habitat for some macroinvertebrate taxa, offering opportunities for adaptable species to colonise, and resulting in a community distinct from those living on natural substrates (Czarnecka *et al.,* 2009). Anthropogenic litter with complex physical structure (e.g. items like bottles which have interiors: Czarnecka *et al.,* 2009; or items with rough surfaces: Boyero, 2003) may also be able to support a greater diversity of organisms through increasing available niche space. This may be especially true where the natural substrate is relatively inhospitable to macroinvertebrates, such as sandy or silty estuarine rivers and harbours with low bed stability (e.g. Hoggart *et al*., 2011; García-Vazquez *et al*., 2018). The quality and quantity of macroinvertebrate food resources are also affected by microhabitat conditions (Wallace and Webster, 1996) so changes in the abundance or diversity of food resources caused by anthropogenic litter may have cascading effects in macroinvertebrate communities. In addition, anthropogenic litter may preferentially support non-native species that may be better able to take advantage of the novel habitat than native species (Tyrrell and Byers, 2007; Katsanevakis, 2008).

The limited number of published studies examining fauna living on anthropogenic litter have been focussed on marine (e.g. Chapman and Clynick, 2006; Katsanevakis *et al.,* 2007; García-Vazquez *et al*., 2018; Rech *et al*., 2018) rather than freshwater environments. These studies report that experimentally introduced anthropogenic litter on a sandy sea bed may locally increase the abundance and diversity of benthic communities, where it provides habitat for hard-substratum dwelling species that are otherwise absent (Katsanevakis *et al*., 2007). But where comparable natural habitats are present, such as natural rocky reefs, there may be limited differences in patterns of colonisation of anthropogenic litter and natural substrates (Chapman and Clynick, 2006). So far, only two studies have considered pre-existing in situ anthropogenic litter as a component of freshwater habitats (Jatulewicz, 2007; Czarnecka *et al.,* 2009), where it was reported that the macroinvertebrate communities found on anthropogenic litter in Polish reservoirs were more diverse and considerably different in taxonomic composition to those on the surrounding sand bed, but were similar in diversity to those recorded on macrophytes. However, so far there have been no investigations of in situ anthropogenic litter undertaken within non-tidal river systems.

5.1.1. Research approach

In this study, I compared macroinvertebrate communities inhabiting anthropogenic litter with those on natural rock substrates in three urban rivers to provide a direct evaluation of the role of anthropogenic litter as riverine habitat. I anticipated that distinct communities would be recorded on the two substrate types, and that faunal diversity would be higher on anthropogenic litter given its heterogeneity relative to natural mineral substrates. If anthropogenic litter provides novel habitats, understanding how it affects macroinvertebrates is important in informing future urban stream management.

5.2. Methods

5.2.1. Study sites

Sampling was conducted in three small (1st or 2nd order) urban gravel-bed rivers in Leicestershire and Nottinghamshire, UK: the River Leen, Black Brook, and Saffron Brook (Figure 5.1). The sites were located near to BB3, RL1, RL2, and SB2 sites in Chapter 4. Each river was sampled over two consecutive days in September and October 2018. Straightened reaches with homogeneous substrate grain-size and morphology were selected to minimise any effect of natural morphological heterogeneity. Sites were similar in dimension, water quality and discharge, but differed in urbanisation intensity (Table 5.1).

Table 5.1: River characteristics at each sampling site. Water Framework Directive (WFD) classification in 2016 from the Catchment Data Explorer (Environment Agency, 2021b,c,d). Average width was determined during sampling. Distance from source was measured as river length from sampling site to source. Percentage of upstream catchment which is urban (% urban) was calculated by measuring the river length upstream of the sampling site and calculating the proportion of this which lies within the urbanised area on a 1: 50 000 scale OS map.

	Black Brook	**River Leen**	**Saffron Brook**
GPS Coordinates	52° 47’ N 01° 14’ W	52° 59’ N 01° 11’ W	52° 36’ N 01° 07’ W
Distance from source (km) (% urban)	15.3 (15%)	11.4 (25%)	7.9 (80%)
Average width at site (m)	4.0	5.5	3.8
WFD overall classification (2016)	Moderate	Moderate	Moderate

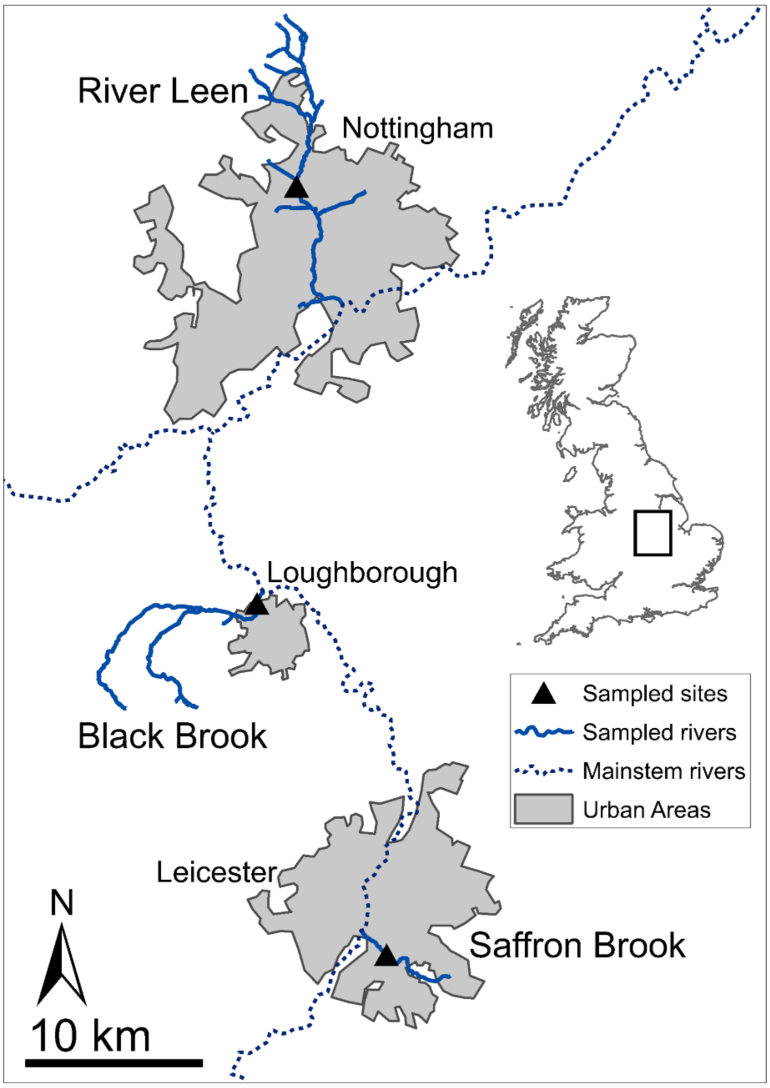

Figure 5.1: Map showing the three sampling sites (shown as triangles) on the River Leen, Black Brook and Saffron Brook relative to the urban areas they flow through and the mainstream rivers.

The predominant natural substrate (substrate is defined here as river bed material on which an organism lives) at all sites was gravel and cobbles (hereafter ‘rocks’), with some interstitial fine sediment (sand and silt). Rocks were comparable in size to anthropogenic litter pieces and could be easily isolated from the river bed to collect the macroinvertebrates inhabiting them (similar to anthropogenic litter). Hence rocks were chosen for comparison with anthropogenic litter. Both rocks and anthropogenic litter were sampled from the river bed surface for consistency. There was not any discernible structure to the bed sediments, such as armouring, as subsurface sediments were visually similar to those on the surface.

5.2.2. Field methods

Anthropogenic litter density was assessed at each site by measuring the area of river bed (average channel width x river length surveyed) containing 100 pieces of anthropogenic litter. Rock and anthropogenic litter samples were collected from the full width of the channel and the surface layer of the river bed. Whilst moving upstream in a grid pattern, I collected alternately encountered anthropogenic litter items (providing 50 samples at each site), and a representative sub-sample of 50 rocks by pacing through the sampling area and taking the rock immediately at the sampler's foot (Wolman, 1954). Only items larger than 1 cm in their b-axis length were sampled as smaller items were difficult to consistently collect and macroinvertebrate numbers would be low on such items. Items were described in terms of their material composition (fabric, glass, metal, plastic [including rubber], masonry, rock or other). Pieces of masonry (e.g. brick, concrete, and roofing tiles) were classified as rocks in comparisons of all anthropogenic litter types against all rocks, as it was thought that they may function like natural mineral substrates. However, masonry and rock samples were considered as separate materials in analyses of material types to test this assumption.

Macroinvertebrates were collected by transferring items (anthropogenic litter or rocks) from the river bed into a 1 mm mesh kick net held directly downstream (following Benke and Wallace's (2003) methodology for sampling macroinvertebrates on large wood). The contents of the net were placed into a sampling bag, along with the item, and preserved with Industrial Methylated Spirit. Large or embedded items were cleaned of macroinvertebrates in the field by scrubbing a set area of 0.03 or 0.06 m^2 depending on their exposed area (0.03 m^2 was roughly equivalent to the median surface area of anthropogenic litter pieces) with a brush to dislodge macroinvertebrates into a kick net held downstream (Pilotto *et al.,* 2016).

5.2.3. Laboratory methods

All anthropogenic litter and rock items were individually washed through a 500 µm mesh sieve, then manually processed to collect macroinvertebrates. Macroinvertebrates were identified to species or genus level where possible. Exceptions were Diptera and Sphaeriidae which were identified to family, Oligochaeta to subclass, and Acarina to order. Taxonomic levels were consistent between samples and sites. Identification followed Holland (1972), Macan (1977), Ellis (1978), Friday (1988), Wallace *et al.* (1990), Edington and Hildrew (1995), Reynoldson and Young

(2000), Killeen *et al.* (2004), Elliott and Humpesch (2010), Cham (2012), Dobson *et al.* (2012), and Elliott and Dobson (2015). Trichoptera (caddisfly) pupae (0.9% of total individuals), unlike larvae, could only be identified to family level so were excluded from further analysis. The data analyses outlined in Section 5.2.4 were repeated with family level data, which included caddisfly pupae, and findings were qualitatively identical (Table 5.S 1).

The surface area of each item (anthropogenic litter or rock) was approximated by wrapping the item in tin foil and weighing the resultant foil pieces (1g: 0.0214 m^2) (Dudley *et al.*, 2001). The surface area of flexible materials or items with complex shapes (e.g. plastic bags) was determined using equations for the surface area of the approximate geometric shape (Bergey and Getty, 2006). Items that were too large or embedded to be collected from the field were measured in situ.

5.2.4. Data analysis

All statistical analysis was conducted using R statistical software (version 3.6.3; R Core Team, 2020). Completeness of sampling was assessed by calculating coverage for anthropogenic litter and rocks at each site. This measure of sample completeness estimates the proportion of total individuals in a community that belong to taxa in the sampled community (Chao and Jost, 2012). Macroinvertebrate density was calculated by dividing the total macroinvertebrate abundance across taxa by the sampled surface area of an item (0.03 or 0.06 m^2 for partially sampled items). Macroinvertebrate diversity was assessed by calculating Hill's numbers in *vegan* (Oksanen *et al.,* 2020). ${}^0D$ is equivalent to observed taxon richness which places greater emphasis to rare taxa as it is insensitive to relative frequencies (i.e. evenness), ${}^1D$ is equivalent to the exponential of Shannon's Diversity index which is weighted towards common taxa, and ${}^2D$ to the inverse of Simpson's Diversity index which is weighted towards highly abundant taxa (Tuomisto, 2010). Each point in the series therefore provides complementary information on taxon richness and evenness.

The mean surface area of rocks (including masonry) was four times smaller than that of anthropogenic litter items (rocks: 0.03 m^2 ± 0.01 (SE), anthropogenic litter: 0.12 m^2 ± 0.02; two-sample Wilcoxon $W = 16899$, $p < 0.001$). Given that a strong positive relationship exists between item surface area and total macroinvertebrate abundance (Spearman's Rank (R_s) = 0.80, $p < 0.001$), as well as between surface area and observed taxon richness (${}^0D$) ($R_s = 0.79$, $p < 0.001$), all subsequent analysis controlled for surface area (by including area in Linear Mixed Effect models and Generalised Linear Models) to account for this difference between substrates.

To test for differences in macroinvertebrate density and diversity (^{0}D, ^{1}D and ^{2}D) between anthropogenic litter and rocks, linear mixed effects (LME) analysis was performed using *lme4* (Bates *et al.,* 2015) with significance calculated for parameter estimates using *lmerTest* (Kuznetsova *et al.,* 2017). To compare diversity, substrate (anthropogenic litter or rock) and sampled surface area were entered as fixed effects, and site (River Leen, Black Brook or Saffron Brook) included as a random effect. LME models for density excluded surface area, as this factor is already incorporated into the calculation of density for each item, but otherwise model structure was identical. Model validation and checking followed the protocol in Zuur *et al.* (2009). Significance values for the effect of substrate type were identified by likelihood ratio tests (distributed as Chi-square) of the full model against a null model without the substrate factor. LME analyses were repeated substituting the substrate factor for material composition using a single factor with seven levels: fabric, glass, metal, masonry, plastic, rock, and other. Significant differences between material types were examined using parameter estimates and associated *p* values calculated using Satterthwaite approximation in *lmerTest*. Thus, I looked for differences between substrates (anthropogenic litter and rock), and between material types (fabric, glass, metal, masonry, plastic, rock, or other) in separate analyses.

Macroinvertebrate community composition was compared using the *manyglm* function in *mvabund* (Wang *et al.,* 2021). The function fits generalised linear models (GLMs) to the raw counts for each taxa assuming a negative binomial distribution, with substrate type, sampled surface area, and site as explanatory variables without interactions. A Sum-of-*LR* test statistic was obtained with significance assigned using randomisation (999 permutations), where the *p* value is adjusted for multiple testing using step-down resampling. This approach deliberately specifies a mean-variance relationship, inherent to count data, meaning it can address the problems of confounded location and dispersion effects and difficulty detecting effects expressed in low-variance taxa, common to distance-based community analysis such as SIMPER and PERMANOVA (Warton *et al.,* 2012). *Manyglm* tests were also repeated substituting substrate for material composition.

Differences between communities were visualised using *boral* (Hui, 2020); a model-based approach to unconstrained ordination which fits a latent variable model to raw abundance data and can be interpreted in a similar way to non-metric multidimensional scaling (NMDS) ordination (Hui, 2015). Ordination assumed a negative binomial distribution, and sample identity effects were included so ordination is based on composition rather than relative abundance. Site was included as a fixed effect. Ordination was repeated for individual sites to visualise differences between material types within each site.

5.3. Results

5.3.1. Anthropogenic litter abundance and composition

Anthropogenic litter was abundant at all sites; 4.2 items m^{-2} in the River Leen, 1.1 items m^{-2} in Saffron Brook, and 0.6 items m^{-2} in Black Brook, comparable to results in Chapter 4. Anthropogenic litter material types included fabric, glass, metal, plastic, ceramic and wood. Fewer than five ceramic and wood items were collected across all sites so these have been collated hereafter as 'other' for simplicity. The proportional abundance of anthropogenic litter materials was similar across all sites, with glass, metal, and plastic the dominant materials across sites. These materials each made up approximately one third of the total anthropogenic litter items (Table 5.2). All fabric items were flexible, as were 5% of metal items, and 69% of plastic items, but no other materials were flexible. Rocks were generally less morphologically complex than anthropogenic litter, having been rounded by fluvial processes. Whilst most rocks were of natural origin, some appeared to be failed bank protection (based on visual comparison with nearby rip-rap), and 10% were masonry (brick, concrete or roofing tiles).

Table 5.2: Number of samples that fit into each material composition category for each river. Anthropogenic litter (fabric, glass, metal, other and plastic) and rock (masonry and rock) samples add up to 50 samples in each river for each substrate. Brackets indicate the percentage of sampled items in this material category which were flexible. Where there are no brackets, 0% of sampled items in this material category were flexible.

River	**Material composition**						
	Rock		Anthropogenic litter				
	Masonry	Rock	Fabric	Glass	Metal	Plastic	Other
Black Brook	2	48	5 (100%)	14	16	14 (93%)	1
River Leen	10	40	1 (100%)	14	12 (8%)	22 (55%)	1
Saffron Brook	3	47	4 (100%)	18	11 (9%)	13 (69%)	4

5.3.2. Differences in macroinvertebrate density and diversity

Across all sites, a total of 16,894 individuals from 46 families (61 taxa) were collected (see Table 5.S 2 for full list of taxa). The completeness of sampling (checked by calculating coverage) was >0.99 for anthropogenic litter and rock at all sites, indicating that sampling was close to completion. As such it is reasonable to compare estimates of diversity between anthropogenic litter and rocks, despite their differences in surface area. The density of macroinvertebrates was not significantly different between anthropogenic litter and rocks (χ^2 (1) = 0.81, $p = 0.37$), or between material types (χ^2 (6) = 7.73, $p = 0.26$) (Table 5.3). However, macroinvertebrate diversity was significantly higher on anthropogenic litter than on rock, indicating a consistent pattern across all sites and for all diversity measures (χ^2 (1) = 24.54 (^{0}D), 22.63 (^{1}D), 12.28 (^{2}D), $p < 0.001$; Figure 5.2). On average, observed taxon richness (^{0}D) was nearly four taxa per item higher on anthropogenic litter than on rocks, with a mean of 8.3 ± 0.5 (SE) for anthropogenic litter and 4.6 ± 0.4 for rocks. This difference was reduced at a higher order of D, suggesting that the higher diversity on anthropogenic litter reflected greater numbers of low abundance taxa with a small number of dominant taxa.

Table 5.3: Results of all statistical tests, showing test statistics, degrees of freedom (*df*) and significance (*p*) values. For full details on how statistical tests were performed see Section 5.2.4. Linear Mixed Effect (LME) models tested for differences in macroinvertebrate density and diversity (^{0}D, ^{1}D and ^{2}D) between substrates and between materials by including substrates/material and surface area as fixed effects (surface area was excluded for density tests), and site as a random effect. The test statistic for LME models is the Chi-square test statistic of a likelihood ratio test. The *manyglm* function tested for differences in community composition between substrates and materials, calculating a Sum-of-*LR* test statistic and associated *p* value with 999 permutations.

Statistical test	**Parameter tested**	**Differences between substrates (anthropogenic litter and rock)**			**Differences between materials (fabric, glass, masonry, metal, plastic, rock, and other)**		
		Test statistic	*df*	*p*	Test statistic	*df*	*p*
LME model	Density	0.81	1	0.369	7.73	6	0.259
	^{0}D (observed taxon richness)	24.54	1	0.001	52.18	6	0.001
	^{1}D (exponential of Shannon's Diversity)	22.63	1	0.001	37.20	6	0.001
	^{2}D (inverse of Simpson's Diversity)	12.28	1	0.001	19.26	6	0.003
manyglm	Community composition	508.5	1	0.001	1329.1	6	0.001

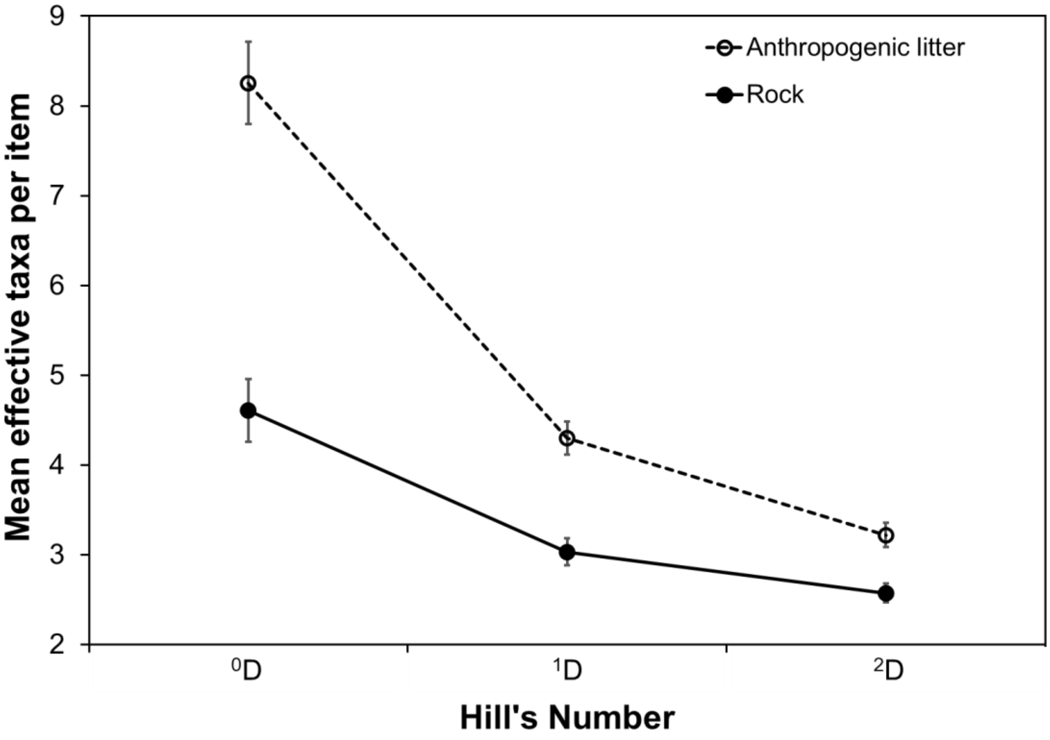

Figure 5.2: Mean Hill's numbers of ^0D (taxon richness), ^1D (exponential of Shannon's Diversity) and ^2D (inverse of Simpson's Diversity) calculated on all anthropogenic litter samples (dashed line) and all rock samples (including masonry; solid line). Error bars represent standard errors.

Material type (fabric, glass, metal, plastic, masonry, rock or other) also significantly affected diversity measures (χ^2 (6) = 52.18 (^0D), 37.20 (^1D), 19.26 (^2D), $p < 0.005$; Figure 5.3). Glass and rock samples were considerably less diverse than other material samples (mean ± SE ^0D per item was 5.1 ± 0.5, and 4.2 ± 0.3 respectively), especially fabric (11.0 ± 1.9) and plastic (10.9 ± 0.9). These differences were significant; rock samples were significantly less diverse than masonry, fabric, plastic and metal samples across all Hill's numbers. Glass samples were less diverse than plastic and metal samples at ^0D and ^1D, but were not different from other materials at ^2D. Plastic, metal, fabric and masonry samples consistently had the highest diversity across Hill's numbers.

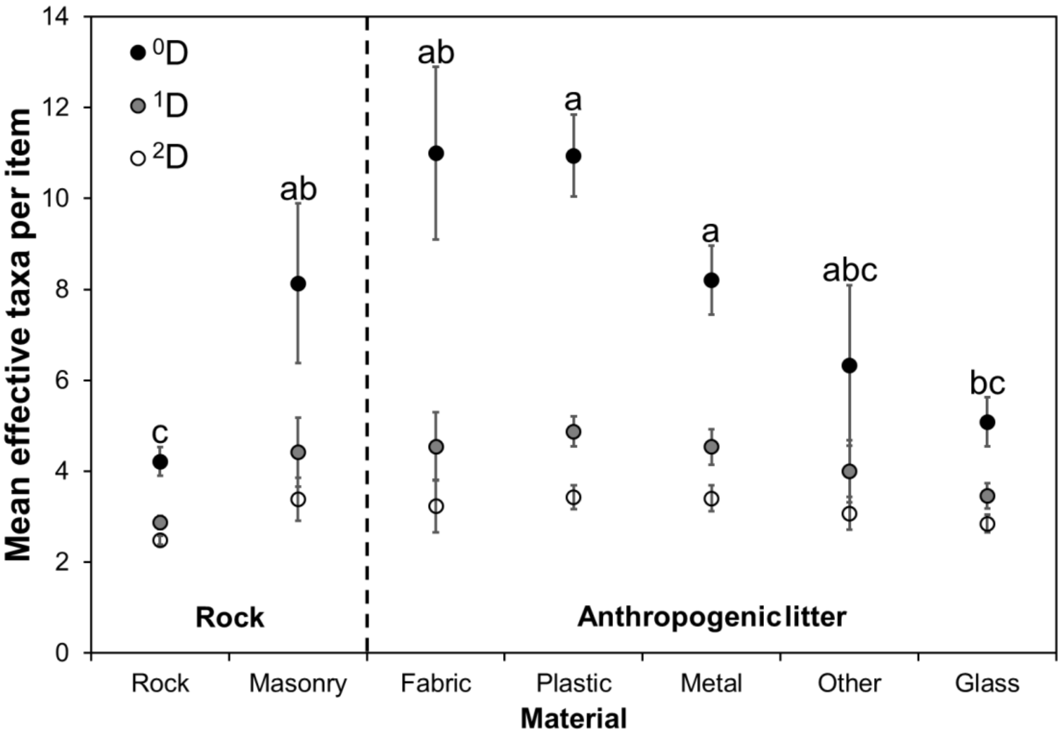

Figure 5.3: Mean Hill's numbers of ^{0}D (taxon richness; black circles), ^{1}D (exponential of Shannon's Diversity; grey circles) and ^{2}D (inverse of Simpson's Diversity; empty circles) calculated on all samples within each material categories (rock, masonry, fabric, plastic, metal, other and glass). Error bars represent standard errors. Materials labelled with the same letter did not differ significantly from one another. Significance tests are only shown for ^{0}D; see Table 5.S 3 for the full model outcomes.

5.3.3. Differences in macroinvertebrate community composition

In checking whether any taxa exclusively inhabited either anthropogenic litter or rocks, taxa that occurred in fewer than five samples were excluded. This was necessary to verify that apparent associations were not due to low abundance of a taxa. Under these conditions, no taxa were recorded only on rocks, but five taxa were recorded exclusively on anthropogenic litter. These were; *Anisus vortex* (Gastropoda: total abundance of 25 across 13 samples), *Theromyzon tessulatum* (Hirudinea: 20 across 14 samples), *Calopteryx splendens* (Odonata: 12 across 6 samples), *Limnophora* sp. (Diptera: 10 across 7 samples), and *Bathyomphalus contortus* (Gastropoda: 10 across

7 samples). Of these, *Limnophora* sp. (80%) and *B. contortus* (90%) were found almost exclusively on flexible anthropogenic litter materials (either fabric or plastic).

Substrate type (anthropogenic litter or rock) significantly influenced macroinvertebrate communities among sites (*LR* = 508.5, $p < 0.001$). The observed differences were substantially driven by eleven taxa, all of which are native species; *Erpobdella octoculata*, *Glossiphonia complanata*, *Helobdella stagnalis*, and *T. tessulatum* (Hirudinea: leeches), Oligochaeta, Sphaeriidae (Bivalvia), *Asellus aquaticus* and *Gammarus pulex/fossarum* agg. (Crustacea), Chironomidae (Diptera), *Mystacides azurea* (Trichoptera: caddisfly), and *A. vortex*. These taxa were all more abundant on anthropogenic litter than rock, with more than 85% of occurrences on anthropogenic litter. Ordination indicated that although differences between sites were notable, substrate clearly affects communities along the axis of latent variable 1 (Figure 5.4).

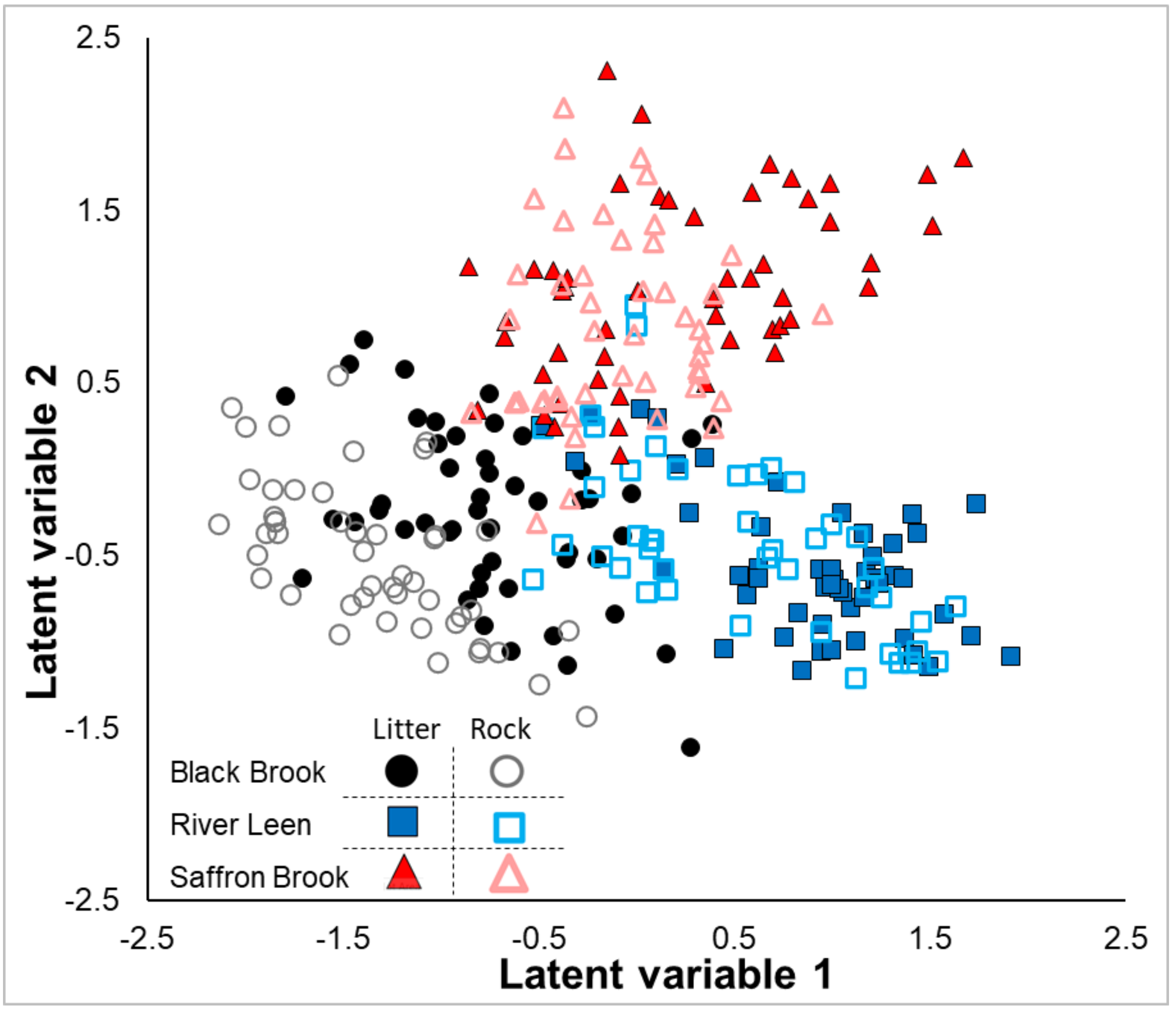

Figure 5.4: Output of latent variable model ordination of macroinvertebrate data for all sites. Symbol shape shows site; Black Brook as circles, River Leen as squares, and Saffron Brook as triangles. Shaded symbols are anthropogenic litter samples, empty symbols are rock samples (including masonry).

Significantly different communities were also recorded between material types (fabric, glass, metal, plastic, masonry, rock or other; LR (6) = 1329.1, $p < 0.001$). Eleven taxa were responsible for the effect, most of which also displayed significantly different occurrences between substrates; *E. octoculata*, *G. complanata, H. stagnalis*, Oligochaeta, Sphaeriidae, *Oulimnius* sp. (Coleoptera: beetles), *A. aquaticus, G. pulex/fossarum* agg., Chironomidae, *M. azurea*, and *P. flavomaculatus* (Trichoptera: caddisfly). All of these taxa were more common on plastic; especially the three leeches (80% of occurrences on plastic items). Separate ordinations for each site indicated that metal, fabric and especially plastic anthropogenic litter items supported the communities which were most dissimilar to those on rocks (Figure 5.5). In contrast, glass samples were similar in composition to those from rocks, as were masonry samples, despite the differences in diversity (^{0}D, ^{1}D and ^{2}D) on these materials. The differences in community composition on different materials were more evident in Black Brook and Saffron Brook than the River Leen.

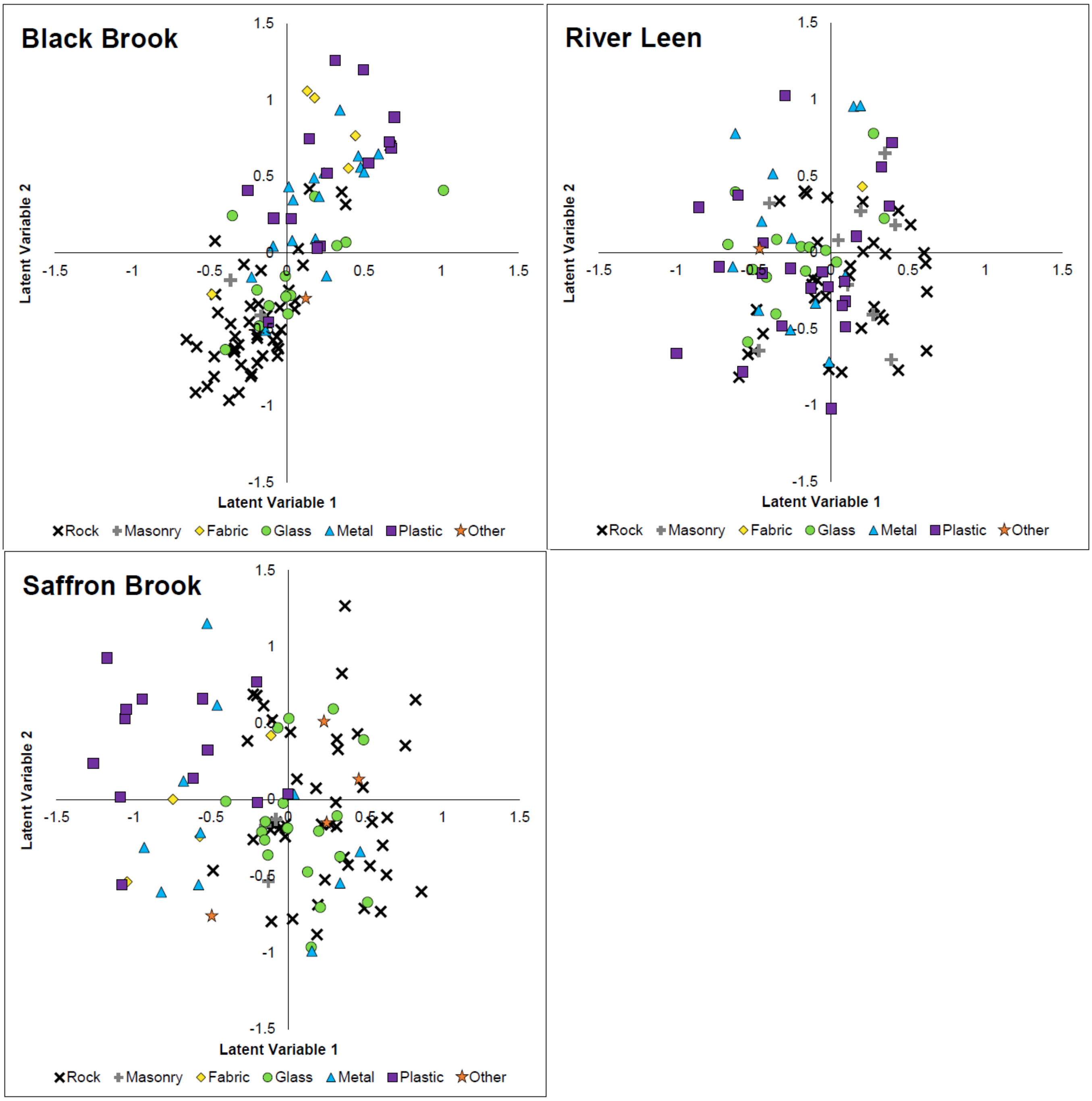

Figure 5.5: Output of latent variable model ordination of macroinvertebrate data for all sites separately to more easily show differences between material types (rock, masonry, fabric, glass, metal, plastic, other), which are shown using different shaped and coloured symbols.

5.4. Discussion

Anthropogenic litter is inhabited by a wide range of macroinvertebrates in the rivers I surveyed, supporting a greater diversity of organisms than rocks (the dominant natural substrate). Macroinvertebrate communities inhabiting these two different substrates were also distinct, indicating that anthropogenic litter typical of urban rivers can significantly alter macroinvertebrate community composition and biodiversity. Additional differences exist between anthropogenic litter material types, suggesting that the physical and chemical characteristics of materials are important controls on macroinvertebrate micro-distribution. Given the prevalence of anthropogenic litter both in this study and reported in other urban rivers (Hoellein *et al.,* 2014; Rech *et al.*, 2014, 2015; McCormick and Hoellein, 2016), it probably affects macroinvertebrate communities in many urban rivers.

5.4.1. Differences between types of anthropogenic litter and rock

Previous research on anthropogenic litter in a Polish reservoir (Czarnecka *et al.*, 2009) and on beaches around the Baltic Sea (García-Vasquez *et al.*, 2018) argued that the greater diversity of macroinvertebrates they recorded on anthropogenic litter reflected the inhospitable nature of the natural substrate (sand) in waterbodies studied. Sand is inherently unstable and provides a poor surface for most macroinvertebrates and their food (Jowett, 2003). Therefore, anthropogenic litter represented a scarce resource (hard and stable substrate) favoured by many macroinvertebrates (Czarnecka *et al.*, 2009). Hard substrates were not lacking in the rivers studied here, but macroinvertebrate communities on anthropogenic litter and rocks were nonetheless significantly different, suggesting that other factors may influence community composition.

Anthropogenic litter was on average larger than rocks. Although surface area was controlled for in statistical analysis, so I can be confident that there is a difference between substrates independent of size, other variables linked to substrate size may be important in structuring macroinvertebrate communities. For instance, the stability of rocks generally increases with size. Stable features in rivers, such as boulders or wood, are known to support high macroinvertebrate abundance and diversity (Death and Winterbourn, 1995; Nakano *et al.*, 2018). These substrates may help macroinvertebrates avoid dislodgment, provide more reliable food resources, and act as flow refuges (Jowett, 2003). Unstable, highly mobile substrates are likely to be inhabited by less diverse communities (Death, 2008), because substrate movement will inhibit colonisation and continually reset successional trajectories (Czarnecka *et al.*, 2009). Predicting the relative stability of anthropogenic litter is complicated by its wide variety of shapes and densities (Williams and Simmons, 1997), and therefore unlike rocks, is it not strongly associated with size. For example, low density plastic bags are easy to entrain but conversely more likely to become stranded on obstacles like vegetation or to become partially buried (McCormick and Hoellein, 2016). An added complication is that recruitment of anthropogenic litter in urban rivers can occur independently of flow stage (McCormick and Hoellein, 2016), making it difficult to estimate its exposure time in the river. Further investigation of these dynamics of anthropogenic litter, and the ways it differs to natural substrates, would help build our understanding of the ways anthropogenic litter may affect macroinvertebrate distribution. Nonetheless, the presence of biofilm on all anthropogenic litter and rocks sampled in this study, and the low flows during summer months prior to sampling, means that most items will have been exposed long enough for the colonisation of macroinvertebrates to have occurred.

Small-scale complexity, at a scale similar to the body length of macroinvertebrates, is known to be an important control of faunal distribution (Robson and Barmuta, 2002; Boyero, 2003). For example, colonisation experiments on introduced substrates of varied complexities have demonstrated that macroinvertebrate diversity increases with greater substrate complexity (Clifford *et al.*, 1989, 1992; Robson and Barmuta, 2002; Boyero, 2003; Adamiak-Brud *et al.*, 2015). Basic life-functions, such as respiration, metabolism, locomotion and reproduction, are affected by the physical characteristics of the habitat (Lancaster and Downes, 2013), so a more structurally diverse habitat is thought to support greater biodiversity. Likewise, complex surfaces allow macroinvertebrates to shelter from hydraulic stress, enabling conservation of energy and preventing accidental entrainment into drift (Brooks *et al.*, 2005), as well as providing shelter from predators (Everett and Ruiz, 1993). In this study, I found that macroinvertebrate communities on smooth and flat glass and rocks were less diverse than those on other material types. Additionally, masonry samples (mostly bricks with complex holes or grooves) supported a much higher macroinvertebrate diversity (for ${}^0D$, ${}^1D$ and ${}^2D$) than rocks, despite there being limited difference in community composition on the two materials. This suggests that though rock and masonry habitats are functionally similar, and so support comparable communities, the greater complexity of masonry samples means they can support more diverse macroinvertebrate communities.

As well as differences in diversity on anthropogenic litter and rock, differences in the macroinvertebrate community composition recorded on different materials suggests that these materials function as distinct habitat types because of their variation in physical structure. For instance, the similar communities found on rocks, masonry and glass could relate to their similar rigid and hard structure. In contrast, plastic and fabric sample communities were clearly distinct from rocks. These distinctions between materials were much less clear in the River Leen, where anthropogenic litter and rock communities were more homogeneous. The reasons for this are not known. A symptom of urban rivers with degraded habitat is community homogenisation (i.e. the Urban Stream Syndrome: Walsh *et al.*, 2005); however, the River Leen had the highest mean ^{0}D per sample (7.5, compared to 7.0 in Black Brook, and 4.8 in Saffron Brook), suggesting that it is not urbanised to the extent of only being able to support disturbance-tolerant taxa. A possible explanation is that the higher density of anthropogenic litter in the River Leen (4.2 items m^{-2}), and therefore greater proximity between materials, has enabled migration of macroinvertebrates between items, and increased community similarity. In Black Brook and Saffron Brook, the anthropogenic litter was more isolated within the river bed, and so communities were more variable, as reported by Czarnecka *et al.* (2009).

Plastic and fabric macroinvertebrate communities were the most diverse and most dissimilar to those on rocks. The most obvious difference between fabric, plastic, and rocks, is that all fabric and most plastic items were flexible (Table 5.2), suggesting that substrate flexibility may influence and structure macroinvertebrate distributions. The closest natural analogue for this type of habitat is macrophytes or organic detritus. It is possible that anthropogenic litter could provide a substitute for this type of habitat, which is commonly removed and thus absent from many urban rivers (Walsh *et al.*, 2005; Old *et al.*, 2014). For instance, flexible anthropogenic litter could replicate the geomorphic role of macrophyte stands in lowland rivers (e.g. Folkard, 2011), where macrophytes locally reduce flow velocity and encourage the deposition of the fine sediments and organic detritus.

There were no nearby macrophytes to sample in the urban river reaches studied, but taxa which are typically associated with aquatic vegetation were abundant on flexible materials. Of those taxa found only on anthropogenic litter, three tend to live on macrophytes: *C. splendens, A. vortex* and *B. contortus*. *C. splendens* larvae have strong preferences for complex vegetation, which provides cover and plentiful prey species (Goodyear, 2000). The gastropods *A. vortex* and *B. contortus*, which were primarily found on plastic, generally inhabit plants which provide shelter, oxygenate flows, and provide a surface for biofilm development which they feed on by scraping (Boycott, 1936). Other taxa also showed associations with flexible materials, although they were also on rocks and other types of anthropogenic litter. *Asellus* spp. and *Gammarus* spp. are omnivorous detritivores which live amongst and feed on decomposing plant material (Gledhill *et al.*, 1993). These taxa are unable to consume anthropogenic litter but were still strongly associated with it in this study (especially plastic; 53% and 48%, and fabric: 31% and 30% of occurrences respectively), suggesting that the accumulation of fine organic matter around flexible materials could attract shredders and collectors. The fine sediment collected around flexible materials may also provide habitat for organisms that prefer soft sediments (e.g. Sphaeriidae, Oligochaeta, and some Chironomidae, all of which were recorded in greater numbers on plastic and fabric). However, if macrophytes were present nearby in these rivers, it is possible that many of these taxa would preferentially inhabit this natural substrate, especially those taxa which directly feed on macrophytes. Past studies have recorded different macroinvertebrate communities on plastic and natural leaves, with lower macroinvertebrate abundance and diversity on plastic leaves (Quinn *et al.*, 2000; Hofer and Richardson, 2007).

As well as interacting with fine sediments and organic matter, anthropogenic litter may also affect other macroinvertebrate food resources. I observed that biofilm, an important food for scrapers, had developed on the exposed surface of anthropogenic litter, as well as on rocks. If this biofilm is of a different quality or quantity to that which develops on rocks, this could influence macroinvertebrate distribution. For instance, it has been shown that more complex surfaces are more quickly colonised by biofilm and that this in turn will attract macroinvertebrates (Clifford *et al.*, 1992). Distinct biofilm communities have been found to colonise different materials in marine environments (studies tested a range of plastic polymers and glass; Oberbeckmann *et al.*, 2014; Pinto *et al.*, 2019), meaning biofilm quality could be affected by material composition. However, a more comprehensive study of different materials in an urban river only found differences in biofilm composition and gross primary production between solid surfaces (plastic, glass, aluminium, and ceramic tiles) and soft organic materials (leaf litter and cardboard), rather than between all material types (Hoellein *et al.*, 2014). Further research into a wider range of food resources and materials in rivers is necessary to expand our understanding of this topic.

The differences in community composition between substrates were driven by the taxa that differed most in abundance between anthropogenic and rock samples. These tended to be generalist taxa which are tolerant of poor habitat conditions. Anthropogenic litter samples were dominated by Chironomidae, Oligochaeta, and *A. aquaticus*, which dominate communities where there is organic enrichment and low dissolved oxygen concentrations (Pennak, 1978; Armitage *et al.* 1995). The corresponding high abundances of macroinvertebrates which feed on these taxa may be an indirect response to the increase in prey availability. This includes *Limnophora* sp. (which was only found on anthropogenic litter), the four leech species (especially *E. octoculata* and *H.stagnalis*; Elliott and Dobson, 2015), and *P. flavomaculatus* (Edington and Hildrew, 1995). All of these taxa were recorded more frequently on fabric or plastic than on rocks in this study. During sample collection it was noted that some plastic bags were associated with organic-rich fine sediments and accompanying signs of anoxia. In marine and estuarine environments, plastic bags have been linked to localised anoxia through preventing gas and nutrient exchange process at the sediment-water interface (Green *et al.*, 2015), although this effect is moderated when they are regularly in motion (Clemente *et al.*, 2018), which possibly explains why I still found diverse communities on such materials. The reduced difference between anthropogenic litter and rock diversity at higher Hill's numbers suggests that although the complexity of anthropogenic litter can support a diverse fauna, the taxa which are best able to exploit anthropogenic litter are those of lower conservation value, which can tolerate reduced habitat quality, and so numerically dominate the communities.

5.4.2. Conclusions and implications

The rivers studied here were limited in habitat heterogeneity, as is typical of urban rivers globally (Walsh *et al.*, 2005). In such cases, the habitat provided by anthropogenic litter may support biodiversity, both by providing complex and stable habitat for a wide range of organisms, and by representing a unique habitat distinct from rock substrates. Although rocks were the more abundant substrate in the rivers studied here, anthropogenic litter supported novel and more diverse communities, including five unique taxa. In urban rivers where it is not possible to restore instream large wood or macrophytes, anthropogenic litter may accidentally offset a lack of habitat diversity by providing an analogue for these natural substrates which are typically absent. In particular, these results suggest that flexible materials may replicate in-channel macrophyte habitat. It is possible that where anthropogenic litter provides a novel habitat structure, such as in sandy habitats (García-Vazquez *et al.*, 2018), it may enable colonisation by non-native taxa that would not inhabit these environments under natural conditions (Tyrrell and Byers, 2007). Although this was not observed in the rivers studied here, it should be considered in future investigations, especially as urban areas are key sites for the establishment and spread of non-native and invasive species (Francis *et al.*, 2019).

Anthropogenic litter removal and reduction of inputs should be the aim of management strategies, given that rivers are a key source of marine anthropogenic litter (Rech *et al.*, 2014), anthropogenic litter is environmentally damaging (Rochman, 2015), and because of the negative societal and social impacts of littered waterways (Williams and Deakin, 2007). However, the results presented herein suggest that the removal of anthropogenic litter from urban rivers may not lead to biodiversity improvements in the immediate area and may even reduce biodiversity at the local scale. Anthropogenic litter removal efforts should therefore be carefully managed to reduce disturbance to the wider environment (Chapman and Clynick, 2006). This could mean preferentially removing some materials rather than others or improving habitat complexity following the clearance of anthropogenic litter to replace the removed habitat.

Importantly, these responses to anthropogenic litter suggest that even small-scale enhancement of river habitat complexity (even if the habitats are artificial) could have positive effects on the local-scale biodiversity and ecological health of urban rivers (Francis and Hoggart, 2008). Understanding the types of habitat provided by anthropogenic litter and comparison to a wider range of natural substrates such as macrophytes and large wood, could help inform mechanisms to provide these functions using alternative and less damaging materials.

5.5. Supplementary materials

Table 5.S 1: Results of all statistical tests on family level taxonomic data, showing test statistics, degrees of freedom (*df*) and significance (*p*) values. For full details on how statistical tests were performed see Section 5.2.4. All tests were the same as detailed in the text but were performed on family level data which included caddisfly pupae. Linear Mixed Effect (LME) models tested for differences in diversity (^{0}D, ^{1}D and ^{2}D) between substrates and between materials by including substrates/material and surface area as fixed effects, and site as a random effect. The test statistic for LME models is the Chi-square test statistic of a likelihood ratio test. The *manyglm* function tested for differences in community composition between substrates and materials, calculating a Sum-of-*LR* test statistic and associated *p* value with 999 permutations.

Statistical test	**Parameter tested**	**Differences between substrates (anthropogenic litter and rock)**			**Differences between materials (fabric, glass, masonry, metal, plastic, rock, and other)**		
		Test statistic	*df*	*p*	Test statistic	*df*	*p*
LME model	^{0}D (taxon richness)	25.67	1	0.001	53.09	6	0.001
	^{1}D (exponential of Shannon's Diversity)	23.31	1	0.001	37.61	6	0.001
	^{2}D (inverse of Simpson's Diversity)	13.72	1	0.001	21.08	6	0.002
Manyglm	Community composition	430.4	1	0.001	1145.2	6	0.001

Table 5.S 2: List of the 61 taxa recorded during the study. Asterisks denote non-native species.

PLATYHELMINTHES
RHABDITOPHORA
Tricladida
- *Dendrocoelum lacteum*
- *Dugesia* sp.
- *Planaria torva**
- *Polycelis* sp.

NEMATOMORPHA
- Nematomorpha

MOLLUSCA
GASTROPODA
Hygrophila
- *Radix balthica*
- *Physa fontinalis*
- *Ancylus fluviatilis*
- *Anisus vortex*
- *Bathyomphalus contortus*
- *Gyraulus crista*
- *Gyraulus albus*
- *Planorbis carinatus*

Littorinimorpha
- *Bithynia tentaculata*
- *Potamopyrgus antipodarum**

BIVALVIA
Veneroida
- Sphaeriidae

ANNELIDA
- **OLIGOCHAETA**

CLITELLATA
Arhynchobdellida
- *Erpobdella octoculata*

Rhynchobdellida
- *Glossiphonia complanata*
- *Helobdella stagnalis*
- *Hemiclepsis marginata*
- *Theromyzon tessulatum*

ARTHROPODA
ARACHNIDA
- Hydrachnidae

MALACOSTRACA
Amphipoda
- *Crangonyx pseudogracilis**
- *Gammarus pulex/fossarum* agg.

Isopoda
- *Asellus aquaticus*

INSECTA
Ephemeroptera
- *Baetis rhodani*
- *Caenis luctuosa*
- *Ephemera danica*

Odonata
- *Calopteryx splendens*

Neuroptera
- Sisyridae

Trichoptera
- *Brachycentrus subnubilus*
- *Agapetus fuscipes*
- *Glossosoma boltoni*
- *Goera pilosa*
- *Hydropsyche pellucidula/angustipennis*
- *Hydropysche siltalai*
- *Hydroptila* sp.
- *Lepidostoma hirtum*
- *Athripsodes cinereus*
- *Ceraclea nigronervosa*
- *Mystacides azurea*
- *Polycentropus flavomaculatus*
- *Psychomyia pusilla*
- *Tinodes* (cf.) *waeneri*
- *Rhyacophila dorsalis*

Coleoptera
- *Halticinae* sp.
- *Nebrioporus elegans*
- *Elmis aenae*
- *Limnius volckmari*
- *Oulimnius* sp.
- *Orectochilus villosus*

Diptera
- Ceratopogonidae
- Chironomidae
- Empididae
- Ephydridae
- *Antocha* sp.
- *Limnophora* sp.
- *Dicranota* sp.
- Simuliidae
- Stratiomyidae

Table 5.S 3: Mean effective taxa per sample for each Hill's number (^{0}D: taxon richness; ^{1}D: exponential of Shannon's Diversity; and ^{2}D: the inverse of Simpson's Diversity) with results divided by material type. Materials labelled with the same letter did not differ significantly from one another based upon comparison of parameter estimates and associated *p* values produced using *lmerTest*.

Substrate	**Material**	**^{0}D mean (±SE)**		**^{1}D mean (±SE)**		**^{2}D mean (±SE)**	
Rock	Rock	4.2 ± 0.3	c	2.9 ± 0.1	c	2.5 ± 0.1	b
	Masonry	8.1 ± 1.8	ab	4.4 ± 0.8	ab	3.4 ± 0.5	a
Anthropogenic litter	Fabric	11.0 ± 1.9	ab	4.5 ± 0.8	ab	3.2 ± 0.6	ab
	Plastic	10.9 ± 0.9	a	4.9 ± 0.3	a	3.4 ± 0.3	a
	Metal	8.2 ± 0.8	a	4.5 ± 0.4	a	3.4 ± 0.3	a
	Other	6.3 ± 1.8	abc	4.0 ± 0.7	abc	3.1 ± 0.4	ab
	Glass	5.1 ± 0.5	bc	3.5 ± 0.3	b	2.9 ± 0.2	ab

Chapter 6: Anthropogenic litter affects macroinvertebrates in the surrounding river bed as well as those inhabiting its surface

6.1. Introduction

Aquatic macroinvertebrates are known to colonise a wide variety of artificial materials and structures in rivers and lakes, including engineered bank protection (Way *et al.*, 1995; Brunke *et al.*, 2002; Weber *et al.*, 2017) and river walls (Attrill *et al.*, 1999; Hoggart *et al.*, 2011). This propensity of macroinvertebrates to colonise any non-toxic surface has long been utilised by researchers using artificial substrate samplers (Beak *et al.*, 1973; De Pauw *et al.*, 1986), or by those investigating macroinvertebrate settlement preferences through experimental manipulation of substrate properties (Jeffries, 1993; Robson and Barmuta, 1998; Molokwu *et al.*, 2014; Adamiak-Brud *et al.*, 2015). More recently, researchers have discovered that anthropogenic litter is also frequently inhabited by freshwater macroinvertebrates (Chapter 5; Czarnecka *et al.*, 2009).

Anthropogenic litter has been found to negatively affect organisms through entanglement, ingestion, inhibition of gas exchange between sediment layers, and the release of toxic chemicals bound to the litter (see reviews by: Bergmann *et al.*, 2015; Gall and Thompson, 2015; Green *et al.*, 2015; Gunaalan *et al.*, 2020). As well as these negative effects of anthropogenic litter, some researchers have also found that anthropogenic litter can create new and novel habitat and increase the space available for organisms to colonise. Several researchers found distinct and diverse communities inhabiting pieces of anthropogenic litter in shallow seas (Katsanevakis *et al.*, 2007; Crocetta *et al.*, 2020), on beaches (García-Vazquez *et al.*, 2018; Rech *et al.*, 2018), on the deep sea floor (Song *et al.*, 2021), in tidal estuaries (Chapman and Clynick, 2006), in reservoirs (Jatulewicz, 2007; Czarnecka *et al.*, 2009), and in rivers (Chapter 5). Research on anthropogenic litter has predominantly been conducted in the marine environment (Bletter *et al.*, 2018), but focus is starting to shift towards freshwater ecosystems and organisms (Windsor *et al.*, 2019). In this study I focus on rivers, where anthropogenic litter remains an understudied aspect of human influence, despite reported densities of anthropogenic litter which are comparable to, or exceed concentrations found in the marine environment (Hoellein *et al.*, 2014; McCormick and Hoellein, 2016; Rech *et al.*, 2014, 2015).

The few surveys comparing the macroinvertebrates inhabiting anthropogenic litter with those on natural substrates in freshwater, found a greater diversity and significantly different community composition of macroinvertebrates on anthropogenic litter than on the reservoir bottom (Jatulewicz, 2007; Czarnecka *et al.*, 2009), or on river gravels and cobbles (Chapter 5). The higher diversity of macroinvertebrates inhabiting anthropogenic litter was attributed to its more complex structure which provides diverse microhabitat conditions and shelter, and can therefore support more species (Chapter 5). Additionally, the different types and materials of anthropogenic litter have distinctive physical and chemical properties that are unlike natural substrates, for instance most plastics are long-lasting, buoyant and hydrophobic (Bond *et al.*, 2018). Because the distribution of macroinvertebrates is strongly related to substrate properties (Death, 2000; Jowett, 2003), these novel characteristics have been causatively linked to differences in the community composition of macroinvertebrates inhabiting anthropogenic litter and natural substrates. For example, Chapter 5 found that taxa living on flexible plastics and fabrics would typically be associated with macrophytes, suggesting the physical properties of the anthropogenic litter mimicked submerged plants. However, this research has so far only addressed the ways in which anthropogenic litter affects macroinvertebrates directly inhabiting its surface.

In perennial rivers, a key control on aquatic organisms is the fact that water is constantly flowing. Any obstacles that protrude from the river bed interact with this flow, thereby changing habitat conditions. By this mechanism, large anthropogenic litter (e.g. tyres, shopping trolleys, traffic cones) could influence habitats and organisms in the surrounding river bed, analogous to the effects of similarly-sized natural obstacles like large wood, macrophytes, or boulders. These natural structures have been shown to reduce flow velocity within their roughness projection area and accelerate flow around, over, or through themselves (Abbe and Montgomery, 1996; Mutz, 2000; Shamloo *et al.*, 2001; Green, 2005; Afzalimehr *et al.*, 2019). Areas of concentrated flow expose coarser bed material and scour pools (Gurnell and Sweet, 1998; Montgomery *et al.*, 2003), whereas regions of slower flow velocity deposit and retain fine sediment and organic matter (Daniels, 2006; Gurnell *et al.*, 2006; Osei *et al.*, 2015a). These processes create predictable landforms, introducing and maintaining notable habitat diversity (Gurnell, 2013; Pilotto *et al.*, 2014; Osei *et al.*, 2015a; Schlömer *et al.*, 2021).

Changes to habitat conditions and an overall increase in habitat heterogeneity prompted by natural structures in rivers has been linked to increased macroinvertebrate diversity and adjustments to the community composition in the surrounding river bed (Lester *et al.*, 2007; Wellnitz *et al.*, 2014; Pilotto *et al.*, 2014, 2016; Flores *et al.*, 2017; de Brouwer *et al.*, 2020). For example, large wood has been found to influence macroinvertebrates through the formation of organic matter patches (Entrekin *et al.*, 2009; Pilotto *et al.*, 2014), and pools (Hilderbrand *et al.*, 1997). Structures that protrude from the river bed have also been found to enhance the capture of drifting macroinvertebrates (Kiffney *et al.*, 2014; Wellnitz *et al.*, 2014), as well as to provide shelter from fast flows and predators (Schneider and Winemiller, 2008). The availability and accessibility of food resources have also been linked to structures; for instance, structures may accumulate organic matter, increase the surface area available for biofilm development, or provide better access to floating food particles (Hoffmann and Hering, 2000; Benke and Wallace, 2003; Cashman *et al.*, 2016).

It is possible that large anthropogenic litter performs similar functions to natural habitat structures like large wood, macrophytes or boulders. This is important because these natural structures have commonly been removed from rivers for flood prevention and navigation (Friberg, 2010; DeBoer *et al.*, 2020), but anthropogenic litter is abundant in even highly managed rivers (McCormick and Hoellein, 2016). Where natural structures are absent, anthropogenic litter may provide and create surrogate habitat both by providing a surface for colonisation and by driving changes to habitat conditions in the surrounding river bed. Whether or not the influence of anthropogenic litter extends beyond its surface has not been previously studied, but is important for improving the management of river habitats and anthropogenic litter.

6.1.1. **Research approach**

This study aimed to assess the ways in which large anthropogenic litter items influence river habitats and macroinvertebrates in the surrounding river bed. An experimental approach was taken, using replicates of anthropogenic litter (in this case car tyres) which were installed into rivers and monitored for twelve months. This addresses the limitations of Chapter 5 where the relative exposure time of anthropogenic litter items were unknown, and where a lack of replication in anthropogenic litter shape limited the assessment of the importance of specific physical properties.

The impacts of tyres were compared between two river types: one sand-bed river, and one gravel-bed river. Because of the higher instability of sand river beds, it was expected that tyres would prompt greater sediment movement and therefore greater geomorphic change in the sand-bed river. The instability of sand substrate makes it an unfavourable substrate for most macroinvertebrates as it reduces the amount of available food resources and does not provide a secure platform to live on (Jowett, 2003; Jones *et al.*, 2011). It has been suggested that large wood structures have a greater impact on macroinvertebrates in sand-bed rivers as they can counter the effects of bed instability by securing the river bed and providing otherwise scarce solid substrate (Benke and Wallace, 2003; Pitt and Batzer, 2011; Nakano *et al.*, 2018). It is possible that the same is true for anthropogenic litter.

A ‘bottom-up’ approach was taken to understand how tyres affect habitats by comparing macroinvertebrate diversity and community composition on and around the tyres. Samples were taken from the upstream, downstream, sides, in the middle, and on the surface of tyres, because I expected the tyre to differentially influence habitat conditions in these different locations. These were compared to samples from the wider river where the tyres had negligible influence on habitat conditions. As macroinvertebrate distributions are strongly linked to habitat (Jowett, 2003), differences in the sampled communities can be used as indicators of ecologically relevant differences between locations. This study asks the following questions:

1) How do macroinvertebrate communities vary between those on the tyre surface and those inhabiting the surrounding river bed?
2) How do these tyre-associated communities compare to those in the rest of the river?
3) How do observed patterns compare between the sand- and gravel-bed rivers?

It was expected that different macroinvertebrate communities would inhabit different locations on and around tyres, and that these communities would differ to those found elsewhere in the river. This would suggest that large litter affects macroinvertebrates within its vicinity by interacting with the flow, driving sorting and redistribution of sediment, and changing patterns of vegetation and organic matter in the surrounding river bed. This study is the first known investigation of the impacts of experimentally introduced anthropogenic litter items on macroinvertebrates in rivers, and the first investigation of whether the impacts of anthropogenic litter extend into the surrounding river bed.

6.2. Methods

6.2.1. Study sites

Twenty-four tyres were installed into two rivers (twelve in each) in February and March 2019: the sand-bed River Maun and the gravel-bed River Idle (Figure 6.1). Both are lowland rivers (2nd and 3rd order respectively) in North Nottinghamshire, located downstream of large towns (Mansfield and Retford). The Maun is part of the Idle catchment (it joins the Idle at Elkesley 21 km river length downstream from the Maun field site); a predominantly agricultural catchment draining 842 km^2 (Downs and Thorne, 1998). The upper Idle catchment geology is a mixture of Sherwood sandstones, coal measures and magnesium limestone, whilst the lower catchment is underlain by Keuper Marls and alluvial sands and gravels (Downs and Thorne, 1998).

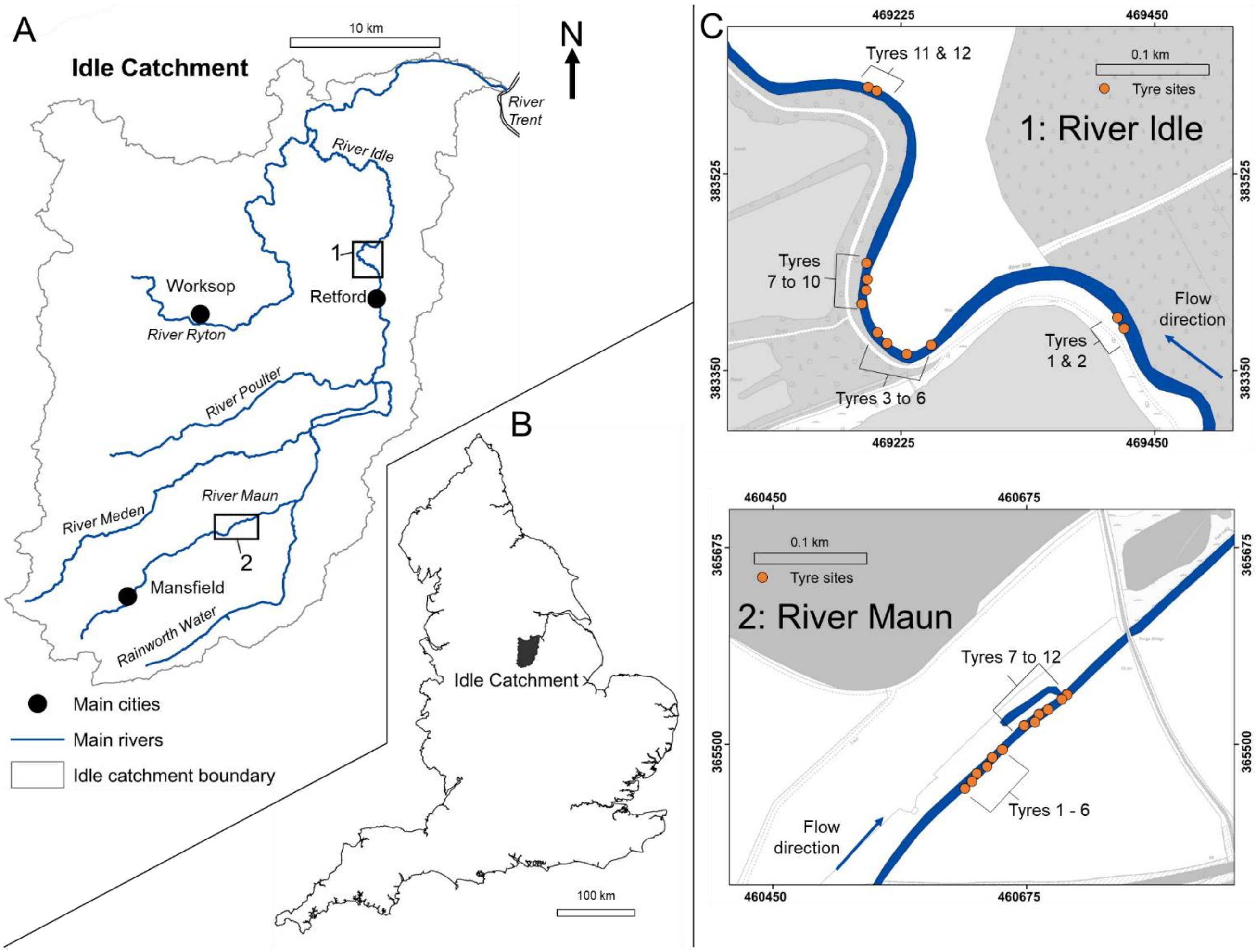

Figure 6.1: Maps showing the location of field sites on the Rivers Idle and Maun with respect to: A) the Idle Catchment; and B) England. C) Shows the locations of individual tyres at each field site.

The River Idle field site is bordered on the left bank by the Idle Valley Nature Reserve (managed by Nottinghamshire Wildlife Trust), with agricultural fields on the right bank. The reach is managed by the EA to mitigate flood risk in the upstream town of Retford. This involves annual macrophyte cutting and the clearance of large wood from the river. The river had been disconnected from its floodplain by the raising of flood embankments and enlargement of the channel cross-section which occurred in response to severe flooding in 1977. Hallcroft weir, located between tyres 2 and 3, impounds the upstream river for approximately 100 m so I avoided placing tyres within this impounded section. The river has pool-riffle sequences and some meandering, but instream habitat features are limited. The bed substrate was predominantly gravel with large stands of submerged macrophytes (range of taxa including *Ranunculus*: Water Crowfoot and *Potamogeton*: Pondweed). Silt, organic matter, and sand patches were frequent but small in the riffles where tyres were installed. However, the river bed in pools and the impounded section was composed of mixed sand, silt and gravel.

The River Maun field site has been straightened extensively and receives substantial agricultural runoff from adjoining arable fields on both banks. Large quantities of anthropogenic litter were found in the river, including sewage waste which probably came from stormwater overflow discharges at upstream sewage treatment works (two are located upstream of the field site). Despite this, its Water Framework Directive (WFD) classification for invertebrates was good in 2019 (Table 6.1), suggesting that water quality problems do not limit macroinvertebrate populations at this site. There is very limited habitat diversity (glide flow type predominates) at the field site due to channelisation. Previously, the EA introduced several cobble patches (three within the sampled reach) with the aim of introducing flow diversity and riffle habitat to help fish populations. These are still present and affecting flow in small areas, but they have been mostly broken up and buried by sand. The bed substrate was predominantly bare sand, with some patches of fine organic matter (leaf litter and twigs) and silt, especially in the margins. In the summer, the sand-bed was overlayed by extensive submerged macrophyte cover (mostly *Callitriche:* Water Starwort).

Both sites have been heavily managed and so retain limited instream habitat features. Car tyres were introduced to these rivers to see how macroinvertebrates respond to the artificial habitat created over a twelve-month period. Conducting this study in rivers with different dominant substrate types (gravel and sand) allows comparison of the tyres influence in contrasting river systems.

Table 6.1: River characteristics for field sites on the Rivers Idle and Maun. Mean width was determined from channel width at each tyre location. Water Framework Directive (WFD) classification was taken from the Catchment Data Explorer (Environment Agency, 2021e,f). Mean discharge was taken from the National River Flow Archive (NRFA, 2021a,b); Idle at Mattersey (11.9 km downstream of site) and Maun at Mansfield the Dykes (5.6 km upstream).

	River Idle	**River Maun**
GPS Coordinates	53° 20' 34'' N 00° 57' 42'' W	53° 10' 59'' N 01° 05' 38'' W
Mean width at site (m)	9.4	8.1
WFD classification (as of 2019)	Overall: Moderate Invertebrates: Good Fish: High Macrophytes/ phytobenthos: Moderate	Overall: Moderate Invertebrates: Good Fish: Moderate Macrophytes/ phytobenthos: Good
Mean discharge (m^3s^{-1})	2.324 (1982-2019)	0.687 (1992-2019)

River Idle

Looking downstream from Tyre 3 April 2019

River Maun

Looking downstream from Tyre 8 Sept 2019

All installed tyres were standard car tyres. Tyres were of various makes, but were similar in size and shape. The mean diameter was 0.64 m, and mean height 0.25 m. They were secured to the river bed with stakes and rope (Figure 6.2) into uniform patches of the dominant substrate type in each river. Tyres had to be located in similar habitats in order to act as replications, but they also needed to be independent of one another. Therefore, they were positioned with a minimum of 5 m between tyres (outside of their zone of visible influence on flow) to prevent interacting effects. Tyres were located within riffles, runs or glides as water depth in pools restricted safe access. They were removed after twelve months.

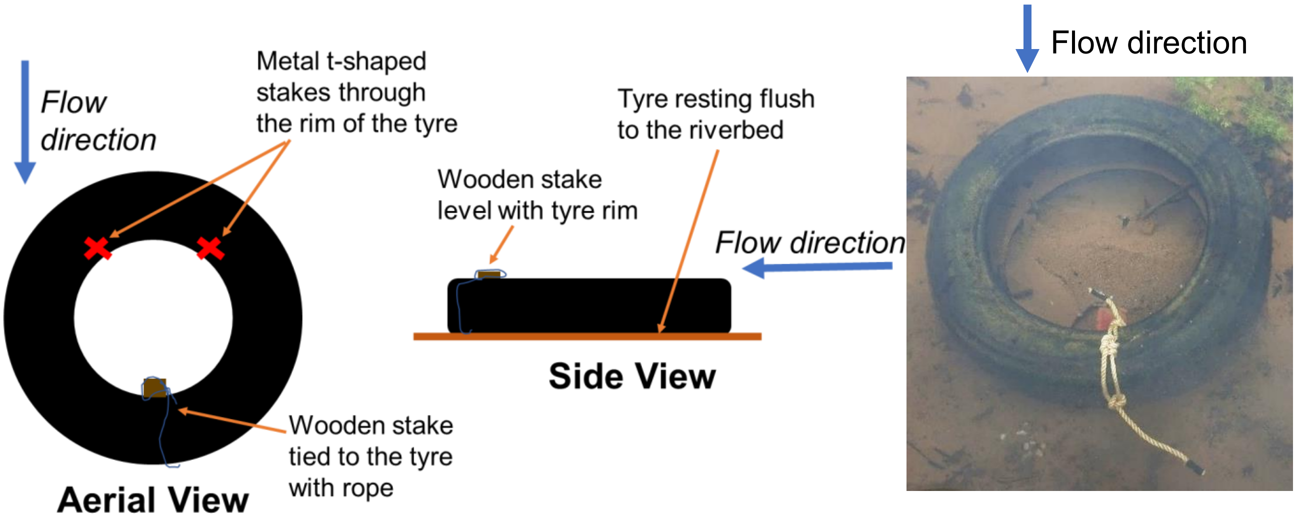

Figure 6.2: Diagram showing how tyres were installed into the river bed. Photo shows Tyre 10 in the River Maun just after installation.

6.2.2. Field methods

6.2.2.1. *Benthic macroinvertebrate sampling*

Benthic macroinvertebrates were sampled before (zero months), and six and twelve months after installation. Pre-installation macroinvertebrate samples were collected from the area of river bed that would become the tyre mid-points. Six of the tyres from each river were randomly selected for sampling after six months (in September 2019), and all tyres were sampled after twelve months (in February 2020; excepting tyres 3 and 12 in the Maun which were removed earlier than planned due to exceptional scour which dislodged these tyres). Three categories of post-installation macroinvertebrate samples were collected from: 1) the tyre's surface, 2) the river bed surrounding the tyre, and 3) the wider river bed.

The surface of tyres were sampled using a brush to scrub 0.04 m^2 of its surface into a Surber net (mesh = 250 µm) held downstream to collect dislodged macroinvertebrates (following Pilotto *et al.*, 2016). Samples from the river bed surface around the tyre were collected from the tyre mid-point and four positions immediately outside of the tyre rim: upstream, downstream and to both sides. These areas of river bed were sampled using a 0.04 m^2 Surber sampler (200 mm x 200 mm, mesh = 250 µm) to match the area of tyre surface sampled. These samples from on and around tyres (downstream, middle, side, tyre surface, and upstream samples) are collectively referred to as being from tyre microhabitat zones hereafter.

Background river samples of macroinvertebrates were collected from the main meso-habitat types found in the wider river bed pre-installation, and during six- and twelve-month monitoring periods. The four main meso-habitat types at each site were visually assessed following definitions from Pardo and Armitage (1997; gravel, sand, silt, and macrophytes in the Idle, and cobbles, sand, silt, and macrophytes in the Maun). Between 15 and 18 macroinvertebrate Surber samples were collected during each sampling period (again over 0.04 m^2 of river bed so they could be quantitatively compared to tyre microhabitat zone samples). These samples were spread across the range of meso-habitat types, with the majority of samples being taken from the dominant habitat type (sand for the Maun, and gravel for the Idle). The spread of samples across meso-habitat types was not proportional to the spatial coverage of these meso-habitats. This decision was made because it was important to have multiple samples from habitats that had limited spatial coverage but that might provide analogous function to tyres, such as cobble substrates in the Maun. This means I could assess whether tyre microhabitat zones were similar to meso-habitats within the wider river, or if the conditions within different zones were outside of the range of meso-habitat types found in the background river.

6.2.2.2. *Measuring habitat conditions around the tyres*

To get an indication of the impact of the tyre on flow patterns in the river bed surrounding the tyre, one dimensional flow velocity measurements (mean velocity over 30 s) were taken immediately before and after removal of each tyre using an electromagnetic flow meter. As flow velocity is highly variable over time, this data does not show the absolute impact of the tyre on flow velocity, except at the time of measurement, but it does allow relative comparison between conditions in each of the tyre microhabitat zones. Measurements were taken from the tyre mid-point and at four positions immediately outside of the tyre rim, like the macroinvertebrate samples (downstream, middle, upstream, and to both sides). Flow velocity was measured at both 60% depth, to approximate the average flow velocity of the water column (Herschy, 1995), and at the river bed to better estimate the flow velocities experienced by benthic macroinvertebrates (Jowett, 2003).

Substrate type and depth of fine sediment were also recorded for tyre mid-points pre-installation, and from all tyre microhabitat zones at six and twelve months, to assess the effect of the tyres on bed sediment. Sketch diagrams of meso-habitat types around the tyre twelve months after installation were also created prior to removal.

6.2.3. Laboratory methods

All macroinvertebrate samples were washed through a 500 µm mesh sieve, then manually processed to collect macroinvertebrates. Macroinvertebrates were identified to mixed taxonomic levels, as shown in Table 6.2. This was because of taxonomic difficulties in identifying some invertebrates (e.g. early instar Limnephilidae and Coenagrionidae), because of limited knowledge of the functional significance of differences between genus/species of some families (e.g. Oligochaeta), and to follow common convention in the literature (e.g. Chironomidae to tribe). Across all samples, 62% of individuals were identified to tribe, genus or species level (if Simuliidae and Oligochaeta are excluded this rises to 99%). Chironomidae pupae were included as a separate taxon. Taxonomic levels were kept consistent between samples and sites. Identification followed Cham (2012), Dobson *et al.* (2012), Edington and Hildrew (1995), Elliott (2009), Elliott and Dobson (2015), Elliott and Humpesch (2010), Ellis (1978), Friday (1988), Holland (1972), Killeen *et al.* (2004), Macan (1977) Reynoldson and Young (2000), and Wallace *et al.* (1990). Chironomidae that could not be identified to tribe (because they were missing their heads; 61 individuals) and first instar Trichoptera that could not be identified to family (4 individuals) were excluded from analysis.

Table 6.2: Table showing the taxonomic level used in macroinvertebrate identification where taxa were not identified to species or genus. Brackets show number of individuals found of each taxa.

Taxonomic level	Taxa
Tribe or sub-family	Ceratopogoninae (226), Chironomidae (6402)
Family	Chironomidae pupae (237), Coenagrionidae (1), Crambidae (2), Gerridae (1), Limnephilidae (19), Psychodidae (10), Rhagionidae (1), Simuliidae (6168), Tipulidae (6),
Subclass	Oligochaeta (4869)

6.2.4. Data analysis

Data analysis was performed with the full dataset of mixed taxonomic resolution using R statistical software (version 4.0.4: R Core Team, 2021).

6.2.4.1. *Geomorphological effects of tyres*

To evaluate the effect of the tyres on flow velocity, I conducted a paired *t*-test on flow velocities (across all tyre microhabitat zones) which were measured before and after tyre removal twelve months after installation. The difference in flow velocity caused by tyres, for each tyre microhabitat zone, was also calculated by subtracting the flow velocity without the tyre in place from that with the tyre in place.

The change in fine sediment depth was calculated by subtracting the fine sediment depth at installation from that measured six or twelve months later. This provides an estimate of sediment accumulation or scour, as fine sediment depth likely changed multiple times during the twelve-month period, and particularly because winter 2019/20 (just before twelve month sampling) was a period of relatively high flows at both sites. Linear Mixed Effect (LME) models were used to compare fine sediment depth at installation to that measured six or twelve months later using *lme4* (Bates *et al.* 2015). Models were produced for each river separately, and used time of sampling (zero, six, or twelve months) and microhabitat (downstream, middle, side and upstream), as fixed effects, and tyre number as a random effect. This random effect was included to account for the fact that each tyre was repeatedly sampled and so repeat measurements from the same tyre are therefore not independent. Significance values for the effect of time were identified by likelihood ratio tests of the full model against a null model without the time factor. This showed whether the fine sediment depth was significantly different between sampling periods, whilst taking into account the differences between microhabitats.

6.2.4.2. *Macroinvertebrate communities in tyre microhabitats*

To answer question 1 I looked for differences in the macroinvertebrate abundance, diversity and community composition between tyre microhabitat zones (downstream, middle, side, tyre surface, and upstream). As the area sampled was consistent, the macroinvertebrate abundance (total number of individuals in a sample) could be directly compared between samples. Macroinvertebrate diversity was investigated by calculating Hill's numbers using *vegan* (Oksanen *et al.*, 2020), as detailed in Chapter 4 (Section 4.2.4.).

Differences in the abundance and diversity in tyre microhabitat zones were examined using Generalised Linear Models (GLMs). Maximal GLM models were constructed relating each response variable (abundance, ${}^0D$, ${}^1D$ and ${}^2D$) with river (Idle or Maun) and microhabitat (downstream, middle, side, tyre surface, or upstream) as fixed effects with an interaction term. Both six- and twelve-month sampling periods were analysed separately, as repeat measurements of tyres meant that data would be non-independent. Models were validated by examination of residuals against fitted values, and a normal Q-Q plot. GLMs with Gaussian error distribution models were a good fit for diversity models (^0D, ^1D and ^2D) but not for abundance data. Abundance data was positively skewed due to exceptionally high abundance in one sample. Tests of abundance were therefore repeated with and without this sample to check that it did not affect results (Table 6.S 1). Significance values for the effects of these factors were identified via F-tests of the maximal model against nested models without the factor of interest. Comparison of parameter estimates and associated t and p-values between microhabitat categories was used to assess differences between microhabitat zones.

Temporal trends in diversity and abundance were explored to see how tyres changed diversity and abundance from the base level. Samples taken at zero months (before samples) from the area of river bed that would become the tyre's midpoint, were compared to samples taken from tyre microhabitat zones after twelve months. As these samples were taken during the same month of the year comparisons should not be confounded by seasonal differences in communities.

The macroinvertebrate community composition in different microhabitat categories was compared using the *manyglm* function in *mvabund* (Wang *et al.*, 2021). This function fits multiple GLMs to the raw counts for each taxa, with river and microhabitat as explanatory variables with an interaction. Based on examination of plotted residuals against fitted values I used a negative binomial distribution to fit each model. This addresses problems with other distance-based community analysis (e.g. SIMPER and PERMANOVA) by specifying a mean-variance relationship which is common to count data (Warton *et al.*, 2012). A sum of log-Likelihood Ratio (*LR*) test statistic for each model component (river, microhabitat, and their interaction) was calculated based on differences in taxa distribution across all taxa with significance assigned using randomisation (999 permutations), and the p-value adjusted for multiple testing using step-down resampling. These differences between communities were visualised using *boral* (Hui, 2020). Boral ordination can be interpreted in the same manner as NMDS (Non-metric Multi-Dimensional Scaling), but it is a model based approach to unconstrained ordination that fits latent variables to raw count data. Ordination assumed a negative binomial distribution (as *manyglm*), and was carried out on data from each river at six and twelve months separately. Sample identity effects were included so ordination was based on composition rather than relative abundance.

6.2.4.3. *Macroinvertebrate traits in tyre microhabitats*

Trait analysis was performed only on data taken during the twelve-month sampling period as this was when the greatest number of samples were taken. Macroinvertebrate functional traits were derived from Tachet *et al.* (2010). Individual traits represent modalities (e.g. scraper, shredder) within trait groups (e.g. feeding types) (Schmera *et al.*, 2015). The database adopts a "fuzzy-coding" approach (ordinal scale), whereby macroinvertebrate affinities for individual traits are scored from zero (no affinity) to a maximum score of three or five (indicating strong affinity) (Chevene *et al.*, 1994). This method captures variation in the affinity of taxa to individual traits. Four trait groups were selected for analysis (Table 6.3); substrate and flow velocity preference to see of patterns matched the expected changes to sediment and flow conditions, and locomotion and feeding type to see whether the effects of tyres selected for certain macroinvertebrate adaptations.

Table 6.3: Macroinvertebrate traits and trait groups investigated within this study.

	Trait group	Trait
Ecological traits	Flow velocity preference	Null Slow Medium Fast
	Substrate preference	Coarse substrates Gravel Sand Silt Macrophytes Microphytes Twigs/roots Organic detritus Mud
Biological traits	Locomotion	Flier Surface swimmer Full water swimmer Crawler Burrower Interstitial Temporarily attached
	Feeding type	Deposit feeder Shredder Scraper Filter feeder Piercer Predator Parasite

Trait information in the database was not always recorded at the same taxonomic level as taxa were identified to, so taxa abundances in the sample data matrix were adjusted to account for this (i.e. coarsening taxa resolution from species to genus where necessary). After adjustment of taxonomic resolution, any taxa with fewer than five individuals recorded were removed, as were taxa for which there was limited trait information or which were not identified to a high enough taxonomic level (i.e. Chironomidae pupae, Limnephilidae, Oligochaeta and Simuliidae; this left 62% of individuals). Trait information was then processed by standardising affinity scores so that the sum of a given grouping feature equals one, thereby giving the same weight to each grouping feature. A trait-abundance array was calculated by multiplying the standardised trait values by ln(x+1) transformed taxa abundances for each sample. Transforming abundances like this prevents an over emphasis of highly abundant taxa (Gayraud *et al.*, 2003). Trait scores across taxa were then averaged to get the community-weighted mean trait values for each sample and these values standardised (as above) to account for differences in abundance between samples (White *et al.*, 2017).

Principal Component Analysis (PCA) using *vegan* (Oksanen *et al.*, 2020) was conducted on the community weighted mean trait value for substrate and flow velocity preferences to see whether there were differences between tyre microhabitat zones, and whether any traits were associated with different zones. The total variation explained by each axis was calculated by dividing its eigenvalue by the sum of all eigenvalues.

Differences in the community weighted mean value for the tyre microhabitat zones were tested for locomotion and feeding type traits using GLM analysis with microhabitat as the factor of interest. Normal Q-Q plots showed Gaussian error distribution models were an adequate fit. *F*-tests were used to see if traits were significantly different between tyre microhabitat zones by comparing the maximal model with a null model. These tests were conducted on each river separately.

6.2.4.4. *Tyre associated communities compared to those in the background river*

Differences in the abundance and diversity of macroinvertebrates inhabiting tyre-associated habitats were compared with background samples taken from the range of meso-habitat types found elsewhere in each river at zero, six and twelve months. At zero months there was only one tyre sample per tyre as this was taken from the tyre mid-point pre-installation and assumed to be representative of all the river bed that later became tyre microhabitat zones. GLM analysis was repeated, substituting microhabitat with category (tyre surface, around tyre microhabitats, or background river samples), but leaving all other components the same. Significance of factors was again assessed using *F*-tests. Looking for differences between sample categories in this way showed whether tyre-affected zones were still within the range of that found in the different meso-habitat types in the background river.

Macroinvertebrate community composition in tyre associated habitats was also compared with those in the background river. *Manyglm* analysis was repeated using river and category as explanatory variables with an interaction, and significance tested using log-Likelihood Ratio tests with 999 permutations and adjusted *p*-value for multiple comparisons. Ordination via *boral* was also conducted for each river and each sampling period: zero, six and twelve months.

6.3. Results

6.3.1. Geomorphology

Flow velocity was significantly different between tyre microhabitat zones, indicating that tyres significantly impacted flow velocity at 60% depth (Maun: t (49) = 3.712, p = 0.001, Idle: t (59) = 2.172, p = 0.034), and at bed level (Maun: t (49) = 3.359, p = 0.002, Idle: t (59) = 3.330, p = 0.002) (Figure 6.3). Flow velocity downstream and in the middle of tyres was consistently close to zero at bed level, but there was high variation in the flow velocity at 60% depth within these tyre microhabitat zones. Comparison of the flow velocity before and after tyre removal during the twelve-month sampling period in each microhabitat zone shows that tyres slowed flow velocity upstream, downstream and in the middle of the tyre, and slightly increased flow velocity to the sides of the tyre.

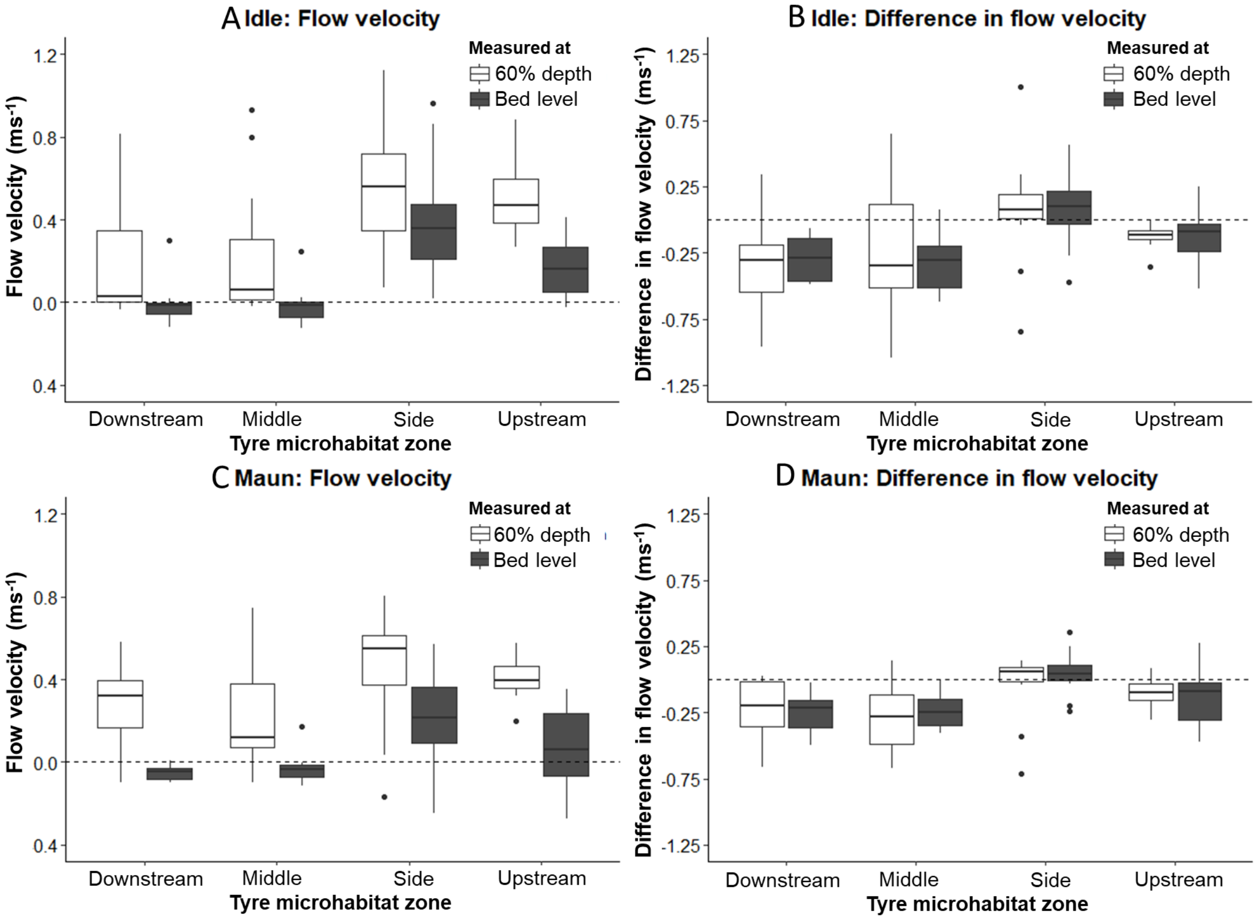

Figure 6.3: Box plots of flow velocity measured twelve months after installation at 60% depth and bed level for A) Idle, and C) Maun. Negative flow velocity measurements indicate that flow was moving upstream. Flow velocity was measured before and after removal of the tyres after twelve months of installation. The difference between these measurements (flow with tyre minus flow without tyre, i.e. the effect of the tyre on flow velocity) is shown in B) Idle, and D) Maun. Data from the River Maun at twelve months is missing tyres 3 and 12 which were removed earlier than planned due to exceptional scour which dislodged these tyres.

Changes in fine sediment depth caused by the presence of tyres were much greater in the Maun than the Idle, because of the greater mobility of sediment in the sand-bed river (Figure 6.4). Twelve months post-installation, fine sediment had accumulated in both rivers downstream (mean ± SE: +0.02 ± 0.05 m in the Maun, and 0.05 ± 0.10 m in the Idle) and in the middle of tyres (-0.01 ± 0.09 m in the Maun, and 0.03 ± 0.05 m in the Idle); although note the high variability, especially in the Maun. In the Maun, fine sediment depth decreased to the side (-0.10 ± 0.06 m) and especially upstream of tyres (-0.24 ± 0.11 m), indicating scour. In contrast, there was little change in fine sediment depth to the side and upstream of tyres in the Idle (< 0.01 m), but there was negligible fine sediment here at installation so any scour that did occur would not have been recorded by measuring fine sediment depth. Differences in fine sediment depth between measurements taken at installation and twelve months later were significant in the Maun (X^2 (2) = 7.538, *p* = 0.023), but not in the Idle (X^2 (2) = 5.333, *p* = 0.069).

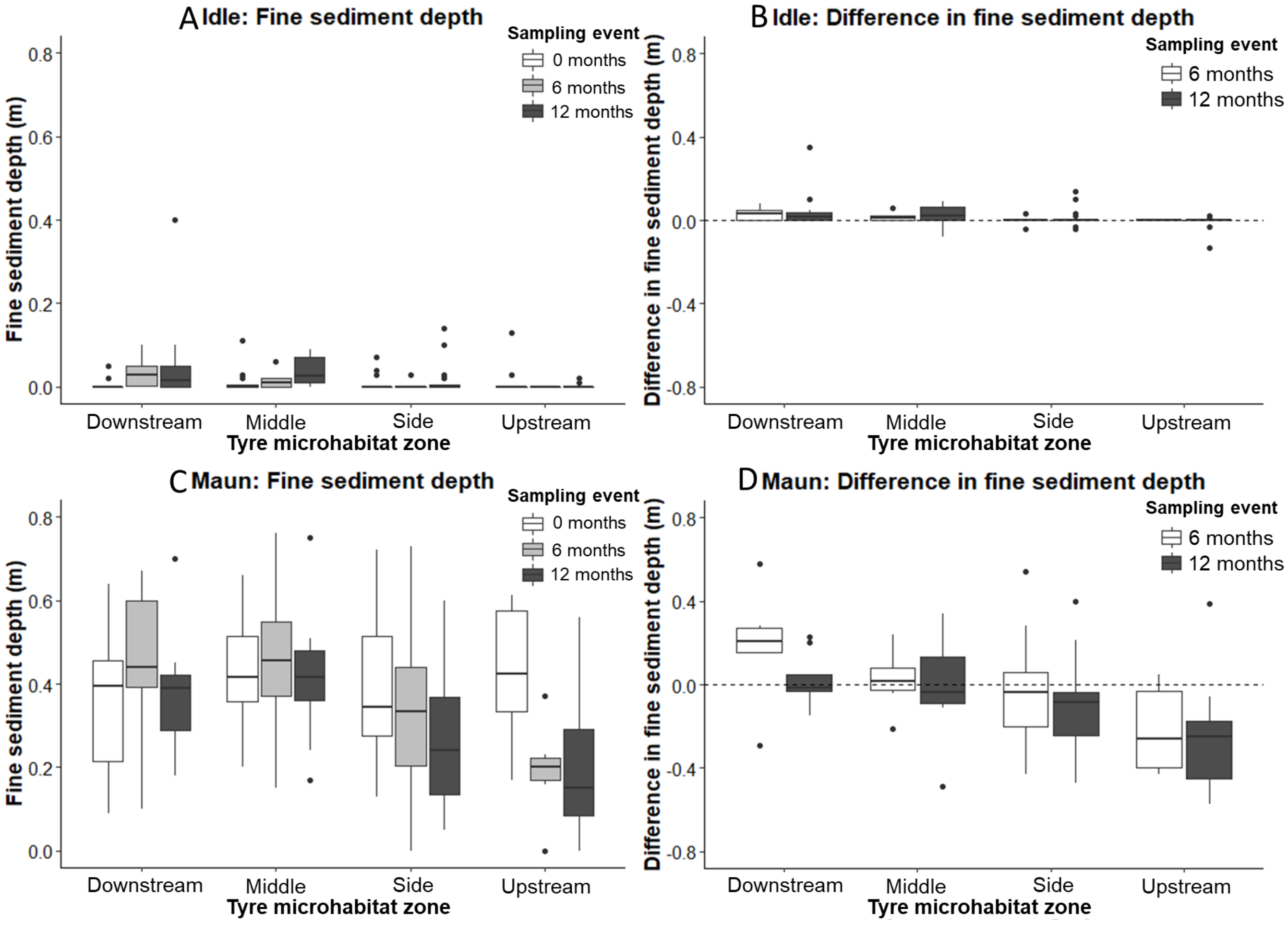

Figure 6.4: Box plots of fine sediment depth measured at installation (zero months) and six and twelve months later for A) Idle, and C) Maun. The difference between the measurements taken during the different sampling events (six or twelve months minus zero months, i.e. the effect of the tyre on fine sediment depth) is shown in B) Idle, and D) Maun. Data from the River Maun at twelve months is missing tyres 3 and 12 which were removed earlier than planned due to exceptional scour which dislodged these tyres.

Sketch maps of tyres showed that in the Idle, tyres contributed to localised redistribution of sediment, with visible scour to the sides of tyres, and deposition of sand and silt downstream and in the middle of tyres. The deposition of sand marked the creation of new habitat patches as tyres were all originally placed in uniform gravel areas. During the six-month sampling period, I observed that some tyres had prompted the establishment of emergent or submerged macrophytes in the middle or downstream of tyres. Most had also accumulated large quantities of macrophytes that had drifted from upstream. These interactions with plants were less apparent after twelve months due to seasonal die-back of vegetation.

The Maun tyres affected the local distribution of sand, with most exhibiting evidence of scour to the sides of tyres, and deposition downstream of the tyres. However, there was no change in sediment type as there was no exposure of gravels or cobbles underneath the sand. Submerged macrophyte growth was extensive after six months, but its distribution was not obviously affected by the tyres. After twelve months, filamentous algae growth on the surface on the tyres was common, but otherwise vegetation was scarce. Tyres accumulated large amounts of racked debris (organic matter and anthropogenic litter) which intensified their impacts on local flow velocity.

6.3.2. Ecology

A total of 29,708 individuals and 85 taxa (58 in the Maun, and 71 in the Idle) from 55 families were recorded across 323 samples (see Table 6.S 2 for the full list of taxa). The mean abundance per sample was 105 (range from 2 to 2137) in the Idle and 78 (range 1 to 491) in the Maun. Three non-native taxa were recorded: *Crangonyx pseudogracilis* (Crustacea), *Potamopyrgus antipodarum* (Gastropoda) and *Planaria torva* (Tricladida). *C. pseudogracilis* was found in substantial numbers in the Idle but rarely in the Maun. *P. antipodarum* was common in both rivers, but *P. torva* was uncommon in both rivers.

Over all samples in the Idle, the most common taxa was Simuliidae (Diptera: 29%), then Chironomidae (Diptera: 15%; mostly Orthocladiinae/Diamesinae), Oligochaeta (12%), *C. pseudogracilis* (9%), *Asellus aquaticus* (Crustacae: 7%) and *Gammarus pulex/fossarum* agg. (Crustacea: 7%). The Gastropoda *Theodoxus fluviatilis* (4%) were common, as well as riffle beetles (Coleoptera): *Oulimnius* sp. (2%) and *Elmis aenea* (1%). Common EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa included *Baetis* sp. (Ephemeroptera: 3%), *Brachycentrus subnubilus* (Trichoptera: 1%), and *Hydropsyche* sp. (Trichoptera: 1%).

Communities in the Maun were dominated by Chironomidae (32% of total abundance; mostly Orthocladiinae/Diamesinae, Tanytarsini or Prodiamesinae), followed by Oligochaeta (23%), *Gammarus pulex/fossarum* agg. (15%), and Simuliidae (9%). *Pisidium* sp. (6%) was the most common Gastropoda, followed by *P. antipodarum* (2%). Common EPT taxa in the Maun include *Baetis* sp. (4%) and *Hydropsyche* sp. (3%).

6.3.2.1. *Macroinvertebrate communities in tyre microhabitats*

In the Idle at six months, macroinvertebrates inhabiting the side and downstream tyre microhabitat zones were more diverse than those in other microhabitat zones (Figure 6.5; mean ± SE ^{0}D side: 22.0 ± 1.8 and downstream: 17.3 ± 1.8, compared to middle: 10.8 ± 1.2, tyre surface: 10.0 ± 2.0, and upstream 9.0 ± 1.6). Idle side samples were also the most diverse at twelve months (mean ± SE: ^{0}D: 14.0 ± 1.2), although differences between microhabitats were smaller than they were at six months. The tyre surface samples were consistently the least diverse in the Idle; they had less than half the taxon richness (D^{0}) of side microhabitat zones during the same sampling periods.

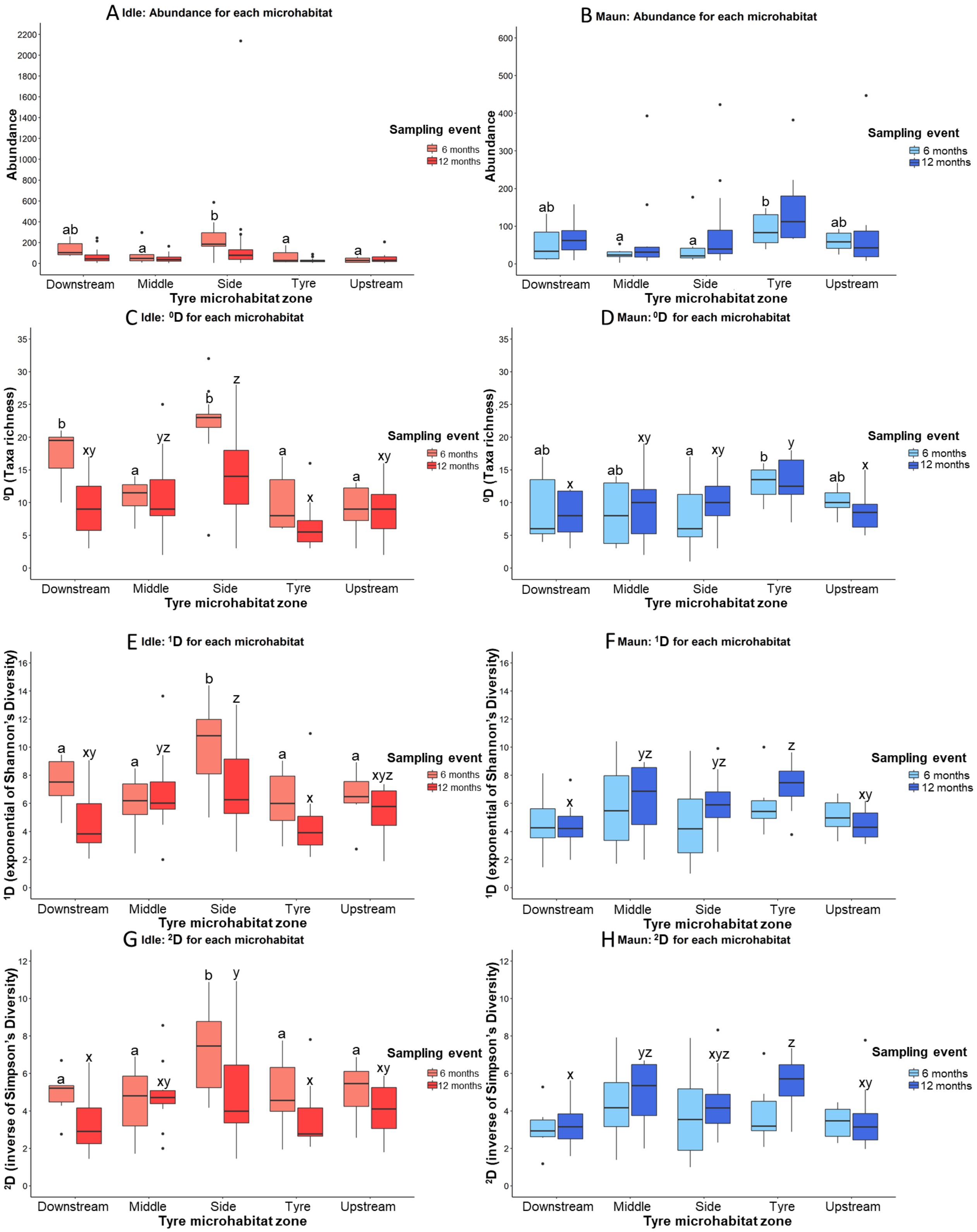

Figure 6.5: Boxplots showing abundance and diversity (⁰D, ¹D and ²D) for the Rivers Idle and Maun during six and twelve month sampling events. Significant differences between tyre microhabitat zones are shown using letters (calculated for six and twelve months separately); microhabitats with the same letter do not differ significantly. No letters indicates that there were no significant differences.

Patterns in the River Maun were different (Figure 6.5). Here the tyre surface had the highest abundance and diversity of macroinvertebrates (mean ± SE ^{0}D tyre surface: 13.3 ± 1.1 at twelve months); significantly higher than that in downstream and upstream microhabitats for all Hill's numbers at twelve months. However, there was little difference in the macroinvertebrate diversity of microhabitat zones surrounding tyres, with the exception of the higher ^{1}D and ^{2}D in middle microhabitat zones and higher ^{1}D in side microhabitat zones at twelve months. There were no significant differences between tyre microhabitat zones for ^{1}D and ^{2}D during the six-month sampling event.

Tyre microhabitat zone was found to significantly affect macroinvertebrate diversity at twelve months after installation (^{0}D, ^{1}D and ^{2}D; Table 6.4). Significant interactions between microhabitat and river for ^{0}D and ^{1}D indicates that patterns of diversity across microhabitats were significantly different between rivers. Abundance was significantly affected by microhabitat, river and their interaction at six months, but was unaffected by all factors at twelve months.

Table 6.4: Results of Generalised Linear Models (GLMs) comparing the differences between tyre microhabitat zones (downstream, middle, side, tyre surface, and upstream) and the interaction with river (Idle or Maun). Models included abundance or diversity (^{0}D, ^{1}D or ^{2}D) as the response variable, with river, microhabitat, and their interaction as fixed effects. Significance was obtained using an *F*-test comparing the model with and without the factor of interest. Asterisks are used to mark significant results.

Sampling event	**Metric**	**Results of GLM analysis**								
		River*Microhabitat			Microhabitat			River		
		df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Six months	Abundance	4	5.723	<0.001*	4	2.637	0.042*	1	13.401	<0.001*
	^{0}D	4	10.002	<0.001*	4	2.471	0.053	1	18.633	<0.001*
	^{1}D	4	3.693	0.009*	4	1.355	0.259	1	17.320	<0.001*
	^{2}D	4	1.856	0.130	4	1.600	0.185	1	17.587	<0.001*
Twelve months	Abundance	4	1.230	0.302	4	0.802	0.526	1	0.034	0.855
	^{0}D	4	4.750	0.001*	4	2.972	0.022*	1	0.416	0.520
	^{1}D	4	2.789	0.029*	4	3.688	0.007*	1	0.123	0.726
	^{2}D	4	2.055	0.091	4	3.040	0.020*	1	0.016	0.898

Comparing the abundance and diversity of samples taken after zero and twelve months shows how tyres affected macroinvertebrates over time (Figure 6.6). As samples were taken during the same season the differences can be assumed to primarily relate to the presence of tyres. After twelve months in the Idle, only tyre surface and downstream samples were different in terms of diversity to before (zero months) samples (^{0}D for tyre surface, and ^{1}D for both); both were less diverse than before samples. Side microhabitat samples had greater abundance than before samples, but this result was skewed by extremely high abundance in one sample. However, in the Maun, all tyre microhabitat zones samples after twelve months were considerably more diverse than before samples for all diversity measures (mean increase in ^{0}D over all samples was 125%). This suggests that tyres positively affected macroinvertebrate diversity in the surrounding river bed. There was also a much greater abundance of macroinvertebrates on the tyre's surface compared to before samples in the Maun.

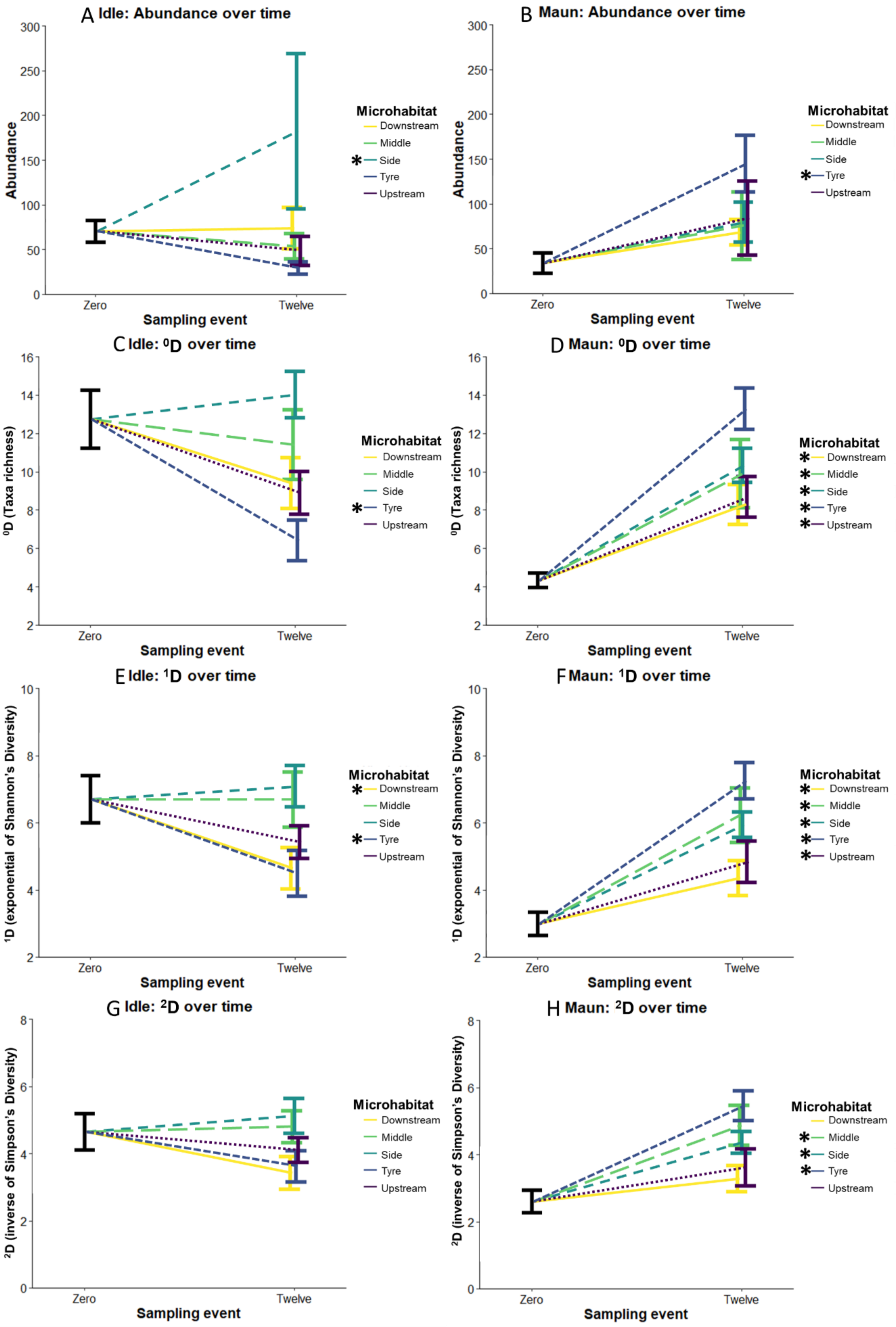

Figure 6.6: The temporal change in mean and standard error (shown as error bars) of diversity and abundance for each tyre microhabitat zone. Zero months values for tyre microhabitat zones were taken from samples of the area of river bed that would become tyre mid-points. Asterisks indicate microhabitats where the standard error around the mean does not overlap with the mean ± the standard error of before samples.

There were significant differences between the composition of macroinvertebrate communities in different tyre microhabitat zones, rivers and their interactions at both six and twelve months (Table 6.5). At six months, Orthocladiinae/Diamesinae and *T. fluviatilis* were significantly different between microhabitats, as was *Baetis* sp. at twelve months (Table 6.6). Taxa that showed different patterns between tyre microhabitat zones in the two rivers were Orthocladiinae/Diamesinae and Chironomidae pupae at six months, and *P. antipodarum* and Oligochaeta at twelve months. At six months, Orthocladiinae/Diamesinae and Chironomidae pupae were most common on tyre surfaces in the Maun, but were respectively most common downstream and to the side of tyres in the Idle. *T. fluviatilis* was only found in the Idle and was mostly found to the side of tyres. At twelve months, *P. antipodarum* and *Baetis* sp. were much more common on tyre surfaces than in other tyre microhabitat zones in the Maun, but were most common to the side of tyres in the Idle. Oligochaeta were relatively common in all microhabitat zones, but were less common on the surface of tyres in the Idle than anywhere else. No taxa in either river (excluding those that occurred only once) were found only on the surface of tyres. Seventeen taxa expressed significant effects for river indicating that their abundance was significantly different between rivers (Table 6.S 3).

Table 6.5: Results of *manyglm* analysis comparing differences in the community composition of samples from different rivers and tyre microhabitat zones. Significance was calculated using 999 replications so a significance of 0.001 is the minimum possible. Asterisks are used to mark significant results.

Sampling event	**Results of *manyglm* analysis**									
		River*Microhabitat			Microhabitat			River		
	n	*df*	*LR*	*p*	*df*	*LR*	*p*	*df*	*LR*	*p*
Six months	72	5	270.6	0.001*	5	561.0	0.001*	1	535.5	0.001*
Twelve months	132	5	280.7	0.001*	5	522.0	0.001*	1	592.5	0.001*

Table 6.6: Mean abundance per sample for taxa which showed significant effects for River*Microhabitat and/or Microhabitat.

	Taxa	**Six months**			**Twelve months**		
		Orthocladiinae /Diamesinae	Chironomidae pupae	*Theodoxus fluviatilis*	*Potamopyrgus antipodarum*	Oligochaeta	*Baetis* sp.
	Significant effect	River* Microhabitat & Microhabitat	River* Microhabitat	Microhabitat	River* Microhabitat	River* Microhabitat	Microhabitat
Idle	Downstream	1.8	2.3	1.0	0.7	13.1	0.4
	Middle	0.8	0.7	0.0	0.0	10.7	0.2
	Side	10.4	0.7	9.3	2.7	14.0	2.4
	Tyre	4.0	0.2	0.0	0.1	1.3	1.2
	Upstream	1.2	0.0	0.8	0.6	8.3	0.3
Maun	Downstream	2.5	0.5	0.0	0.3	25.8	0.8
	Middle	1.0	0.2	0.0	0.9	11.1	1.5
	Side	2.0	0.1	0.0	0.5	14.4	6.2
	Tyre	48.2	4.3	0.0	5.9	21.6	19.9
	Upstream	9.2	0.7	0.0	0.2	16.6	3.9

Ordination of macroinvertebrate community composition in the Maun at six and twelve months, and in the Idle at twelve months shows that the sample communities taken from the surface of tyres were different from those in the surrounding benthic microhabitat zones along the axis of latent variable 1 (Figure 6.7). The community composition of tyre surface samples was most similar to side and upstream samples, which are located in the middle of the ordination plots, but were distinct from those of samples from downstream or the middle of tyres. The consistency in this pattern across rivers suggests that the direction of change to habitat conditions caused by the tyres was similar between rivers even though the source community differed. However, this pattern was not present in the Idle at six months.

In the Idle, the relative location of taxa coefficients compared to samples suggests that the effects of the tyre on flow velocity were important in structuring macroinvertebrate distribution between microhabitats. The tyre surface, side and upstream microhabitat zones, where flow speed was fastest, were associated with rheophilic taxa (taxa which prefer fast flow velocities) including: *Baetis* sp., *Hydropsyche* sp., riffle beetles (*E. aenea* and *Oulimnius* sp.), *T. fluviatilis*, Simuliidae, and *B. subnubilus*. In contrast, the samples from sheltered microhabitats downstream and in the middle of tyres were associated with taxa that like fine sediments, organic matter and slow flows, including: *Bithynia tentaculata*

(Gastropoda), *A. aquaticus*, *G. pulex/fossarum* agg.*, C. psuedogracilis*, and several Chironomidae tribes (Prodiamesinae and Chironomini).

In the Maun, gastropods which prefer slow-flowing habitats, including Sphaeriidae mussels (*Sphaerium* sp. and *Pisidium* sp.), *Bathyomphalus contortus*, and *Radix balthica*, were more common in downstream and middle microhabitat zones; as were leeches (*Erpobdella octoculata* and *Glossiphonia complanata*) and Prodiamesinae. In contrast, the surface of tyres were associated with some Chironomidae tribes (Orthocladiinae/Diamesinae, Tanytarsini, and Chironomidae pupae) and *P. antipodarum*. Several caddisfly and mayfly taxa were also more common on the surface of tyres, including: *Baetis* sp. and *Serratella ignita* (Ephemeroptera), and *Hydropsyche* sp., *Athripsodes* sp., *Mystacides* sp., *Polycentropus flavomaculatus*, *Psychomyia pusilla* and *Rhyacophila dorsalis* (Trichoptera). These EPT taxa, with the exception of *Mystacides* sp.*,* are all sensitive to fine sediment according to the Proportion of Sediment-sensitive Invertebrates (PSI) biotic index (Extence *et al.*, 2013). Mean EPT taxa abundance on the surface of tyres in the Maun was 26.7 at twelve months and 10.8 at six months. This was much higher than that for around tyre samples (7.9 at twelve months and 3.3. at six months). Only cobbles had a similar density of EPT taxa (mean EPT taxa was 10.7 at twelve months); the mean EPT taxa across other background river meso-habitats was only 2.1 at twelve months.

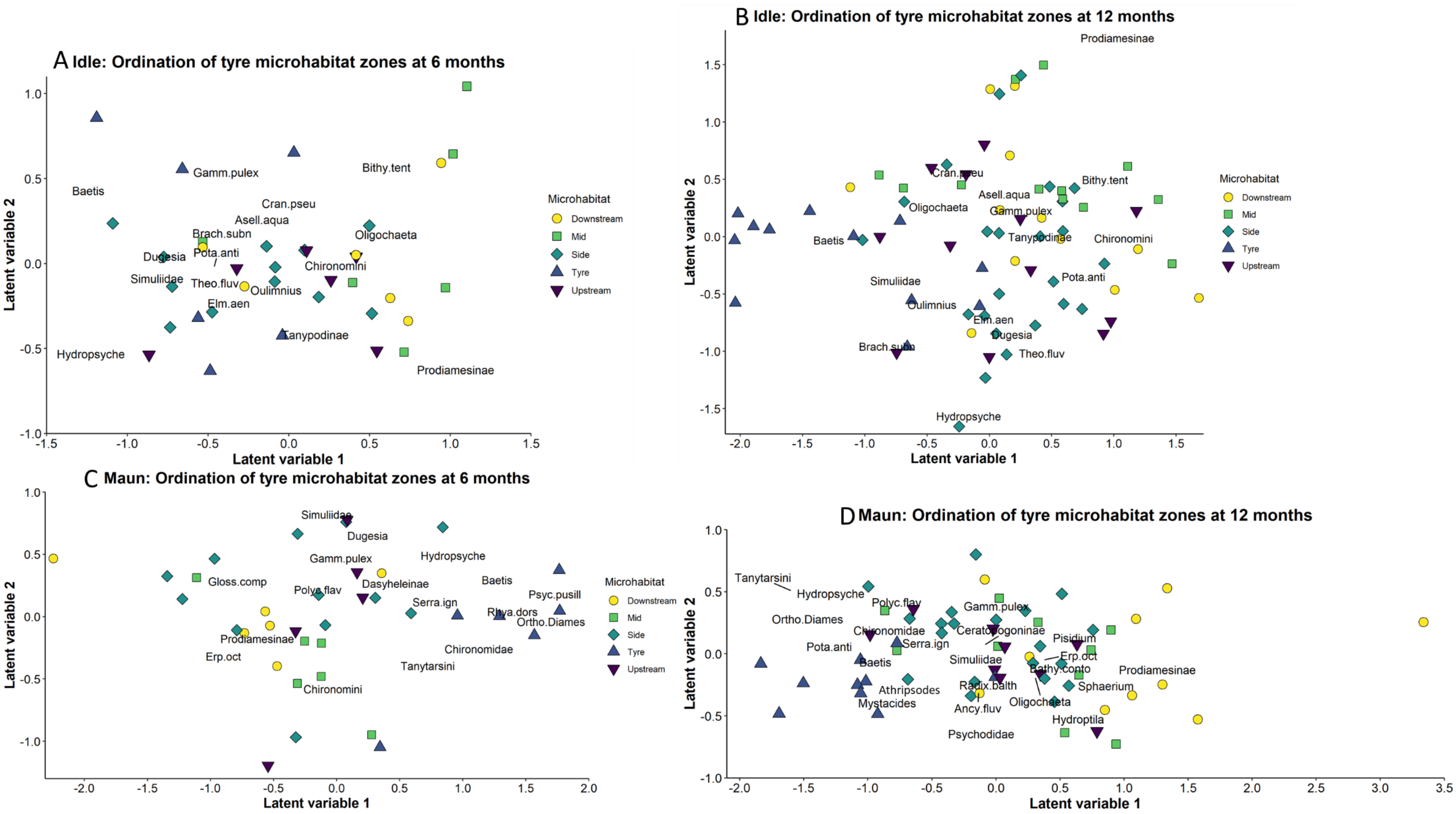

Figure 6.7: Boral ordination with two latent variables for six and twelve month samples from tyre microhabitats for the River Idle (A and B) and the River Maun (C and D). Taxa with the largest *LR* test statistics (based on *manyglm* test for each river and sampling event) are overlayed to show how taxa coefficients relate to samples. Full taxa names for codes are in Table 6.S 4.

6.3.2.2. *Macroinvertebrate traits in tyre microhabitats*

Ordination of the substrate and flow velocity preferences of the taxa found in each tyre microhabitat sample after twelve months provides support for the hypothesis that tyres have created distinct habitat conditions in each microhabitat zone, which relate to flow and substrate conditions (Figure 6.8). Traits showing preferences for fast flow velocity and larger substrate sizes (boulders and gravel) plot alongside tyre surface, side, and upstream samples in both rivers. In the Idle, null and slow flow velocity plot towards the left of the ordination, along with samples from downstream and the middle of tyres. The same is true of the Maun, where downstream and middle samples are also associated with fine sediments (sand, mud, and silt) and organic matter.

The results of GLM tests for locomotion and feeding type are shown in Table 6.7 and Figure 6.9 (only significant results are shown here, full results are in Table 6.S 5 and Figure 6.S 1). The proportion of deposit feeders and scrapers in both rivers, as well as parasites in the Idle, was significantly higher on tyre surfaces than other microhabitat zones. In the Idle this was at the expense of shredders which made up a significantly lower proportion of macroinvertebrates on tyre surfaces than other tyre microhabitats in this river. There was a significantly greater proportion of filter feeders downstream and in the middle of tyres than other microhabitat zones in both rivers, and of parasites downstream of tyres in the Maun. In terms of locomotion, full swimmers made up a greater proportion of taxa on tyre surfaces than other microhabitats in the Idle, but in the Maun they were significantly less common downstream of tyres than other microhabitats. The pattern for crawlers, the most common locomotion trait, contrasted between rivers, with significantly more crawlers on tyres than other microhabitat zones in the Maun, but significantly less on tyre surfaces than other zones in the Idle. Burrowers made up a greater proportion of taxa downstream and in the middle of tyres, but less on the surface of, upstream, or to the sides of tyres.

Table 6.7: Results from Generalised Linear Models (GLMs) comparing the differences in feeding type and locomotion traits between tyre microhabitat zones (downstream, middle, side, tyre surface, and upstream). Models included community weighted mean trait score as the response variable, with microhabitat as a fixed effect. Significance was obtained using an *F*-test comparing the model with and without the factor of interest. Only significant results are shown here, full results are in Table 6.S 5.

		Results of GLM analysis					
Trait group	Trait	Idle			Maun		
		df	*F*	*p*	*df*	*F*	*p*
Locomotion	Full water swimmer	4	3.565	0.011*	4	7.320	<0.001*
	Crawler	4	2.894	0.029*	4	4.097	0.006*
	Burrower	4	9.187	<0.001*	4	4.799	0.002*
Feeding type	Deposit feeder	4	4.332	0.004*	4	3.842	0.008*
	Shredder	4	3.825	0.008*			
	Scraper	4	7.786	<0.001*	4	3.973	0.007*
	Filter-feeder	4	3.766	0.008*	4	4.916	0.002*
	Parasite	4	4.020	0.006*	4	4.320	0.004*

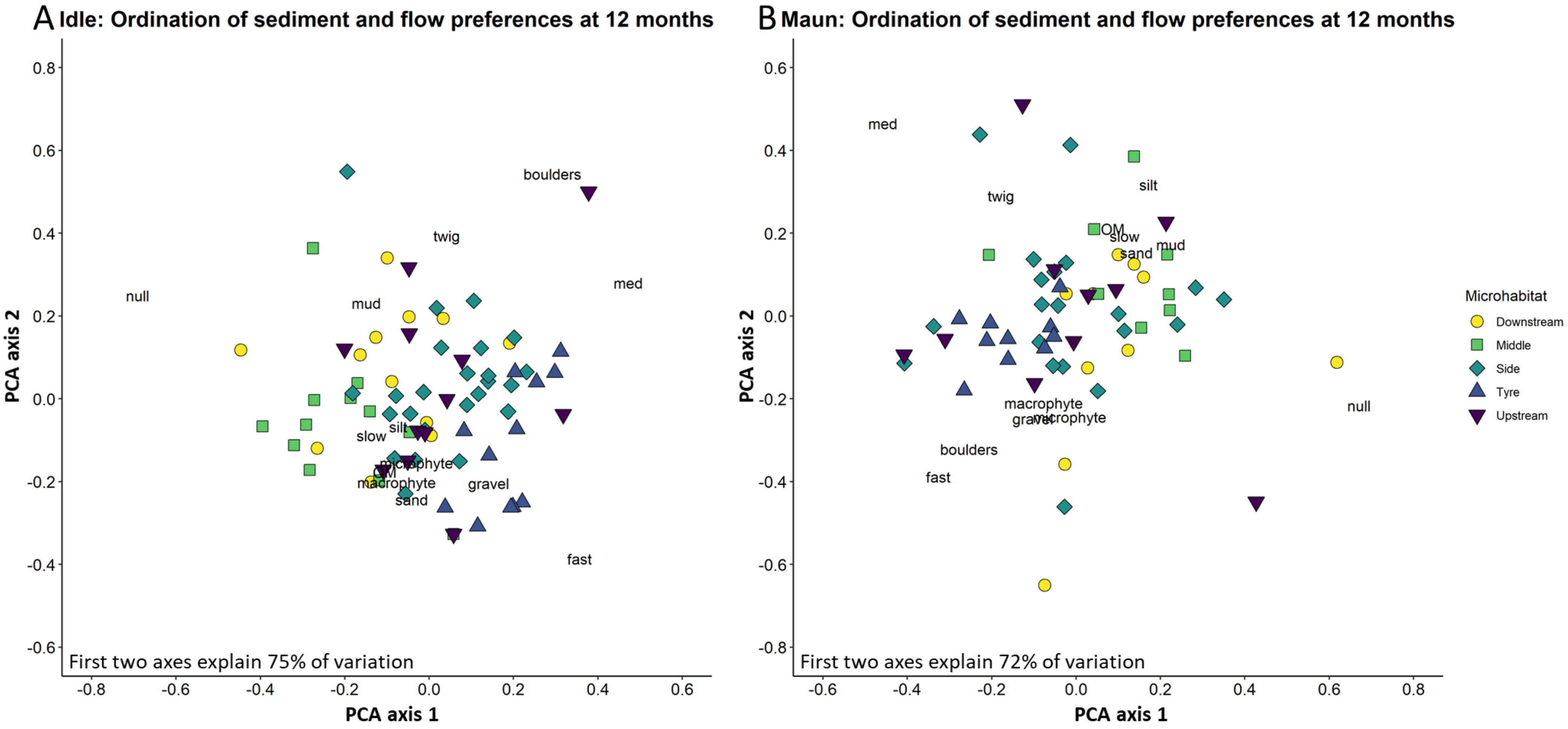

Figure 6.8: Principal Component Analysis (PCA) plot for the Idle (A) and Maun (B). PCA used community weighted mean trait values for the substrate and flow velocity preferences of the community sampled in each tyre microhabitat zone after twelve months. Traits are overlayed to show how trait coefficients relate to samples. Codes for traits are: Flow velocity preferences (null, slow, medium, fast), and substrate preferences (boulders: flags/boulders/cobbles/pebbles, gravel, sand, silt, macrophytes, microphytes, twig: twigs/roots, OM: organic detritus/leaf litter, mud).

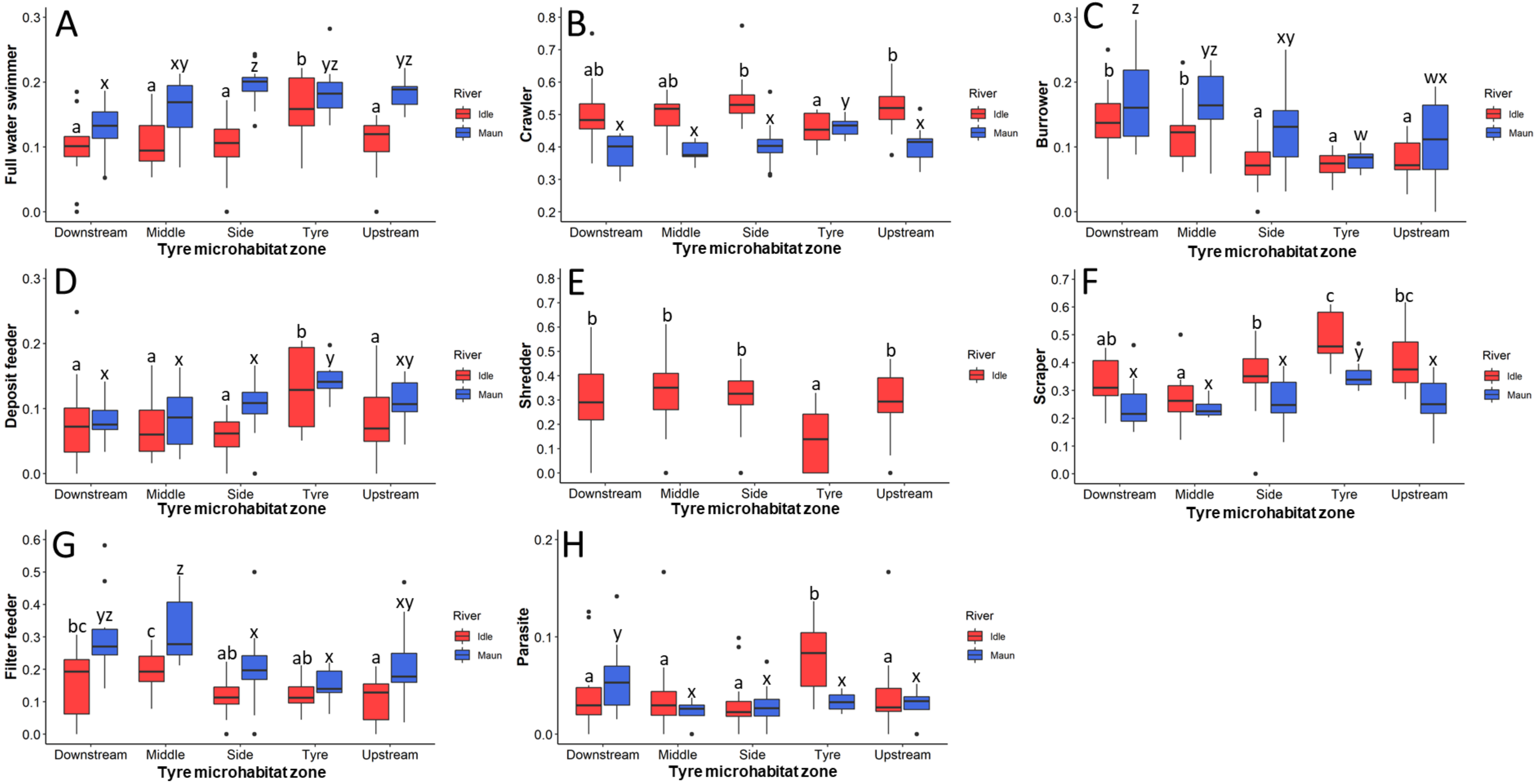

Figure 6.9: Boxplots of community weighted mean trait values for locomotion (A to C) and feeding type (D to H) trait groups that are significantly different between tyre microhabitat zones for twelve month data. Significant differences between tyre microhabitat zones are shown using letters (calculated for Idle and Maun separately); microhabitats with the same letter do not differ significantly. Plot scales are different to emphasise differences between tyre microhabitat zones.

6.3.2.3. *Tyre associated communities compared to those in the background river*

Pre-installation (at zero months) samples taken from tyre mid-points had lower macroinvertebrate diversity than background river samples in the Maun, but similar diversity in the Idle (Figure 6.10). This was because tyres were installed within patches of the main meso-habitat type in each river (sand for the Maun, and gravel for the Idle). Pre-installation samples therefore reflected typical abundance and diversity in these meso-habitat types, whereas background river samples inflate/deflate their relative diversity by not standardising for spatial coverage of different meso-habitat types (the reasons for this are explained in Section 6.2.2.1). Sand substrates typically support low macroinvertebrate diversity and so as tyres were located on sand patches in the Maun it makes sense that pre-installation tyre sites had relatively low diversity. In comparison, Idle tyres were placed on gravel (a habitat type which supports relatively high diversity) so the diversity of these samples was closer to that found in the rest of the river.

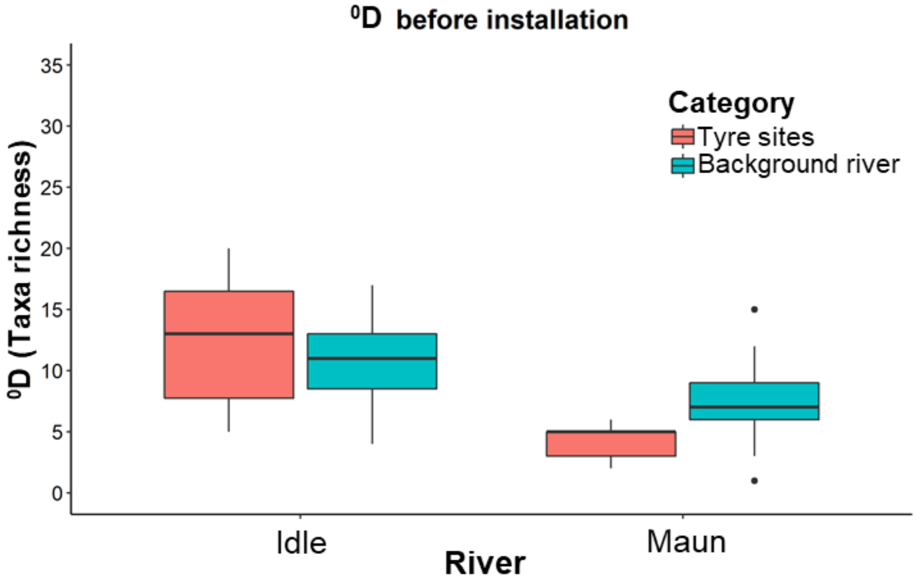

Figure 6.10: Diversity (⁰D) differences between categories for the Rivers Idle and Maun during the zero month sampling event which took place pre-installation. Tyre site samples were taken from the area of river bed which would become the tyre midpoint. Boxplots for other diversity measures and abundance at zero months are in Figure 6.S 2.

In the Idle, there were no significant differences in abundance between sample categories at six and twelve months (Figure 6.11). The three sample categories are: background river, tyre surface, and around tyre microhabitats (i.e. downstream, middle, side, and upstream microhabitats). The macroinvertebrate diversity of background river and around tyre samples were also similar suggesting that, although I have shown that tyres do affect the surrounding river bed (see Section 6.3.2.1), this is within the range of diversity found in existing habitats elsewhere in the river. Both categories of samples, however, had significantly higher ${}^{0}D$ than that found on tyre surfaces at twelve months. This difference is not significant for ${}^{1}D$ and ${}^{2}D$, implying that it is the loss of low abundance taxa on the surface of tyres that is responsible for the distinction. At six months, however, there was no difference between categories.

Contrary to patterns of diversity in the Idle, the tyre surface had significantly greater diversity than other sample categories in the Maun for all diversity measures at twelve months (Figure 6.11), although diversity at six months was not significantly different between categories. The significance of the river and category interaction terms (Table 6.8) reflects this contradictory pattern between rivers. Unlike in the Idle, the ${}^{1}D$ and ${}^{2}D$ of around tyre microhabitat samples are also significantly higher than that in the background river at twelve months. This suggests that tyres created conditions outside of the range found in the rest of the river, and that this resulted in more diverse communities with greater evenness around tyres. Differences in abundance were not significant at twelve months, although tyre surfaces had lower abundance than the other categories.

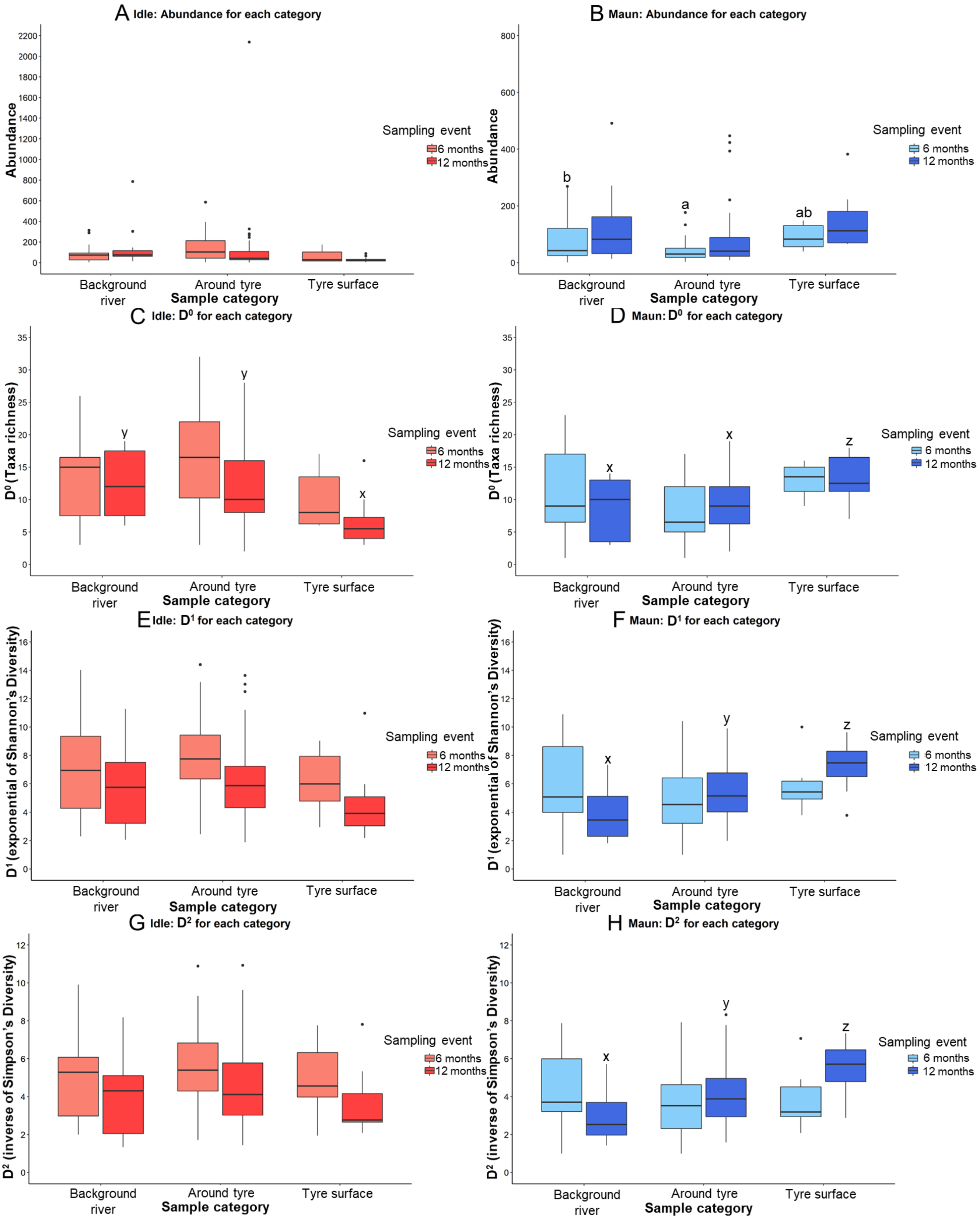

Figure 6.11: Boxplots showing abundance and diversity (^{0}D, ^{1}D and ^{2}D) differences between categories for the Rivers Idle and Maun during six and twelve month sampling events. Significant differences between sample categories (tyre surface, around tyre microhabitats, and the background river) are shown using letters (calculated for each sampling event separately); categories with the same letter do not differ significantly. No letters indicates that there were no significant differences.

Table 6.8: Results of Generalised Linear Models (GLMs) comparing the differences between abundance and diversity of macroinvertebrates on and around tyres, and in the background river. Models included abundance or diversity (^{0}D, ^{1}D or ^{2}D) as the response variable, with river and category (tyre surface, around tyre microhabitats, or background river) as fixed effects with an interaction effect. Significance was obtained using an *F*-test comparing the model with and without the factor of interest. Asterisks are used to mark significant results.

Sampling event	**Metric**	**Results of GLM analysis**								
		River*Category			Category			River		
		df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Zero months	Abundance	1	0.406	0.527	1	5.057	0.028*	1	3.158	0.081
	^{0}D	1	6.875	0.011*	1	0.442	0.509	1	30.617	<0.001*
	^{1}D	1	7.890	0.006*	1	0.226	0.636	1	14.186	<0.001*
	^{2}D	1	4.416	0.040*	1	0.359	0.552	1	5.823	0.019*
Six months	Abundance	2	4.071	0.020*	2	0.154	0.857	1	9.198	0.003*
	^{0}D	2	4.725	0.011*	2	0.092	0.912	1	14.726	<0.001*
	^{1}D	2	2.078	0.131	2	0.123	0.885	1	14.830	<0.001*
	^{2}D	2	1.185	0.310	2	0.124	0.884	1	14.857	<0.001*
Twelve months	Abundance	2	1.304	0.274	2	0.502	0.606	1	0.070	0.790
	^{0}D	2	9.307	<0.001*	2	0.442	0.644	1	2.244	0.136
	^{1}D	2	6.244	0.002*	2	1.824	0.165	1	1.57	0.246
	^{2}D	2	4.722	0.010*	2	2.157	0.119	1	0.809	0.370

There were significant differences in macroinvertebrate community composition between sample categories, as well as between rivers and their interaction, during all sampling events (Table 6.9). The mean abundance per sample for taxa which show significant effects are reported in Table 6.S 6. At six months Chironomidae pupae were significant for the interaction term. They were rare in the Idle, but in the Maun they were more common on tyres or in the background river than in around tyre samples. At twelve months, *P. antipodarum* and Oligochaeta were significant for the interaction term, and Oligochaeta and Prodiamesinae were significantly different between sample categories. *P. antipodarum* was most common on tyres, but both only showed differences in abundance between categories in the Maun. Oligochaeta was rarer on tyre surfaces than other categories in the Idle, but was most common in the background river for both rivers. Prodiamesinae was more common in the background river for both rivers. Taxa which show significant differences between rivers are recorded in Table 6.S 3.

Table 6.9: Results of *manyglm* analysis comparing differences in the community composition of samples from different rivers and categories (tyre surfaces, around tyre microhabitats, and background river samples). Significance was calculated using 999 replications so a significance of 0.001 is the minimum possible.

Sampling event		**Results of *manyglm* analysis**								
		River*Category			Category			River		
	n	*df*	*LR*	*p*	*df*	*LR*	*p*	*df*	*LR*	*p*
Zero months	59	1	85.5	0.001*	1	107.2	0.004*	1	269.0	0.001*
Six months	102	2	138.3	0.002*	2	255.2	0.002*	1	682.4	0.001*
Twelve months	162	2	172.4	0.001*	2	247.4	0.002*	1	708.0	0.001*

The differences in community composition at zero months (pre-installation) again reflect the fact that the tyres were placed in patches of the dominant meso-habitat type in each river. An ordination of zero months data shows that in the Idle, the community composition of tyre surface samples was similar to gravel background river samples. In the Maun, tyre surface samples were similar to sand samples from the background river (Figure 6.12).

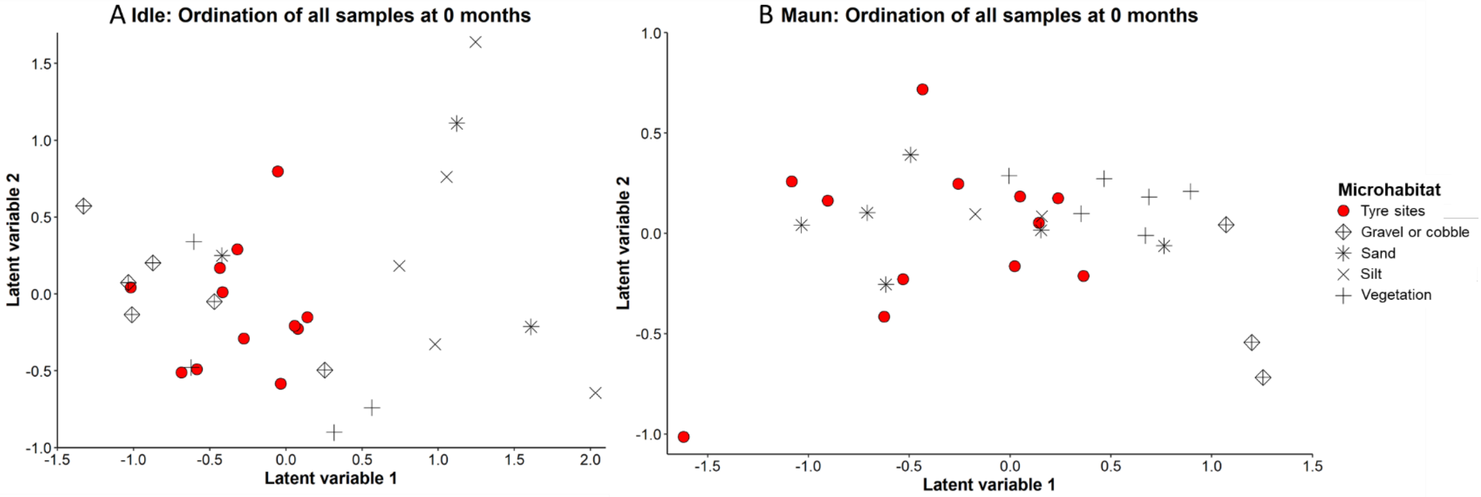

Figure 6.12: Boral ordination of A) Idle and B) Maun samples with two latent variables using data from the zero-month sampling period from background river meso-habitats and samples taken pre-installation from what would become the tyre mid-points (tyre sites).

Ordination of River Idle sample communities from on and around tyres, and background mesohabitat types shows a similar trend to Figure 6.7. Samples from the surface of tyres are positioned to one side of the plot, with side and upstream samples in the middle, and downstream and middle of tyre samples on the opposite side to the tyre surface samples (Figure 6.13). Silt and sand sample community composition was most similar to downstream and middle of tyre samples, which makes sense as tyres caused fine sediment deposition in these zones. The spread of background river samples in the Idle encompasses the range of around tyre microhabitat samples, suggesting that although tyres created locally different microhabitat conditions which supported different macroinvertebrate communities, these were not outside of the range of that in the background river. At twelve months, however, tyre surface samples are further left on the ordination than the background river samples, suggesting that the tyre surface community was distinct from that in the rest of the river.

In the Maun at six months, cobble and tyre surface samples were closely associated to each other on the right-hand side of the plot, separated from the rest of the samples along latent variable 1. Sand and silt samples plot to the left-hand side with samples from downstream and the middle of tyres. Upstream and side microhabitats plot in the middle alongside vegetation samples. Patterns were similar at twelve months, except there was a separation of sand and some silt background river samples further to the right of the plot than all tyre microhabitats save a few downstream samples. Given that sand is the dominant substrate type in the Idle, this suggests that as well as tyre surfaces providing a distinct habitat (only similar to cobbles which cover a small portion of the river bed), tyres have caused a shift in the community in the surrounding river bed away from that found elsewhere.

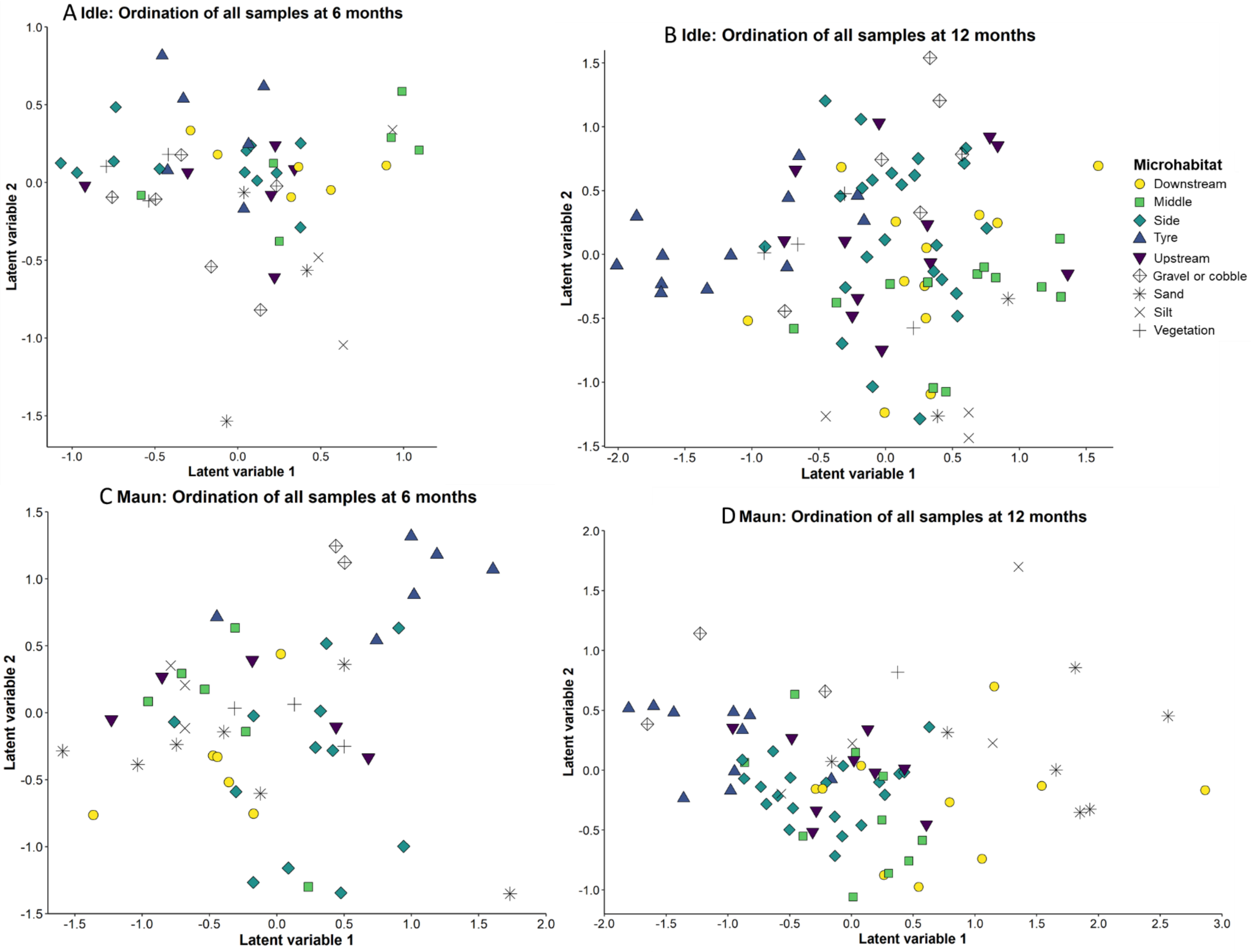

Figure 6.13: Boral ordination with two latent variables for six and twelve month samples for the River Idle (A and B) and the River Maun (C and D). Plots compare samples from on and around tyres (coloured symbols) to the different meso-habitat types represented by background river samples.

6.4. Discussion

6.4.1. Macroinvertebrate communities in tyre microhabitats

6.4.1.1. *The surface of tyres was a distinct habitat type*

The surface of car tyres in both the sand-bed River Maun and the gravel-bed River Idle were colonised by distinct macroinvertebrate communities that were different to those found elsewhere in the rivers. In both rivers, the tyre surface communities had greater proportional abundance of rheophilic taxa and those which typically prefer large mineral substrates (boulders or gravel). The tyres protruded into the flow and so macroinvertebrates occupying the tyre's surface were exposed to faster flow velocities than that generally experienced in the river bed. Similarly, the hard structure of tyres was akin to the characteristics of large mineral substrates, therefore tyre surfaces provided habitat for taxa which typically inhabit boulders, cobbles or gravel and prefer fast flows like *Baetis* sp., *T. fluviatilis*, *Athripsodes* sp., and *Hydropsyche* sp. Scraper taxa were also more common on tyre surfaces in both rivers, probably because of the extensive biofilm that developed on the tyres. Biofilm will grow on any submerged and non-toxic artificial materials (Cattaneo and Amireault, 1992), and its biomass is known to be inversely proportional to bed instability (Segura *et al.*, 2011); hence tyres provided an excellent surface for scrapers, like *Baetis* sp. and several gastropods, to graze. The greater proportional abundance of deposit feeders on tyre surfaces suggests tyres also accumulated sufficient quantities of the fine particulate organic matter upon which they feed.

Trait information in the literature is limited for some taxa and it was also not possible to identify several families (i.e. Chironomidae pupae, Simuliidae, Oligochaeta, and Limnephilidae) to high enough taxonomic level for investigation of their traits. These taxa were therefore excluded from trait analysis. Only a few Chironomidae pupae and Limnephilidae were found, so it is unlikely that ignoring these taxa in trait analysis has changed the results; however, Simuliidae and Oligochaeta made up large proportions of the community in both rivers. Simuliidae are generally filter feeders that temporarily attach to substrate and use their cephalic fans to capture suspended food particles (Wallace and Merritt, 1980). They were fairly evenly distributed across tyre microhabitats in the Maun but were more common downstream, to the side, and on the surface of tyres in the Idle. In contrast, Figure 6.9G suggests that filter feeders were most common downstream and in the middle of tyres, probably because the filter-feeding snails *Bithynia* spp. and Sphaeriidae (*Pisidium* sp. and *Sphaerium* sp.), which were most common in these zones, preferred the slow flows found here. Oligochaeta are mostly burrowers and deposit feeders, and in this study they were relatively uncommon on tyre surfaces. This matches the pattern for other burrowers, but contrasts with the distribution of deposit feeders in Figure 6.9C and D.

The egg masses of some macroinvertebrates were found on tyres (Figure 6.14). Ovipositing taxa are known to exhibit preferences for the different sides of rocks (Reich and Downes, 2003), so different tyre surfaces may have created favourable conditions for certain taxa (e.g. the eggs pictured in Figure 6.14A were located only on the sheltered downstream edge of the tyre). Emergent rocks and other structures are essential ovipositing habitats for some egg-laying macroinvertebrates (Lancaster *et al.*, 2021). During low flows in the spring and summer many of the tyres stuck out from the water's surface, providing an opportunity for taxa that crawl down emergent structures for oviposition (Miller *et al.*, 2020).

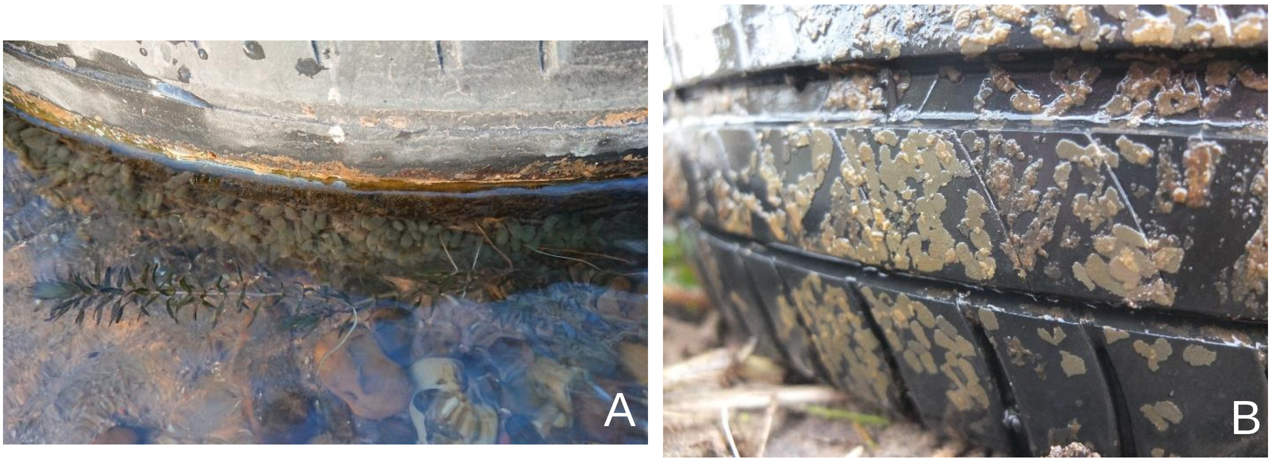

Figure 6.14: Photos of eggs found on tyre surfaces. A) Unknown eggs on downstream edge of a tyre in the Idle, B) *Baetis* sp. eggs photographed after removal, as were found on tyres in both the Idle and the Maun.

However, although patterns in the habitat preferences and feeding types of macroinvertebrates on the surface of tyres were similar between rivers, patterns of diversity were distinctly different. In the Maun, tyre surfaces were inhabited by highly diverse communities comprising of a greater number of sensitive taxa. However, in the Idle, the diversity of macroinvertebrates colonising the surface of tyres was considerably lower than that found in the surrounding river bed.

In the Maun, this difference in the diversity of macroinvertebrate communities living on tyres compared to the surrounding river bed is especially remarkable because the tyre supported taxa which were uncommon in the river bed. The only other meso-habitat type in the Maun where I found macroinvertebrate communities of a similar composition and diversity to tyre surface samples was cobbles. Patches of cobbles, however, only made up a small proportion of the river bed as they were introduced as part of an old habitat enhancement scheme and have now largely been buried or separated. Tyres, therefore, provided an otherwise scarce solid and stable habitat in the Maun.

This finding is similar to previous reports that large wood provides a particularly important hotspot for macroinvertebrate diversity in rivers with abundant fine sediment due to its relative stability and solid structure (Hoffmann and Hering, 2000; Benke and Wallace, 2003; Pitt and Batzer, 2011). The sand-bed of the Maun is highly mobile, even during periods of low flow. This inhibits the locomotion of some taxa, does not provide a secure platform for macroinvertebrates to hold onto, inhibits their ability to take refuge in the interstices between particles, and affects the availability of food resources (Gibbins *et al.*, 2007; Jones *et al.*, 2011). Hence, there were greater numbers of EPT taxa, which are sensitive to fine sediment, inhabiting the surface of tyres. These taxa score highly in biotic indices for river quality, like the Whalley, Hawkes, Paisley and Trigg index (WHPT) (Water Framework Directive - United Kingdom Advisory Group [WFD-UKTAG], 2014) that is used to classify a rivers' WFD status. This means the presence of tyres could affect reach scale environmental quality assessment as they increase the chance of these high scoring taxa appearing in samples.

In contrast, tyre surfaces in the Idle were inhabited by relatively impoverished macroinvertebrate communities compared to the river bed. As opposed to studies based in sand-bed rivers, several researchers have found limited difference between the abundance and diversity of macroinvertebrate communities on large wood compared to gravel or cobble substrates, despite finding differences in the composition of communities (Wallace *et al.*, 1996; Dossi *et al.*, 2018). This may be because taxa that require hard substrates to fix to, or feed from, are already able to find suitable conditions in the river bed. For instance, many of the tyre-associated taxa in the Maun like *Hydropsyche* sp. and *P. antipodarum* were, by contrast, more common in side microhabitats than on the surface of tyres in the Idle. The surface of tyres was much smoother and simpler in surface texture than the gravel-bed, and so it accordingly supported fewer taxa. Structurally complex habitats have greater niche space and surface area, support short distance dispersal of taxa from neighbouring habitats, provide shelter, and reduce the likelihood of competitive exclusion. Thus, more complex surfaces can generally support greater biodiversity (O'Connor, 1991; Douglas and Lake, 1994; Robson and Barmuta, 1998; Boyero, 2003). Upon removal of tyres, it was clear that macroinvertebrates were concentrated within the tread where their exposure to flows would be lower (Figure 6.15). The filter-feeders *Hydropsyche* sp. and *B. subnubilus* were particularly common here, presumably because they could gain better seston access by occupying elevated positions on the tyres, whilst using the tread for shelter and to fix their nets or cases to. These filter feeders have also been reported to use large wood in a similar way to increase their capture of drifting food particles (Hoffmann and Hering, 2000).

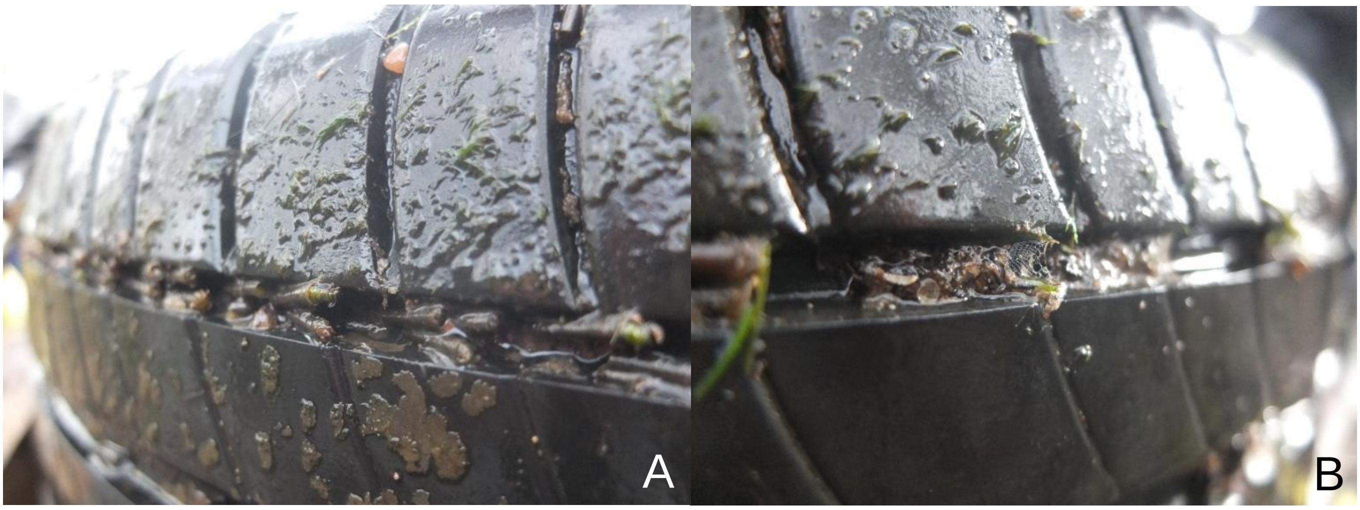

Figure 6.15: Photo of *Brachycentrus subnubilus* (A) and *Hydropysche* sp. caddisflies (B) occupying the tyre tread on one of the tyres removed from the River Idle.

It is also possible that the lower diversity and abundance of macroinvertebrate communities inhabiting tyres in the Idle is in part a reflection of the sampling methods. Surber sampling of the river bed collects macroinvertebrates living up to a few cm in depth. However, macroinvertebrates could not burrow into tyres, and so tyre samples represent a smaller 3D area. I do not believe that this invalidates the conclusions as the sampled surface area was as consistent as could be achieved (and is a commonly used method), and consider it simply a different characteristic of the two substrates. In fact, it makes the high diversity of tyre surface samples in the Maun all the more remarkable, as these communities were still significantly more diverse than river bed samples.

6.4.1.2. *The tyres created different microhabitat zones in the surrounding river bed*

There were differences in the diversity and the composition of macroinvertebrate communities in different microhabitat zones on and around tyres in both rivers. These patterns reflect the changes to flow and sediment patterns caused by tyres and show that the effects of large anthropogenic litter extend into the surrounding river bed. The consequences of a tyre's presence on the surrounding river bed was therefore an increase in the variety of habitat conditions within the vicinity of the tyre.

By protruding into the flow, tyres influenced hydraulic conditions in their immediate surroundings; slowing flow speeds in the middle and downstream of tyres and accelerating flow along their sides, as has been described in detail for individual submerged boulders and other simple obstacles (e.g. Baki *et al.*, 2017; Zexing *et al.*, 2020). Accordingly, there was a greater abundance of rheophilic taxa to the sides of tyres and on tyre surfaces, and greater numbers of taxa that prefer slow flows downstream and in the middle of tyres. In the Idle, the faster flow speeds that characterised side microhabitats were related to significantly higher macroinvertebrate diversity compared to the other tyre microhabitats. The flow acceleration adjacent to tyres could be important for macroinvertebrate resilience if it provides locally high velocities during low flows when these conditions become more scarce.

Changes to local flow velocity have implications for sediment distribution, which in turn affects macroinvertebrates. In lowland rivers, accelerated flow velocities associated with large wood have been shown to clear patches of fine sediment (Osei *et al.*, 2015a), coarsening bed material in proximity to the wood (Mutz, 2000; Pilotto *et al.*, 2014). Patches of scour were visible around tyres in both rivers, but in the Maun where there is no gravel layer underlying the sand-bed, there was no corresponding change to sediment size. Sheltered zones, like those downstream and in the middle of tyres, encouraged the deposition of fine sediment and organic matter (Koljonen *et al.*, 2012; Flores *et al.*, 2011, 2017; Osei *et al.*, 2015a); hence there were a greater proportion of taxa which prefer sand, silt, twig and organic matter substrates in these zones. This included burrowing taxa, filter-feeding gastropods: *B. tentaculata*, *Pisidium* sp. and *Sphaerium* sp., and shredders, which accounted for the greater proportional abundance of these traits downstream and in the middle of tyres. This fine sediment deposition could lead to additional repercussions for macroinvertebrates, as seeds and other plant propagules are also deposited around obstacles and because sheltered zones and fine sediment provides ideal conditions for vegetation establishment (Osei *et al.*, 2015a,b). Some changes to vegetation were observed even in the relatively short time span of this experiment; for example, the reedbed pictured in Figure 6.16B expanded approximately 1 m into the river throughout the monitored period because reeds grew downstream and in the middle of the tyre where flow velocity was slower. Over time this would increase the hydraulic effect of the tyres and potentially increase geomorphic response, as well as changing the habitat type by introducing vegetation.

Figure 6.16: Photos taken of vegetation responses at A) tyre 7 and B) tyre 5 in the Idle. Photos were taken in August and September 2019, around six months after the tyres had been in place. Arrows indicate the flow direction for each image.

6.4.2. Tyre associated communities compared to those in the background river

In the Maun, the diversity of macroinvertebrate communities inhabiting the river bed around tyres was significantly greater than that found elsewhere in the river, except on the surface of tyres or on cobbles. Diversity was significantly greater around tyres after twelve months compared to pre-installation samples, and there was a clear shift in community composition away from that of sand meso-habitat samples. These findings suggest that the tyres acted as focal points for diversity by increasing habitat quality of the surrounding river bed and providing opportunities for taxa with different habitat preferences. Similar results have been reported for large wood structures in other lowland sand-bed rivers (Pilotto *et al.*, 2014, 2016; Nakano *et al.*, 2018), but I did not find the same pattern in the Idle. This is likely to relate to the difference in river bed stability between the two rivers.

Despite destabilisation of two of the tyres in the Maun by scour, most remained stable elements of the river bed, slowing flow velocities and prompting local deposition and accumulation of fine sediment and organic matter. The ability of structures to stabilise the surrounding river bed has previously been found to support greater macroinvertebrate diversity within its vicinity in other sand-bed rivers where the majority of river bed was highly mobile (Pilotto *et al.*, 2014, 2016; Nakano *et al.*, 2018). It has been suggested that large wood can provide sheltered and stable zones from which macroinvertebrates could recolonise after high flows (Palmer *et al.*, 1996), or that it could bolster populations by enhancing capture of drifting organisms, which then disperse into surrounding habitats (Wellnitz *et al.*, 2014). Sand-bed rivers have limited interstitial space for macroinvertebrates to take refuge in, so recovery after high flows may be relatively slow compared to gravel-bed rivers (Lake, 2000), and therefore stable obstacles may be more significant as refuges.

As a related consequence of localised river bed stabilisation around obstacles, accumulations of organic matter and debris are known to occur around structures like large wood (Gerhard and Reich, 2000; Flores *et al.*, 2011). Where large wood was found to enhance accumulation of organic matter, the areas of river bed around the wood was reported to support more productive and more diverse communities of macroinvertebrates in several sand-bed rivers (Entrekin *et al.*, 2009; Pilotto *et al.*, 2014, 2016), because organic matter accumulations are known to be important sources of habitat and food (Palmer *et al.*, 2000). Tyres accumulated wood pieces, leaf litter, anthropogenic litter, and, particularly in the Idle, drifting macrophytes (Figure 6.17). This rafting of material intensified the tyre's effects on local flow velocity and thereby on sediment distribution, as has been found for large wood jams (Wellnitz *et al.*, 2014). For example, a large (0.6 by 1.4 m) wooden board caught on a tyre in the Maun caused exceptional scour (estimated at 0.3 m depth during the six-months sampling event). Drifting macrophyte accumulation was especially prevalent during six-month sampling in the Idle, as annual cutting of *Ranunculus* stands occured near to the time of sampling. This may explain why tyre surface samples were more diverse and more similar in community composition to around tyre microhabitat samples during the six-month sampling event. It is therefore more likely that taxa inhabiting the macrophytes and other debris would be collected in tyre surface samples at this time, elevating their diversity and changing their composition.

Figure 6.17: Photos taken of A) board racked against tyre 8 in the Maun, B) drifting weed collected at tyre 4 in the Idle, C) wood and vegetation caught on tyre 6 in the Idle. Photos were taken in August and September 2019, around six months after the tyres had been in place. Arrows indicate the flow direction.

However, although local habitat distribution was affected by tyres in the Idle, comparison with background river macroinvertebrate samples suggests that tyres did not create unique habitats in the surrounding river bed. Neither macroinvertebrate diversity or composition in the vicinity of tyres was outside of that found elsewhere in the river, and similar patches of sand, silt, gravel and macrophytes existed within the study section. Similarly, restoration of boulders or large wood which fails to create new and novel habitat types, like deep pools or depositional zones, or to significantly enhance the quality of existing habitats, has been found to have limited reach-scale effect on macroinvertebrate communities post-restoration (Hilderbrand *et al.*, 1997; Lepori *et al.*, 2005; Miller *et al.*, 2010). In this study, the tyres' influence was limited to only small areas of the River Idle, so changes to the proportional coverage of habitats were minimal. Where there are large concentrations of anthropogenic litter, or where large jams are able to build up around the anthropogenic litter (see Figure 6.18 for examples), it could be a more significant driver of geomorphology and ecology. Future measurement of the geomorphological role of anthropogenic litter would be useful to extend these findings.

Figure 6.18: Fly-tipped debris in Alfreton Brook which has caused notable change to the surrounding habitats.

6.4.3. Conclusions and implications

These results expand on the findings of Chapter 5 and previous research which shows that abundant and diverse communities inhabit anthropogenic litter in a range of environments (Chapman and Clynick, 2006; Katsanevakis *et al.,* 2007; Czarnecka *et al.*, 2009; Song*, et al.,* 2021). Not only does anthropogenic litter itself provide a surface for colonisation, but in rivers it interacts with flow, sediment and organic matter, thereby changing habitat conditions in the surrounding river bed and influencing macroinvertebrates. The specific taxa and traits associated with the different microhabitats around tyres, as well as the geomorphological measurements and observations, showed that the effects of anthropogenic litter were similar to the impacts of similarly sized natural structures like large wood and boulders (Zexing *et al.*, 2020; Pilotto *et al.*, 2016). Where stable habitat is lacking, as in the sand-bed River Maun, large anthropogenic litter can provide and create especially valuable habitat. In particular, it supported more taxa which are sensitive to fine sediment, which could have implications for reach-scale environmental quality assessment.

Therefore, in rivers where large wood, boulders or macrophytes cannot be used for habitat enhancement, it may be possible to design non-toxic artificial materials to create and provide valuable habitat (Nakano *et al.*, 2018). Natural materials like large wood are preferable as it has a more complex structure (twigs and branches) and the ability to provide nutrients, both important factors for macroinvertebrates (Hoffmann and Hering, 2000; Benke and Wallace, 2003; Gurnell, 2013). These is also a risk that anthropogenic litter could leach harmful chemicals with health or mortality consequences for aquatic organisms (Degaffe and Turner, 2011; Gunaalan *et al.*, 2020). As such, tyres should not be deliberately installed as artificial habitat, however, clearance of existing anthropogenic litter should also be considered in terms of the habitat that would be lost through its removal (Backhurst and Cole, 2000; Chapman and Clynick, 2006), and this habitat should be replaced with other non-environmentally damaging alternatives where possible.

6.5. Supplementary materials

Table 6.S 1: Comparison of results of Generalised Linear Models (GLMs) with and without abundance outlier from the River Idle at twelve months: Tyre 11 left-side sample (abundance 2137 compared to mean of 79 in all other Idle samples). The effect of the outlier is shown for GLM analysis on differences between tyre microhabitat zones (downstream, middle, side, tyre surface, and upstream) and between sample categories (tyre surface, around tyre, or background river). Asterisks are used to mark significant results.

Testing differences by	**Outlier treatment**	**Results of GLM analysis**								
		River*Microhabitat/Category			**Microhabitat/Category**			**River**		
		df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
By microhabitat (tyre samples only)	With outlier	4	1.230	0.302	4	0.802	0.526	1	0.034	0.855
	Without outlier	4	2.387	0.055	4	0.473	0.756	1	2.093	0.151
By category (tyre and background river samples)	With outlier	2	1.304	0.274	2	0.502	0.606	1	0.071	0.790
	Without outlier	2	3.068	0.049*	2	3.103	0.048*	1	0.848	0.359

Table 6.S 2: List of the 85 taxa recorded in the study. Asterisks denote non-native species.

- **PLATYHELMINTHES**
  - **RHABDITOPHORA**
    - **Tricladida**
      - *Dendrocoelum lacteum*
      - *Dugesia* sp.
      - *Planaria torva**
      - *Polycelis* sp.
- **MOLLUSCA**
  - **GASTROPODA**
    - **Caenogastropoda**
      - *Viviparus viviparus*
    - **Cycloneritimorpha**
      - *Theodoxus fluviatilis*
    - **Ectobranchia**
      - *Valvata piscinalis*
    - **Hygrophila**
      - *Acroloxus lacustris*
      - *Physa fontinalis*
      - *Ancylus fluviatilis*
      - *Anisus vortex*
      - *Bathyomphalus contortus*
      - *Gyraulus albus*
      - *Gyraulus crista*
      - *Gyraulus laevis*
      - *Planorbis carinatus*
    - **Littorinimorpha**
      - *Bithynia leachii*
      - *Bithynia tentaculata*
      - *Potamopyrgus antipodarum**
      - *Lymnaea stagnalis*
      - *Radix auricularia*
      - *Radix balthica*
  - **BIVALVIA**
    - **Veneroida**
      - *Pisidium* sp.
      - *Sphaerium* sp.
- **ANNELIDA**
  - **OLIGOCHAETA**
  - **CLITELLATA**
    - **Arhynchobdellida**
      - *Erpobdella octoculata*
      - *Erpobdella testacea*
    - **Rhynchobdellida**
      - *Glossiphonia complanata*
      - *Helobdella stagnalis*
      - *Theromyzon tessulatum*
      - *Piscicola geometra*
- **ARTHROPODA**
  - **MALACOSTRACA**
    - **Amphipoda**
      - *Crangonyx pseudogracilis**
      - *Gammarus pulex/fossarum agg.*
    - **Isopoda**
      - *Asellus aquaticus*
  - **INSECTA**
    - **Coleoptera**
      - Dryopidae
      - *Nebrioporus elegans*
      - *Elmis aenae*
      - *Esolus parallelepipedus*
      - *Limnius volckmari*
      - *Oulimnius* sp.
      - *Orectochilus villosus*
      - *Haliplus* sp.
    - **Diptera**
      - *Dasyhelea* sp.
      - Ceratopogoninae
      - Chironomini
      - Orthocladiinae/Diamesinae
      - Prodiamesinae
      - Tanypodinae
      - Tanytarsini
      - Chironomidae pupae
      - *Hydrellia* sp.
      - *Setacera* sp.
      - *Helius* sp.
      - *Neolimnomyia* sp.
      - *Limnophora* sp.
      - *Dicranota* sp.
      - Psychodidae
      - Rhagionidae
      - Simuliidae
      - Stratiomyidae
      - Tipulidae
    - **Ephemeroptera**
      - *Baetis* sp.
      - *Caenis luctuosa*
      - *Caenis robusta*
      - *Serratella ignita*
      - *Heptagenia sulphurea*
    - **Hemiptera**
      - Gerridae
    - **Lepidoptera**
      - Crambidae
    - **Megaloptera**
      - *Sialis lutaria*
    - **Odonata**
      - *Calopteryx splendens*
      - Coenagrionidae
    - **Trichoptera**
      - *Brachycentrus subnubilus*
      - *Goera pilosa*
      - *Hydropsyche* sp.
      - *Hydroptila* sp.
      - *Lepidostoma* sp.
      - *Athripsodes* sp.
      - *Ceraclea* sp.
      - *Mystacides* sp.
      - Limnephilidae
      - *Polycentropus flavomaculatus*
      - *Lype reducta*
      - *Psychomyia pusilla*
      - *Tinodes waeneri*
      - *Rhyacophila dorsalis*

Table 6.S 3: Table showing taxa which show significant effects for river in *mvabund* tests in Sections 6.3.2.1 and 6.3.2.3.

	Taxa showing significant effects for River
Six months (tyre microhabitat zones only)	*Elmis aenae* *Oulimnius* sp. *Asellus aquaticus* *Crangonyx pseudogracilis* *Theodoxus fluviatilis* *Dugesia* sp. *Polycelis* sp. *Ancylus fluviatilis* Chironomini *Bithynia tentaculata* *Physa fontinalis* *Brachycentrus subnubilus* Prodiamesinae *Caenis luctuosa* *Bithynia leachii*
Twelve months (tyre microhabitat zones only)	*Oulimnius* sp. *Theodoxus fluviatilis* *Polycelis* sp. *Bithynia tentaculata* *Erpobdella octoculata* *Baetis* sp. *Bithynia leachii* *Pisidium* sp.
Zero months (tyre microhabitat zones and background river)	*Crangonyx pseudogracilis* *Pisidium* sp. *Dugesia* sp. *Oulimnius* sp. *Theodoxus fluviatilis* Limnephilidae Simuliidae *Bithynia tentaculata* *Polycelis* sp. *Elmis aenae* *Asellus aquaticus*
Six months (tyre microhabitat zones and background river)	*Elmis aenae* *Oulimnius* sp. *Asellus aquaticus* *Crangonyx pseudogracilis* *Theodoxus fluviatilis* *Ancylus fluviatilis* *Dugesia* sp. *Polycelis* sp. Chironomini *Brachycentrus subnubilus* *Caenis luctuosa* *Physa fontinalis* *Bithynia tentaculata* *Erpobdella octoculata* *Orectochilus villosus* *Piscicola geometra* *Bithynia leachii*

	Tanytarsini *Baetis* sp.
Twelve months (tyre microhabitat zones and background river)	*Oulimnius* sp. *Theodoxus fluviatilis* *Polycelis* sp. *Erpobdella octoculata* *Bithynia tentaculata* *Brachycentrus subnubilus* *Bithynia leachii* *Crangonyx pseudogracilis* *Baetis* sp.

Table 6.S 4: Full names of taxa for codes used in Figure 6.7.

Code	Full name
Ancy.fluv	*Ancylus fluviatilis*
Athripsodes	*Athripsodes* sp.
Asell.aqua	*Asellus aquaticus*
Baetis	*Baetis* sp.
Bathy.conto	*Bathyomphalus contortus*
Bithy.tent	*Bithynia tentaculata*
Brach.subn	*Brachycentrus subnubilus*
Ceratopogoninae	Ceratopogoninae
Chironomidae	Chironomidae pupae
Chironomini	Chironomini
Cran.pseu	*Crangonyx pseudogracilis*
Dasyheleinae	*Dasyhelea* sp.
Dugesia	*Dugesia* sp.
Elm.aen	*Elmis aenae*
Erp.oct	*Erpobdella octoculata*
Gamm.pulex	*Gammarus pulex/fossarum* agg.
Gloss.comp	*Glossiphonia complanata*
Hydropsyche	*Hydropsyche* sp.
Hydroptila	*Hydroptila* sp.
Mystacides	*Mystacides* sp.
Oligochaeta	Oligochaeta
Ortho.Diames	Orthocladiinae/Diamesinae
Oulimnius	*Oulimnius* sp.
Pisidium	*Pisidium* sp.
Polyc.flav	*Polycentropus flavomaculatus*
Pota.anti	*Potamopyrgus antipodarum*
Prodiamesinae	Prodiamesinae
Psyc.pusill	*Psychomyia pusilla*
Psychodidae	Psychodidae
Radix.balth	*Radix balthica*
Rhya.dors	*Rhyacophila dorsalis*
Serra.ign	*Serratella ignita*
Simuliidae	Simuliidae
Sphaerium	*Sphaerium* sp.
Tanypodinae	Tanypodinae
Tanytarsini	Tanytarsini
Theo.fluv	*Theodoxus fluviatilis*

Table 6.S 5: Results from Generalised Linear Models (GLMs) comparing the differences traits between tyre microhabitat zones (downstream, middle, side, tyre surface, and upstream). Models included community weighted mean trait score as the response variable, with microhabitat as a fixed effect. Significance was obtained using an *F*-test comparing the model with and without the factor of interest.

		Results of GLM analysis					
Trait group	**Trait**	**Idle**			**Maun**		
		df	*F*	*p*	*df*	*F*	*p*
Flow velocity preference	Null	4	16.296	<0.001*	4	2.614	0.045*
	Slow	4	3.506	0.012*	4	1.681	0.168
	Medium	4	11.277	<0.001*	4	2.888	0.031*
	Fast	4	11.028	<0.001*	4	2.439	0.058
Substrate preference	Coarse substrates	4	3.456	0.013*	4	2.257	0.075
	Gravel	4	4.108	0.005*	4	2.329	0.068
	Sand	4	0.760	0.555	4	4.897	0.002*
	Silt	4	3.574	0.011*	4	5.437	<0.001*
	Macrophytes	4	3.275	0.016*	4	2.730	0.038*
	Microphytes	4	1.516	0.207	4	0.243	0.913
	Twigs/roots	4	1.375	0.252	4	7.378	<0.001*
	Organic detritus	4	1.757	0.148	4	2.799	0.035*
	Mud	4	8.249	<0.001*	4	2.402	0.061
Locomotion	Flier	4	1.787	0.142	4	1.246	0.303
	Surface swimmer	4	0.928	0.453	4	0.929	0.454
	Interstitial	4	2.025	0.101	4	1.189	0.326
	Temporarily attached	4	0.869	0.487	4	0.975	0.429
Feeding type	Piercer	4	0.714	0.586	4	1.626	0.181
	Predator	4	0.772	0.548	4	0.630	0.643

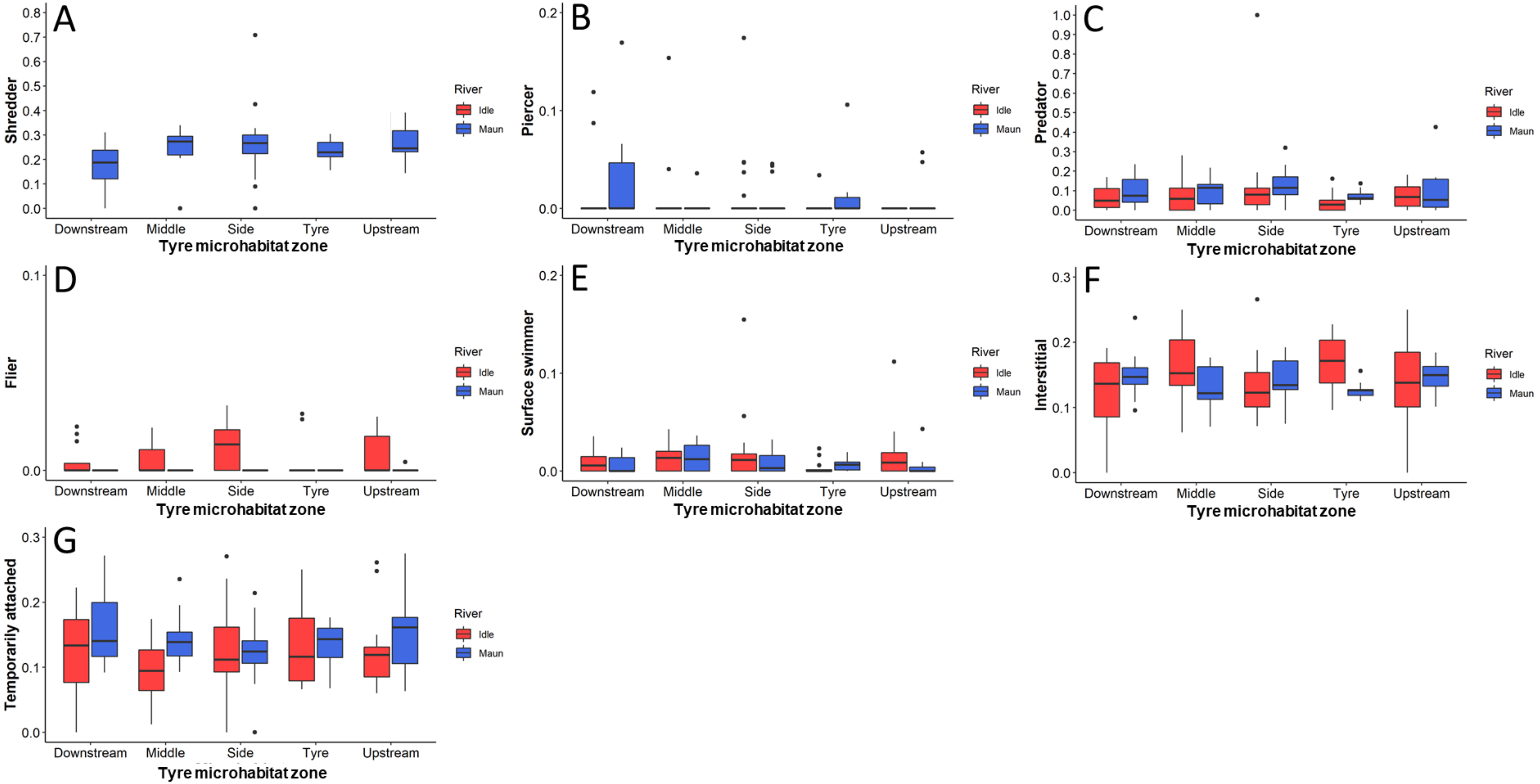

Figure 6.S 1: Boxplots of community weighted mean trait values for locomotion (A to C) and feeding type (D to H) trait groups that were not significantly different between tyre microhabitat zones for twelve month data. Plot scales are different to emphasise differences between microhabitat zones.

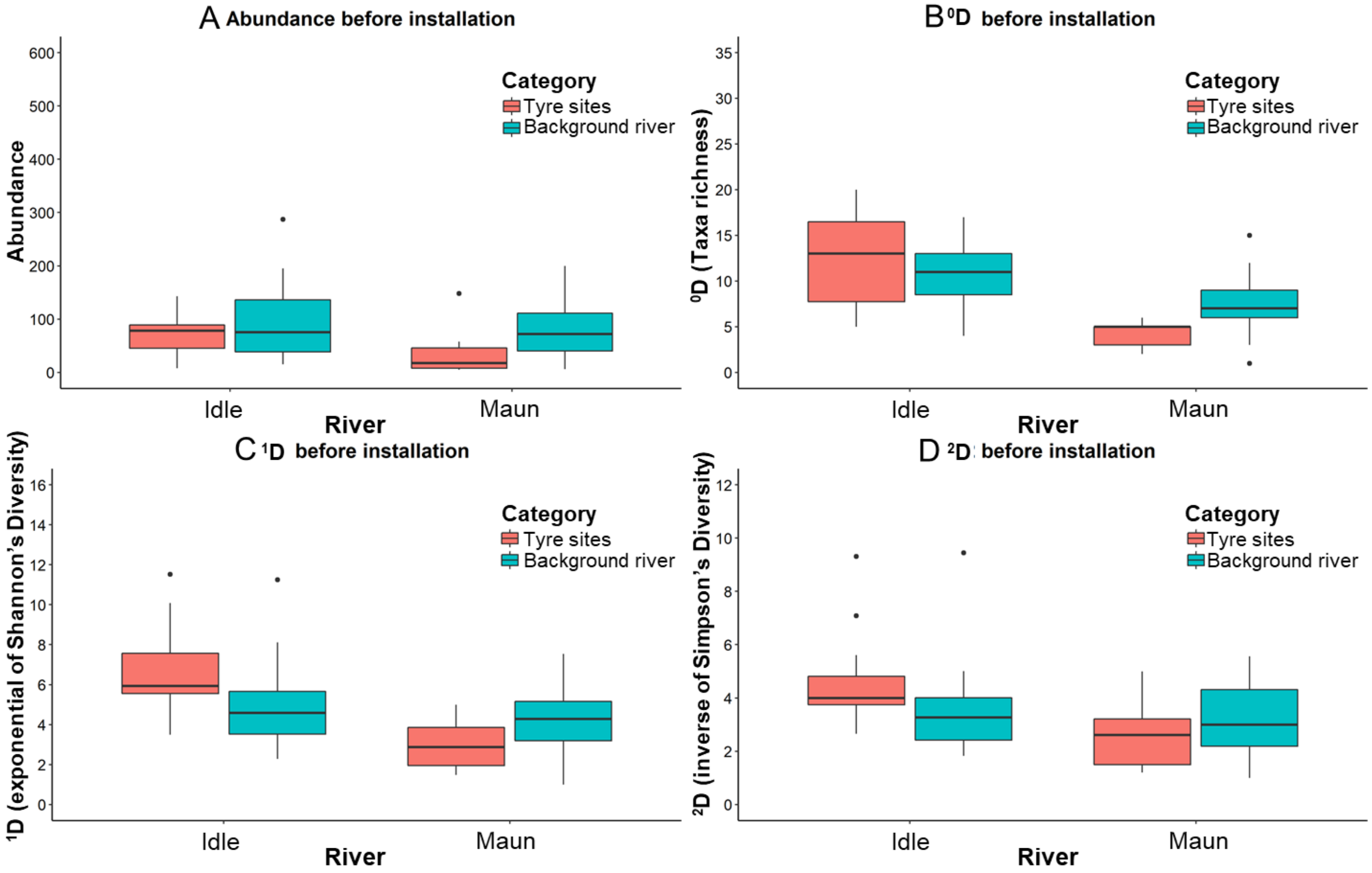

Figure 6.S 2: Boxplots showing abundance and diversity (^{0}D, ^{1}D and ^{2}D) differences between categories for the Rivers Idle and Maun during the zero-month sampling event which took place pre-installation. Tyre samples were taken from the area of river bed which would become the tyre midpoint.

Table 6.S 6: Mean abundance per sample for taxa which show significant effects for either Category (tyre surface/before, around tyre microhabitats, or background river) or River*Category. Around tyre microhabitats are NA at zero months because only before samples (taken from what would become the tyre midpoint) and background river samples were collected during this sampling event.

		Zero months				**Six months**	**Twelve months**		
	Taxa	Simuliidae	*Gammarus pulex/fossarum* agg.	Orthocladiinae/ Diamesinae	Chironomidae pupae	Chironomidae pupae	*Potamopyrgus antipodarum*	Oligochaeta	Prodiamesinae
	Significant effect	Category	River* Category	River* Category	River* Category	River* Category	River* Category	River* Category & Category	Category
Idle	Tyre/Before	6.8	7.25	23.1	1.8	0.2	0.1	1.3	0
	Around tyre microhabitats	NA	NA	NA	NA	0.9	1.3	12.0	0.6
	Background river	41.7	1.9	11.7	0.3	0.7	0.9	18.1	3.6
Maun	Tyre/Before	0.0	1.1	0.7	0.0	4.3	5.9	21.6	0.7
	Around tyre microhabitats	NA	NA	NA	NA	0.3	0.5	16.4	2.8
	Background river	4.2	14.7	13.3	0.8	3.0	0.5	51.5	10.7

Chapter 7: Small fish use anthropogenic litter as habitat structure in rivers

7.1. Introduction

A key control on the distribution of fish is habitat structure (hereafter 'structure') which is defined as a physical feature or entity that supports a community of plants and animals (McCoy and Bell, 1991). Within aquatic environments there are many different types of structure, including those that are made of natural materials (e.g. coral reefs, macrophytes, or boulders; Warfe and Barmuta, 2004; Branco *et al.*, 2013; Richardson *et al.*, 2017) and those which are composed of artificial materials (e.g. artificial rook pools: Morris *et al.*, 2017; Hall *et al.*, 2019). Confusingly, the term 'artificial' is sometimes used to mean constructed structures, even those that are made of natural materials (e.g. Foote *et al.*, 2020), but here I use artificial solely to describe structures made of manufactured materials. Structures may occur naturally, or they could have been introduced into the environment intentionally to provide habitat (e.g. large wood restoration, artificial reefs: Chapman and Clynick, 2006; Roni *et al.*, 2015) or their use as habitat may be unintentional (e.g. anthropogenic litter, sunken vessels, oil and gas infrastructure, or off-shore wind turbines: Feary *et al.*, 2011; Langhamer, 2012; Wilson *et al.*, 2021).

7.1.1. The effects of structures on fish populations

Many studies have demonstrated the importance of structures for fish communities. Positive correlations between the presence of natural structures and local fish abundance, biomass, survival and diversity have been found in many rivers and other aquatic environments (e.g. Lewis, 1969; Lehtinen *et al.*, 1997; García-Charton *et al.*, 2004; Randall *et al.*, 2006; Pettit *et al.*, 2013; Kalogianni *et al.*, 2020). Several researchers have confirmed a mechanistic link between structures and fish by experimentally introducing structures and identifying resultant changes. Local fish abundance, biomass and diversity has been found to increase after the introduction of artificial reefs to marine environments; both for those introduced intentionally (Chapman and Clynick, 2006; Reed *et al.*, 2006; Sargent *et al.*, 2006; dos Santos *et al.*, 2010; Folpp *et al.*, 2020) and unintentionally (Consoli *et al.*, 2013; Scott *et al.*, 2015). In some cases, the density and diversity of fish around artificial reefs were found to be greater than that at nearby natural reefs (Chapman and Clynick, 2006; Reed *et al.*, 2006; Folpp *et al.*, 2013), although differences in the community composition suggests that artificial reefs may not be true surrogates for natural reefs (Perkol-Finkel *et al.*, 2006; Folpp *et al.*, 2013). Artificial reef structures not only attract fish but also increase the secondary production of fish and invertebrates (Steimle *et al.*, 2002; Claisse *et al.*, 2014; Cresson *et al.*, 2014; Smith *et al.*, 2016).

The impacts of introducing structures like macrophytes, boulders, and especially large wood, to rivers and lakes have also been well studied. Researchers have found that installing these natural structures can have positive effects on fish abundance and biomass (Roni and Quinn, 2001; Roni *et al.*, 2006; Louhi *et al.*, 2016; Lyon *et al.*, 2019), especially for salmonids (see reviews by Whiteway *et al.*, 2010; Roni *et al.*, 2015; Foote *et al.*, 2020) which have been more extensively studied due to their higher commercial value and ecological importance in some systems. Fish in rivers tend to move shorter distances and are less likely to leave areas with a high density of natural structures (Harvey *et al.*, 1999) or reaches that have had large wood introduced (Giannico and Hinch, 2003; Roni and Quinn, 2001). Fish are also attracted to stream reaches where wood has been introduced (Riley *et al.*, 1992; Gowan and Fausch, 1996). Conversely homogenised aquatic environments which lack structure, such as rivers that have been cleared or dredged for navigation, flood control, gravel mining, dam construction or timber floating tend to support fewer fish (Kondolf, 1997; Nilsson *et al.*, 2005; Wohl *et al.*, 2014). Experimental removal of structures also reduces fish abundance and biomass, and alters communities (Angermeier and Karr, 1984; Abe and Nakamura, 1999). For instance, Sass *et al.* (2006) found that removing most of the large wood habitat from a lake in Wisconsin led to changes in the lake's food web interactions, causing a dramatic decline in yellow perch (*Perca flavescens)*. Nonetheless, addition of structures has not always led to discernible positive impacts on fish populations (Sass *et al.*, 2012; McLean *et al.*, 2015; Hellström *et al.*, 2019), perhaps because introducing structure does not address the primary factor limiting organisms (Palmer *et al.*, 2010; Verdonschot *et al.*, 2016), or because monitoring was not conducted for long enough to see a response (Roni *et al.*, 2015). Similarly, removal has also not always led to detectable negative impacts (Smokorowski *et al.*, 2021).

7.1.2. The functional roles of structures

7.1.2.1. *Change to flow conditions*

In lotic environments, structures alter the local flow conditions by obstructing the flow of water (Gordon *et al.*, 2004; Plew *et al.*, 2008). This effect has been explored in detail through modelling and laboratory experiments which show that flow obstruction at structures generates turbulence and creates regions of reduced flow velocity downstream of the structure (Shamloo *et al.*, 2001; Smith and Foster, 2007; Lacey and Rennie, 2012; Zexing *et al.*, 2020). This effect is particularly important in rivers because they are characterised by constant and unidirectional flow which is a key control on fish distribution and behaviour (Lamouroux *et al.*, 2001; Pilcher and Copp, 2003), but similar processes occur in lakes and marine environments (Guichard *et al.*, 2001; Shyue and Yang, 2002; Fernando, *et al.*, 2008). As the cost of swimming makes up a significant proportion of a fish's energy use (Brett, 1983), they tend to select habitats that minimise the amount of energy necessary to maintain position (Jackson *et al.*, 2001; Smith *et al.*, 2005). Therefore, the altered hydraulic conditions around structures are often exploited by fish taking advantage of the downstream zone of reduced flow velocity to save energy (Fausch, 1993; Shuler *et al.*, 1994; Webb, 1998; Gerstner, 1998; Przybilla *et al.*, 2010) termed 'flow refuging' (sensu Liao, 2007). Some fish also adjust their orientation and body motions to exploit the turbulent flow patterns associated with structures (Liao *et al.*, 2003a; Przybilla *et al.*, 2010). Over a whole area, the net effect of multiple structures is to slow overall flow velocity (Linstead, 2001; Gurnell *et al.*, 2002; Hafs *et al.*, 2014). In floods this can help fish, especially juveniles, avoid being washed downstream (Booker, 2003). Fish in reaches with a high density of structures are therefore more resistant to spates than those in homogeneous reaches (Heggenes, 1988; Pearsons *et al.*, 1991).

At the local scale, obstructed flow at a structure is forced to accelerate around the obstacle, meaning structures are also associated with nearby zones of accelerated flow (Abbe and Montgomery, 1996; Mutz, 2000). This may make these sites particularly favourable for drift feeding fish as they are attracted to steep flow velocity gradients (Smith *et al.*, 2005; Liao, 2007). This is because the steep gradient enables the fish to minimise their energy use by holding position in slower flows, whilst also allowing them to forage on drifting prey in the nearby fast flows (Bachman, 1984; Fausch, 1984; Liao, 2007).

7.1.2.2. *Change to surrounding habitats*

Concomitant to changes in flow conditions around structures, structures can alter other habitat properties in the surrounding area. In rivers, accelerated flow adjacent to structures has been linked to the exposure of coarser bed material and the creation of pools through scour (Gurnell and Sweet, 1998; Montgomery *et al.*, 2003). Zones with slower flow velocity downstream of structures encourage the deposition of fine sediment and organic matter (Sheng, 2000; Daniels, 2006; Osei *et al.*, 2015a,b). Structures have therefore been found to benefit fish by increasing the overall diversity of habitat (Abbe and Montgomery, 1996; Pilotto *et al.*, 2016) and creating or increasing the surface area of specific beneficial habitats, such as spawning gravels, pools, or rearing habitat (Crispin *et al.*, 1993; House, 1996; Floyd *et al.*, 2008). In the marine environment, artificial reefs are also known to cause the redistribution of sediment through scour associated with waves and currents interacting with the structure (Shyue and Yang, 2002; Düzbastılar *et al.*, 2006).

7.1.2.3. *Shelter from predation and competition*

Structures provide protection from predation by limiting predator manoeuvrability (Mattila, 1992; Crook and Robertson, 1999; Schneider and Winemiller, 2008), as small hiding places within structures can be used by appropriately-sized fish to shelter (Hackradt *et al.*, 2011). For example, openings and cavities are often incorporated into artificial reef designs to restrict the size of fish that can enter them, thereby protecting small fish from predation by larger piscivores (Chua and Chou, 1994). Additionally, structures can visually isolate prey from predators, thereby reducing the hunting success of sight-orientated predators (Mattila, 1992; Beukers and Jones, 1997). Rilov *et al.* (2007), however, found that a reduced visual field can also disadvantage some prey fish; in their study they found it affected mating and feeding behaviours for territorial damselfish. The presence of shelter therefore has implications for predator avoidance behaviour and habitat choice (Schneider and Winemiller, 2008). For instance, prey fish have been found to spend more time in ‘risky’ areas (i.e. areas with predators) if structure is available nearby (Fraser and Cerri, 1982). Shoaling fish will stay in smaller groups when near structures, presumably because there is less need for protection through shoaling in large numbers (Orpwood *et al.*, 2008). Similarly, the distance between predator and prey at which prey will initiate a flight response is greater when prey are further away from a structure (McLean and Godin, 1989; Dill, 1990).

Structures and the shelter they provide are thought to increase the survival of prey fish (Miyamoto *et al.*, 2021), even in some cases enabling the viability of prey persistence where predators are present (Everett and Ruiz, 1993; Sass *et al.*, 2006). This is especially true for juvenile fish, which are particularly vulnerable to predation (Höjesjö *et al.*, 2014). Additionally, Millidine *et al.* (2006) found that the effects of visual isolation meant structures offered metabolic advantages by reducing a fish's need for vigilance and preparedness, meaning fish size and condition may be positively affected by the presence of somewhere to shelter. However, some authors found increased predator abundance at structures (Leitão *et al.*, 2008), which could balance out any benefit of the structure for prey (Janssen *et al.* 2007; DeBoom and Wahl, 2013). This is especially true for relatively simple structures which provide fewer hiding places (Bartholomew *et al.*, 2000; Warfe and Barmuta, 2004; Miyamoto *et al.,* 2021).

Visual isolation between individuals can also reduce competition from conspecifics by reducing animal encounter rates (Sundbaum and Näslund, 1998). This reduces aggressive interactions and enables more individuals to exist in close proximity (Allouche, 2002; Danley *et al.*, 2011). However, aggregation of fish around structures could conversely result in increased competition for food (Höjesjö *et al.*, 2014) and heightened aggression between territorial species which compete for sites (Allouche, 2002). Thus a negative density-dependent effect from the use of structure is possible which could result in slower growth rates and poorer condition of fish (Höjesjö *et al.*, 2014). Certain more competitive species may be able to exclude other species with the same cover requirements (Persson, 1993), so structures are unlikely to benefit all species equally.

7.1.2.4. *Altered food resources*

Structures are associated with greater production of invertebrates, zooplankton and algae, which form essential elements of diet for many fish (Sibbing, 1991). This is because they offer a greater surface area for colonisation (Benke and Wallace, 2003; Dolloff and Warren, 2003; Steimle *et al.*, 2002), provide shelter (Schneider and Winemiller, 2008), change surrounding habitat (Wallace *et al.*, 1995; Hilderbrand *et al.*, 1997; Pilotto *et al.*, 2014, 2016; Chapter 6), and collect and supply food and nutrients (Prince *et* al., 1979; Hoffmann and Hering, 2000; Benke and Wallace, 2003; Falcão *et al.*, 2009). Increased abundance of food around structures is likely to influence fish distributions, with habitat selectivity of fish being strongly affected by a site's potential foraging return (Hill and Grossman, 1993; Milinski, 1993). For instance, hungry fish have been found to prefer more turbulent flows, such as those around structures, than those which are satiated (Pavlov *et al.*, 2000). Trophic relationships studies have proven that some fish feed heavily on taxa living on artificial reefs (Relini *et al.*, 2002; Leitão *et al.*, 2008), thereby benefitting from the increased production of food on the structures. Similar studies have shown the same effect of structures in lakes (Czarnecka *et al.*, 2014) and rivers (Benke *et al.*, 1985).

7.1.3. The context and extent of structures in rivers

Historic river management practices involving the homogenisation of channel morphology and removal of natural structures mean that rivers typically lack structures (Nilsson, *et al.*, 2005; Wohl *et al.*, 2014). This has contributed to the widespread freshwater biodiversity loss and degradation of rivers worldwide (Walsh *et al.*, 2005; Dudgeon *et al.*, 2006). At the same time, the amount of anthropogenic litter in rivers is increasing (Windsor *et al.*, 2019; Hoellein and Rochman, 2021). Due to its ubiquitous nature and longevity, especially in rivers close to urban areas which tend to lack natural structures, it is possible that litter could provide important structural habitat and thereby benefit fish populations. There is precedent for this from the marine environment where marine fish have been found to associate with anthropogenic litter (Katsanevakis *et al.*, 2007; de Carvalho-Souza *et al.*, 2018; Rizzo *et al.*, 2021), and where artificial materials (sometimes including litter such as car tyres: Chapman and Clynick, 2006) have been used to create artificial reefs for decades (Bohnsack and Sutherland, 1985; Baine, 2001). However, apart from research into the harmful effects of toxic chemicals associated with litter (e.g. Rochman, 2013; Adam *et al.*, 2019) or consumption of microplastics (e.g. Sanchez *et al.*, 2014; Faure *et al.*, 2015; Biginagwa *et al.*, 2016; Andrade *et al.*, 2019), the ways in which fish are affected by anthropogenic litter in rivers have yet to be investigated.

7.1.4. Research approach

This study aimed to investigate whether anthropogenic litter provides structure for fish by observing fish around experimentally introduced car tyres in a river. Car tyres were used because they are easily replicable and commonly found in rivers. This complements the experiment detailed in Chapter 6 in which the same tyres were monitored to assess their impacts on macroinvertebrates and surrounding habitats. Although there is a growing understanding of the impacts of natural and intentionally introduced artificial structures, much less is known about the impacts of anthropogenic litter. Understanding how the extensive presence of anthropogenic litter in rivers worldwide affects the organisms that live there is vital to inform future management of the growing problem of litter.

Given the simple shape of tyres and the lack of hiding places they provide, it is reasonable to assume that the main impact of tyres is their effect on flow hydraulics, rather than on biotic interactions. I hypothesised that by blocking flow, tyres would create a downstream zone of low flow velocity, whereas they would have relatively little effect on flow conditions upstream. Other simple structures, such as individual boulders, have been shown to significantly change local flow conditions in this way (Zexing *et al.*, 2020). Fish are known to take refuge in slow flow areas downstream of similar simple structures like boulders (Shuler *et al.*, 1994; Gerstner, 1998; Webb, 1998; Przybilla *et al.*, 2010). Therefore, I expected to find that fish would be more abundant and would spend more time downstream of tyres than upstream. To test this hypothesis, I compared fish presence and behaviour upstream and downstream of tyres using underwater cameras. Although I did not expect tyres to be effective at protecting fish from predation because of their simple shape, the middle of tyres could provide a novel hiding place for prey, which would be difficult for piscivorous fish to enter. Therefore, I also placed cameras in the middle of tyres to see if fish would utilise tyres in this way.

Different species of fish are known to have distinct preferences for habitat conditions (Aadland, 1993; Lamouroux *et al.*, 2001; Huang *et al.*, 2019), and not all have been found to benefit from or associate with structures (Roni *et al.*, 2006; Langford *et al.*, 2012). Therefore, I divided fish by species and size classes to investigate differences between them. Small fish are known to be most limited in their choice of habitat due to their relatively weak swimming ability (Tudorache *et al.*, 2007). Thus, I expected that small fish would show the strongest response to tyres (i.e. that their abundance, duration and behaviours would be more different between upstream and downstream zones than other fish). I predicted that small fish would be more common downstream of tyres, and that they would stay here for longer.

7.2. Methods

7.2.1. Study site

The River Idle is a 3rd order lowland mixed sand and gravel-bed river in North Nottinghamshire draining a catchment of 842 km^2 (Downs and Thorne, 1998). The field site at 53°20’34’’N 00°57’44’’W (Figure 7.1) was located 1 km downstream of the town of Retford (23,000 population estimate for 2019; City Population, 2019), and is regularly managed for upstream flood risk management by weed cutting and clearance of large wood and other structures. Mean discharge at the nearest gauging station (12 km downstream of the field site) was 2.3 m^{3}s^{-1} in the period 1982 to 2019 (NRFA 2021a) and mean width at the site was 9.4 m. Full site details are in Chapter 6 (Section 6.2.1.).

The River Idle (waterbody ID GB104028058091), within which the field site was located, was categorised as good environmental quality for fish according to the 2019 Water Framework Directive (WFD) classification (Environment Agency, 2021e). It is known to exemplify the “Barbel Zone” described by Huet (1949), supporting fish species such as barbel (*Barbus barbus*), roach (*Rutilus rutilus*), chub (*Squalius cephalus*), pike (*Esox Lucius*), European eel (*Anguilla anguilla*) and minnow (*Phoxinus phoxinus*).

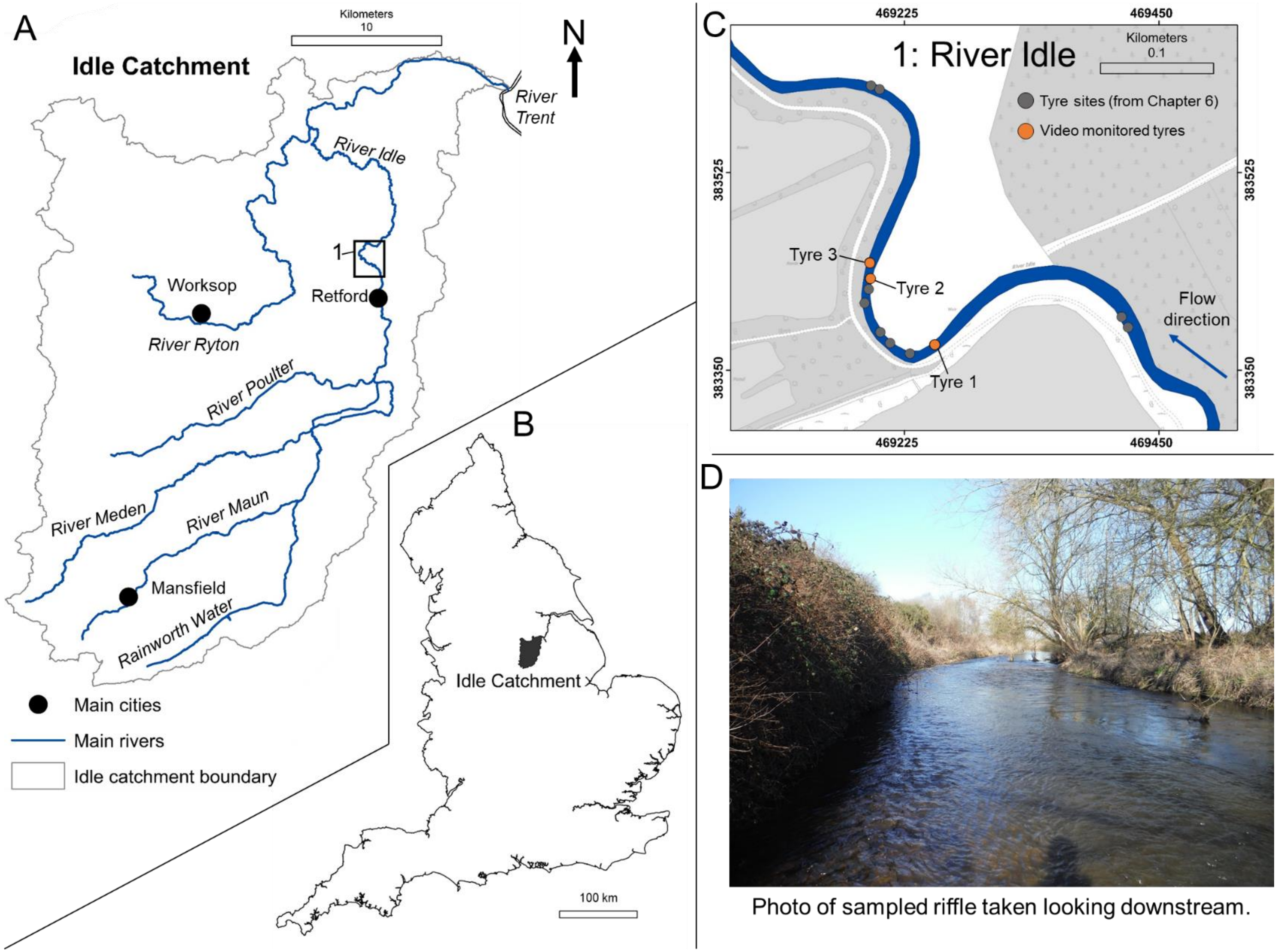

Photo of sampled riffle taken looking downstream.

Figure 7.1: Maps showing the location of the field site on the River Idle with respect to: A) the Idle Catchment; and B) England. C) shows the locations of tyres where video monitoring was completed, and D) is a photo of the riffle where tyres were installed.

EA electrofishing surveys (Environment Agency, 2021g) conducted at the Idle Valley Nature Reserve (0.25 km upstream of the field site) and Tiln (2.25 km downstream) show the types and sizes of fish present within the locality of the field site (Figure 7.2). Analysis of the electrofishing data indicates that eels (mean ± SE across sites: 4.7 ± 3.0 fish per 100 m^2), roach (0.3 ± 2.1 fish per 100 m^2), stone loach (*Barbatula barbatula*; 6.8 ± 3.8 fish per 100 m^2), and minnows (3.7 ± 3.0 fish per 100 m^2) were the most common fish across sites. Other species present include barbel, bullhead (*Cottus gobio*), chub, dace (*Leuciscus leuciscus*), gudgeon (*Gobio gobio*), perch (*Perca fluviatilis*), pike, spined loach (*Cobitis taenia*), and three-spined stickleback (*Gasterosteus aculaeatus*).

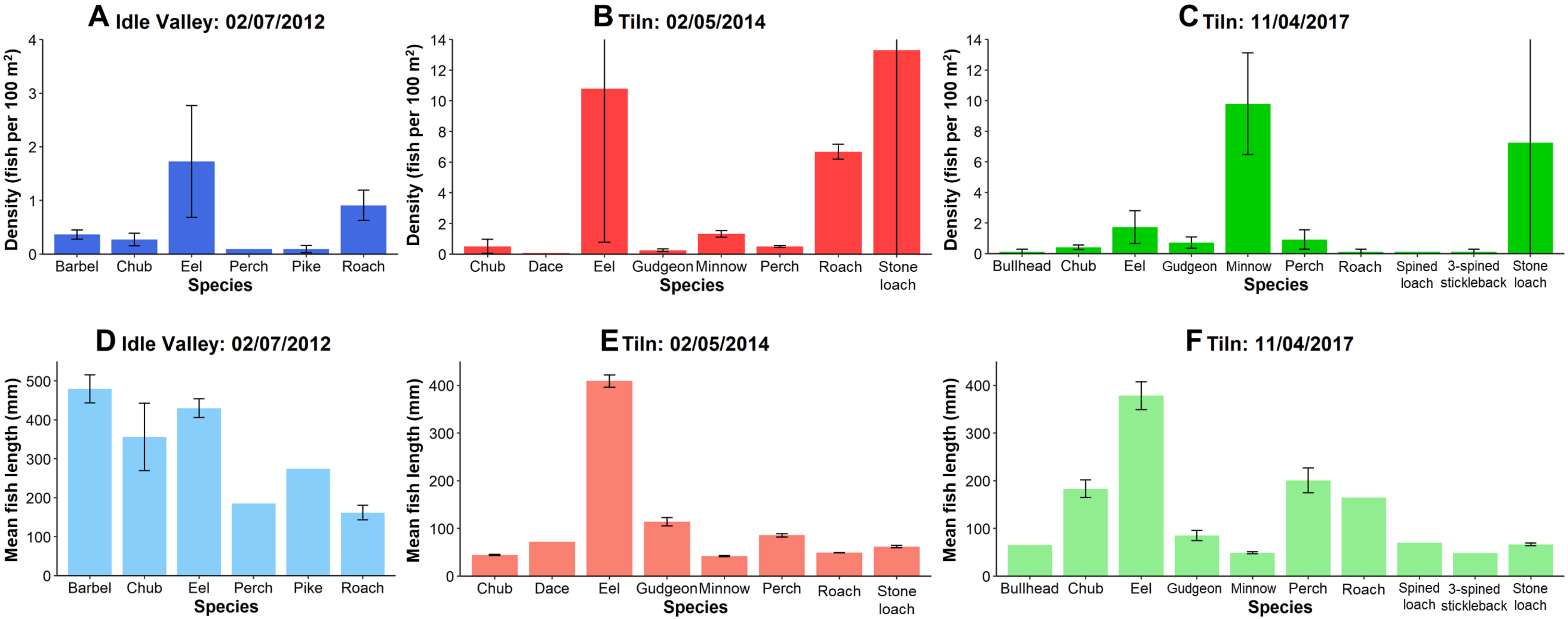

Figure 7.2: Fish communities found during EA electrofishing surveys (Environment Agency, 2021g) using depletion sampling at the Idle Valley Nature Reserve (A and D), and at Tiln (B, C, E and F). Dates of electrofishing survey are shown in individual figure titles. A, B, and C show the estimated density of fish per 100 m2 (estimated by Carle and Strub Maximum Weighted Likelihood) ± the standard error of the population estimate. D, E and F show the mean fish length ± the standard error. Plot scales are different.

Car tyres (mean diameter 0.64 cm and height 0.25 cm) were installed in the River Idle in March 2019 (full details of tyre installation is in Section 6.2.1.). Three of these tyres were selected for video monitoring of fish. Selected tyres were located along the same gravel riffle with a mean depth of 0.24 m at the tyres, as measured on the first day of filming. Tyres were placed into uniform sediment patches so habitat conditions were comparable between upstream and downstream patches, except for changes caused by the tyres themselves (e.g. fine sediment accumulated downstream; see Chapter 6). Selected tyres correspond to tyres numbered 3 (herein tyre 1), 9 (tyre 2) and 10 (tyre 3) in Chapter 6.

In Chapter 6, I demonstrated that tyres affected the local flow velocity in different regions around the tyre. This was ascertained by measuring the average flow velocity over 30 seconds at 60% of depth around the tyre, immediately before and after tyres were removed. To calculate the change in flow velocity caused by the tyre, for each of the twelve tyres that were installed, I subtracted the flow velocity measured after the tyre was removed, from that measured immediately prior to removal. This showed that tyres reduced the flow velocity in the downstream zone by an average of -0.33 ± 0.10 ms^{-1} (mean ± SE). This is a notable reduction given that the mean flow velocity in the downstream zone without tyres was 0.53 ± 0.07 ms^{-1}. Although these measurements were taken in February 2020 (six months after fish monitoring), so do not indicate the absolute effect of tyres during filming, it confirms that tyres were effective at slowing flow velocities in the downstream zone.

7.2.2. Filming set-up

I used small, portable, underwater cameras to record fish as this was a practical method to observe fish presence and behaviour in the absence of an observer that could influence fish activity (Lucas and Baras, 2000; Ebner *et al.*, 2014). This method also enables investigation of fine scale (within 1 to 2 m) patterns in fish behaviour (Porter *et al.*, 2005), suitable to the size of effect being investigated.

The camera set up (Figure 7.3) consisted of three cameras (Apeman Action Cam A80 or A77) at each tyre; one upstream (positioned 1.5 m upstream of the tyre), one in the middle, and one downstream (fixed to the tyre). Cameras were fixed in place in a consistent manner (fixings left in situ between filming sessions), aiming to minimise their protrusion from the river bed, reducing the impact of cameras on flow fields which could influence fish behaviour. They were orientated facing downstream (for consistency in fish identification) towards 0.25 m^2 (0.5 x 0.5 m) standardised monitoring zones. These were marked using coloured stakes and positioned 0.5 m downstream from the cameras. Only fish which passed through these monitoring zones were recorded. All cameras were fully submerged whilst filming and were un-baited to enable investigation of natural fish behaviours without any external attraction (Ebner *et al.*, 2014).

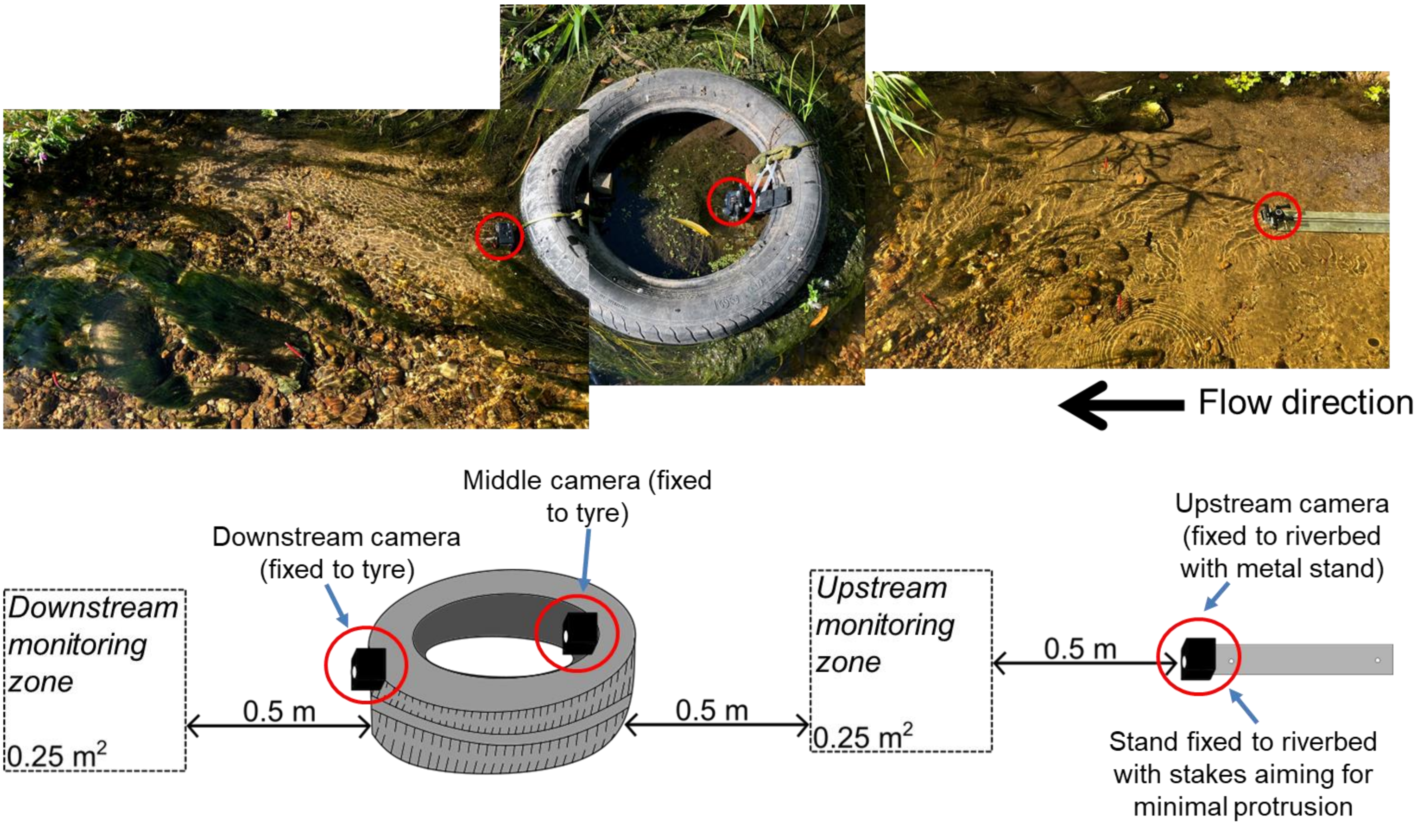

Figure 7.3: Aerial photo (showing tyre 1) and diagram of camera set up. Camera locations are circled in red.

Filming took place over six consecutive evenings under baseflow conditions in late August 2019. This meant that river levels were low (enabling easy installation and removal of cameras with reduced risk of them being washed downstream) and light levels were relatively high (improving camera visibility). Approximate timings were as follows: cameras were set up and started at 17:00, batteries were changed at 18:30, and filming stopped at 20:00. This covered the period before sunset when fish activity is heightened (Helfman, 1986), but before light levels were too low for the cameras (sunset was at 20:10 on the last day of filming). Weather conditions were clear and sunny throughout monitoring, with the air temperature at midday between 17.4 and 25.2 °C at the nearest Met Office weather station (Waddington, 35 km from site; Met Office, 2019).

7.2.3. Video analysis

Over all cameras and all days, 116 hours of video footage was recorded. Footage from each day was split into two periods as batteries had to be changed after approximately 1.5 hours. During the changeover there was a short gap in filming and the river was disturbed by the operator, meaning fish activity was disrupted and effectively ‘reset’. Therefore each section of footage from each day was treated as a separate replicate, making the assumption that fish behaviour was independent of observation period.

Video footage taken at each tyre was considered separately as there were clear differences in the abundance and species of fish recorded at different tyres. My primary inquiry was whether there were differences in fish activity upstream and downstream of the tyres, as these zones are assumed to represent areas where the tyre has negligible effect (upstream) and where it has a strong effect (downstream). Footage taken upstream and downstream of each tyre during corresponding times was therefore paired for comparison. As the middle of tyres was a particularly novel habitat type, and because so few fish were observed in the middle of tyres, footage from the middle of tyres was examined separately.

Mean video duration was 78.3 minutes, but varied between 60 and 94 minutes due to differences in the lifespan of individual batteries. To identify equivalent paired (upstream vs downstream) sections of footage for analysis, 58 minute periods were subsampled from each video replicate. Paired sections for analysis for each tyre were therefore taken from identical time periods, but it was necessary for there to be small differences in start time between days and between tyres as it was impossible to start all cameras at the same time. Analysed periods started at least two minutes after cameras were turned on so that conditions could return to normal and fish could acclimate after the disturbance from putting cameras in place. This acclimation time was deemed sufficient as any disturbed fine sediment had cleared and fish often reappeared in shot within this time. 58 minute periods were also selected for analysis from footage taken by cameras located in the middle of tyres so that the length of time sampled was consistent. However, there were some small differences in the times sampled compared to upstream and downstream pairs.

Videos with inadequate visibility, due to lens blockage by macrophytes or equipment malfunctions, were excluded from analysis (Figure 7.4B). In cases where upstream or downstream videos were omitted, I also removed the corresponding footage from consideration so that only paired videos were analysed. Of footage from the middle of tyres, only videos from the middle of tyres 2 and 3 were analysed, as the shallower river depth at tyre 1 meant that the middle of tyre 1 was inaccessible to fish. For paired upstream and downstream cameras, this left seven recorded video sections for tyre 1, eight for tyre 2, and eleven sections for tyre 3; nearly 54 hours of footage in total. For middle cameras, there were nine usable observation periods for tyre 2 and eleven for tyre 3. Each video section was analysed for the total number of fish observed, the duration that fish were present in frame, and for the proportion of time spent in different behaviours, explained below.

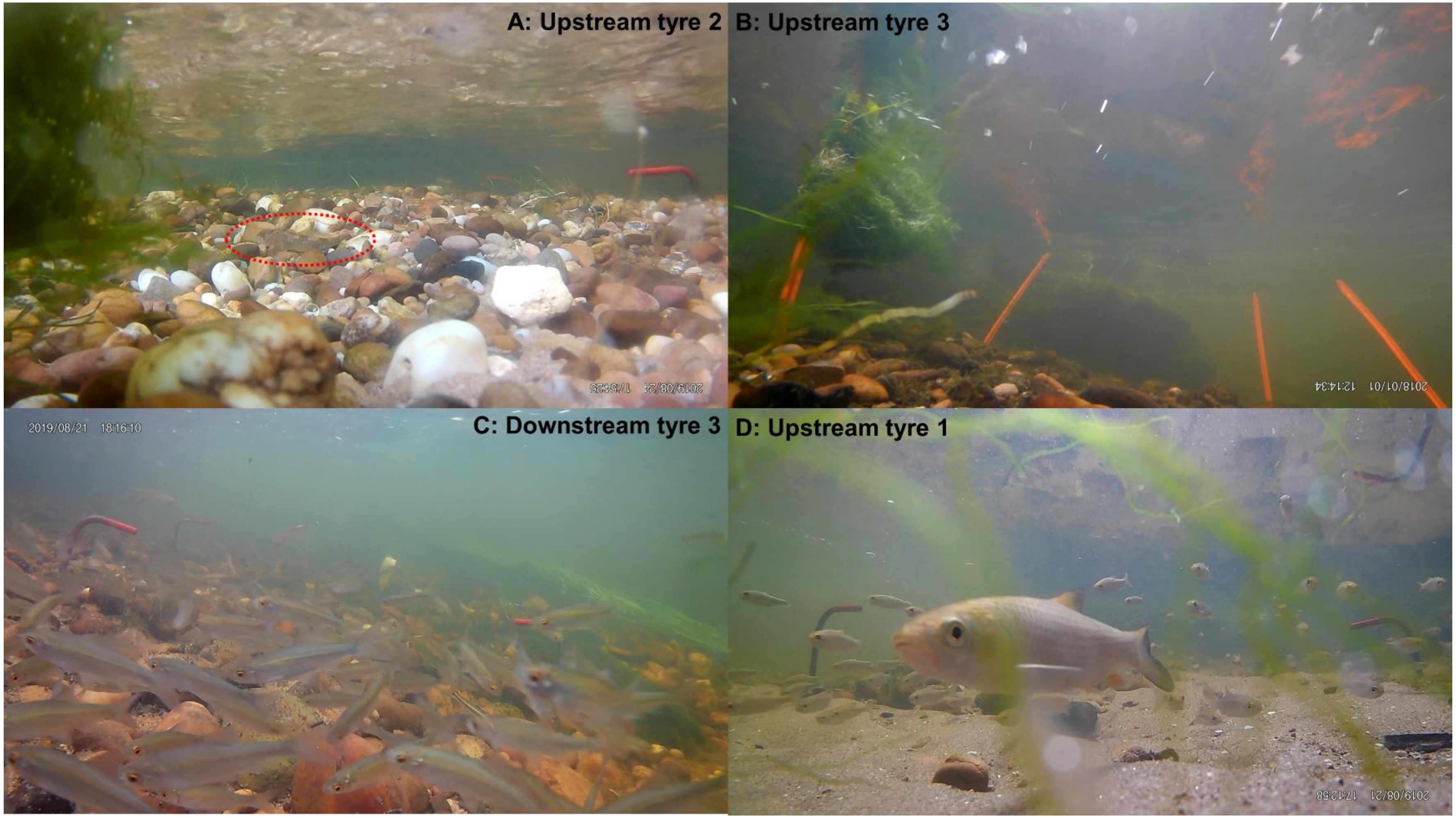

Figure 7.4: Frames from selected videos. A) Bullhead (circled) upstream of tyre 2. B) Camera set up at the wrong angle at tyre 3 which made the video unusable. C) Large shoal of small silver fish downstream of tyre 3. D) A shoal of small silver fish and an individual medium silver fish (chub) upstream of tyre 1.

7.2.3.1. *The abundance and duration of fish at tyres*

All fish which at some point passed through the 0.25 m^2 monitoring zone were counted to enumerate the number of fish which visited the area monitored by the cameras. When fish were present in particularly large shoals (> 100 fish) it was not possible to accurately count the number of fish so their abundance was estimated based on counting fish in a small section and extrapolating this to the whole shoal, assuming that the density of fish is constant. Fish of the same size and species class that disappeared out of view but reappeared within ten seconds were assumed to be the same fish and so were not re-counted. This cut-off period was used because fish repeatedly swam in and out of view for a few seconds and were likely to have remained within the vicinity during this time. It reduces the likelihood of an overestimation in the abundance of fish, whilst also collecting more data about the patterns of shoals than would be recorded if I only counted the MaxN index: the maximum number of fish observed at any one time during an observation period (e.g. Becker *et al.*, 2010). The duration of fish presence was measured by recording the length of time fish were observed until they were out of view for more than ten seconds. This cut-off period was used to match the procedure for counting fish abundance.

All fish were classified by species and size (e.g. large pike or small minnow); hereafter these groups will be referred to as fish classes. Fish species identification followed Maitland (2004) and was based on size, colouration, and body shape diagnostic features to identify where possible to species level. Identification of silver fish, including roach, dace, chub and bream (silver bream: *Blicca bjoerkna* and common bream: *Abramis brama*), was particularly difficult, especially as these fish were generally small, they tended to occur in large groups (see Figure 7.4C), and often moved rapidly past the camera or were far from the lens (as found by: Lowry *et al.*, 2011; Ebner *et al.*, 2014). Therefore, these fish species were grouped together as a unique fish class called ‘silver fish’. This was deemed an acceptable compromise given the similarity of the behaviour and habitat preferences of these species and because of the propensity for these species to form mixed shoals (Allan, 1986).

Size classes were as follows: small fish = < 15 cm, medium fish = 15 to 30 cm, and large fish = > 30 cm. These thresholds were decided on to separate the clearly bimodal age structure of silver fish; most silver fish were approximately 10 cm long, but there were several which were around 20 cm long, with no fish in between these sizes (see Figure 7.4D). Silver fish always shoaled with other similarly sized fish, meaning it was easy to distinguish classes. Only a few fish were longer than 30 cm and these were all pike or eels. Size was estimated based on comparison of fish length (head to tail) relative to the front stakes of the monitoring zone, which were placed 0.5 m apart. Size classes were consistent across species so not all size classes were observed for all species, for instance minnow are rarely longer than 12 cm (Maitland and Campbell, 1992) meaning that all minnow would all be classified as small in this study.

7.2.3.2. *The behaviour of fish at tyres*

Behavioural analysis was only conducted on footage from upstream and downstream cameras as there were very few fish recorded by middle cameras. Only small silver fish were analysed for behaviour as they made up the vast majority of fish observed (97% of all fish). Fish that were observed as individuals were assigned behaviours based on the definitions described below. However, a substantial number of small silver fish appeared in large shoals (mean abundance of fish present at one time was 24 ± 3 (SE) and the maximum was 538). This made it impossible to follow individual fish within shoals, as has been found for other studies using video to study fish behaviour in the natural environment (e.g. Lucas and Baras, 2000). Therefore, when fish were in shoals, behaviour was assessed for the whole shoal based on the predominant behaviour exhibited by the group.

Two categories of behaviour were investigated: locomotion and feeding. The duration of each specific behaviour was standardised by calculating the proportion of the time that fish were present that was spent in each behaviour type.

One of the most commonly described mechanisms by which fish have been shown to exploit the flow conditions downstream of a structure is that fish are able to minimise their energy use by holding station in the area of reduced flow velocity within the lee of the structure (Liao, 2007). A fish that is holding station has no consistent direction of movement, but maintains its position relative to the substrate despite flow and with limited body movements (Gee, 1983; Gerstner, 1998; Liao, 2007). The opposite of this is steady or sustained swimming, defined as continual swimming in a given direction at a near constant speed (Jobling, 1994). Therefore, I measured the proportion of time fish spent either holding or swimming (Table 7.1) to assess whether fish were exploiting the low flow zones downstream of tyres for the function of energy preservation. The duration of time fish spent in one of these two locomotion behaviour states was determined based on when significant changes of activity occurred, i.e. fish switched from holding to swimming (or vice versa) or when fish left the frame. Fish were always either swimming or holding, regardless of whether they were also feeding.

Table 7.1: Definitions and exemplar videos of fish displaying each of the behaviours monitored in this study. Hyperlinks in the example column connect to exemplar videos on Youtube (Wilson, 2021a,b,c,d).

Behaviour category	Behaviour	Definition	Example
Locomotion	Holding	Fish has no consistent direction of movement, but maintains its position relative to the substrate despite flow.	Holding All behaviours
	Swimming	Fish consistently swims in a given direction at a steady pace.	Swimming All behaviours
Feeding	Feeding	Fish show signs of feeding activity e.g. apparently random short darts in varied directions, or touching bed/water surface with mouths.	Feeding All behaviours
	Not feeding	Fish are not feeding.	
	Unknown	It was not possible to definitively tell if fish were feeding or not.	

Feeding behaviours were also assessed for the small silver fish class to see whether fish spent more or less time feeding downstream of tyres, so it is worth understanding something of their diet and feeding behaviour. Roach and dace have very similar diets, feeding extensively on macrophytes and filamentous algae, but can also feed solely on animals, in particular zooplankton, insect larvae/nymphs, and molluscs (Hellawell, 1972; Mann, 1973; Brabrand, 1985; Lammens and Hoogenboezem, 1991). Young roach will feed on small invertebrates, zooplankton and diatoms (Maitland and Campbell, 1992). Bream are primarily benthic feeders, specialising on feeding on Diptera larvae, worms and other invertebrates in the river bed (Lammens and Hoogenboezem, 1991; Maitland, 2004), but they can also efficiently feed on zooplankton, especially when young (Lammens *et al.*, 1987). Chub eat very broad diets including algae, macrophytes, insect larvae/nymphs, molluscs, frogs, crayfish, and small fish (Hellawell, 1971), with young chub (aged 0 + to 5) primarily feeding on plant materials and invertebrates (Maitland, 2004).

Small silver fish species primarily feed through particulate feeding or gulping (Lammens *et al.*, 1987; Lammens and Hoogenboezem, 1991). These feeding mechanisms have standard definitions. During particulate feeding a fish detects an individual prey organism, approaches, and then attacks with fast directed suction (Lammens and Hoogenboezem, 1991). Some fish suck in water as they swim towards particulate prey, thereby reducing its forward push on water which prevents the prey taking evasive action, termed darting by Janssen (1978). A gulping fish swims slowly whilst continually opening and closing their mouths and taking a series of sucks directed at areas of high plankton density (Janssen, 1976; Pledger *et al.*, 2014). Other feeding mechanisms have been described for larger fish and different species (see Sibbing, 1991).

Because the small silver fish observed in this study were small, fast moving, and often in large shoals or far from the camera it was not possible to see their mouths and thereby determine specific feeding mechanisms. Feeding activity was instead judged by looking for alternative signs of feeding, such as a fish making short, sharp and apparently random darts in different directions (with no other obvious cause e.g. the presence of a predator), or swimming towards and touching the river bed or water surface briefly with their mouths (Table 7.1). The time a shoal spent feeding was measured from the first distinguishable feeding activity to when it was no longer possible to see any fish within the shoal feeding. When it was not possible to tell if a fish was feeding, its behaviour was recorded as not feeding.

7.2.4. Data analysis

Statistical analysis was completed using R statistical software (version 4.1.0; R Core Team, 2021). Paired samples Wilcoxon tests were used to statistically compare the abundance and duration of fish recorded by cameras upstream and downstream of tyres, for each tyre separately. These tests included each observation period as a separate replicate and were conducted using the R package *rstatix* (Kassambara, 2021). Tests were repeated on individual fish classes to assess differences in their responses.

Behavioural information was investigated by comparing the mean (± SE) proportion of time that fish were present which they spent exhibiting a particular behaviour type. Proportional data was used rather than duration, as differences in behaviour duration compared between upstream and downstream cameras would likely reflect differences in fish duration independent of behaviour.

Data from the middle of tyres were only assessed visually because there were very few observations of fish from these cameras.

7.3. Results

7.3.1. Fish

I analysed 3016 minutes (26 observation periods) of video footage from paired upstream and downstream cameras, and 1160 minutes (20 observation periods) from cameras placed in the middle of tyres. The fish species observed were silver fish (probably a mix of chub, dace, and roach), minnow, pike, perch, bullhead, gudgeon, and eels (Table 7.2). Only silver fish occurred in more than one size class. In the paired upstream and downstream footage, fish were present for 600 minutes (small silver fish were present for 55.7% of this), and the total abundance of fish was 9900 (97% of which were small silver fish). Due to this predominance of small silver fish, behavioural analysis focused on this fish class.

Table 7.2: Abundance and duration (and the proportion of total abundance or fish duration) of each fish class observed.

	Fish species								
	Small silver	Small minnow	Medium silver	Medium perch	Small bullhead	Small gudgeon	Large pike	Large eel	Total
Abundance of fish	9575	157	129	22	6	6	4	1	9900
% of total abundance	96.72	1.59	1.30	0.22	0.06	0.06	0.04	0.01	
Duration of fish (mins)	334.33	117.40	78.58	3.88	63.15	110.42	20.98	0.25	600.20
% of total duration	55.70	19.56	13.09	0.65	10.52	18.40	3.50	0.04	

7.3.2. Differences in fish abundance and duration

There was considerable variation in the abundance and duration of fish observed, both between tyres and between observation periods (Figure 7.5). Observation periods were named according to the date of filming and whether or not it was the first or second section of filming that day (i.e. period 20.1 refers to the first section of filming on the 20th). There were considerably fewer fish at tyre 2 than at the other tyres, but the duration downstream of tyre 2 in observation periods 24.1 and 24.2 was exceptionally high due to the presence of one gudgeon for nearly the whole period. The extremely high duration of fish upstream of tyre 3 in period 22.1 was likewise caused by one fish, a bullhead, which was present throughout the monitored period.

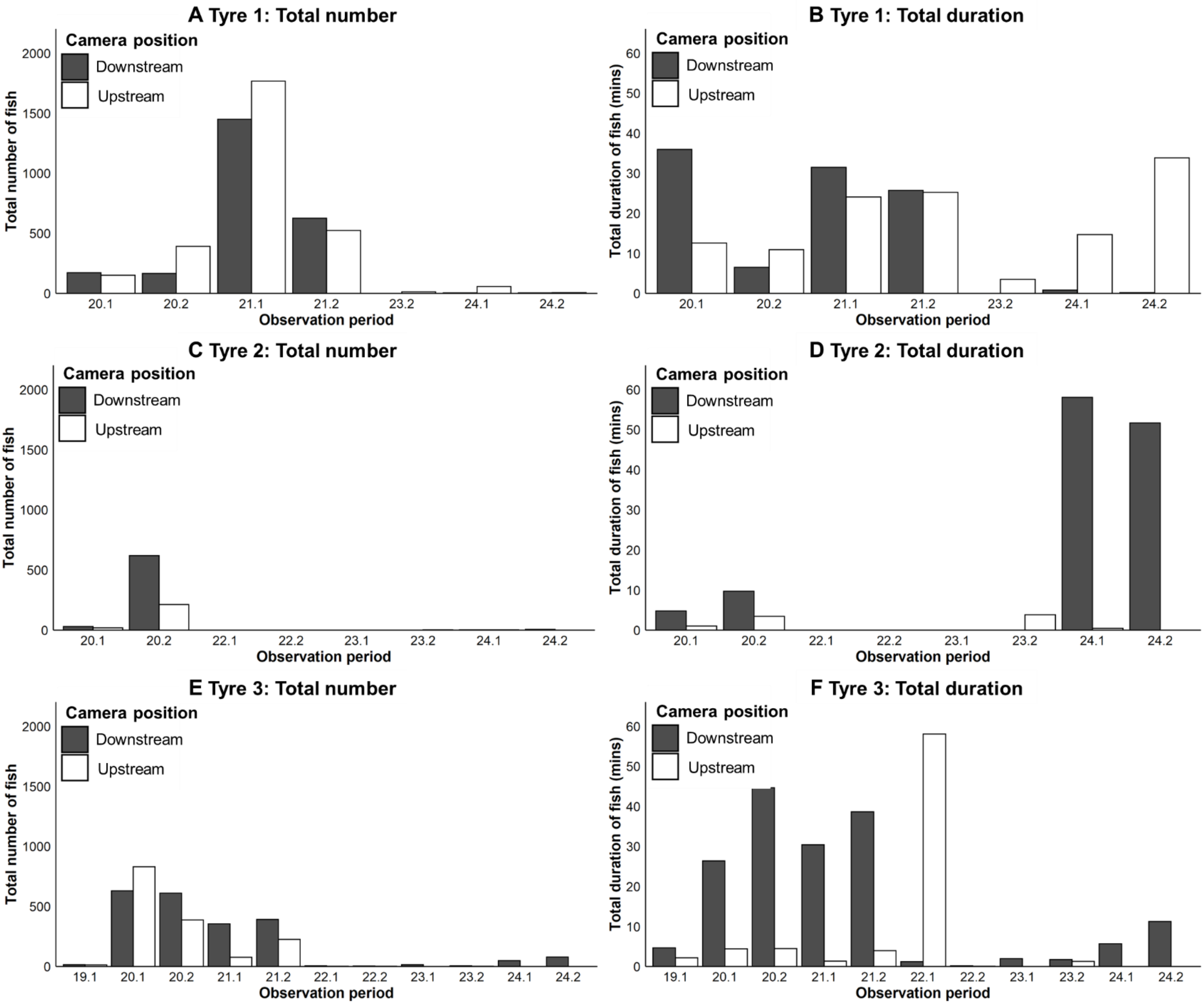

Figure 7.5: Total number (A, C, E) and duration (B, D, F) of all fish species upstream (white) and downstream (grey) of tyre 1 (A, B), tyre 2 (C, D), and tyre 3 (E, F).

Between paired upstream and downstream cameras, there were only a few significant differences in the mean total abundance and duration of all fish together or of individual fish classes (Figure 7.6), and none were consistent across tyres. However, statistical power to detect a difference was low given the relatively few replicates and inconsistency in fish presence (see numbers in brackets on *x* axis). This is especially true when data was divided into fish classes as no classes were observed during all observation periods.

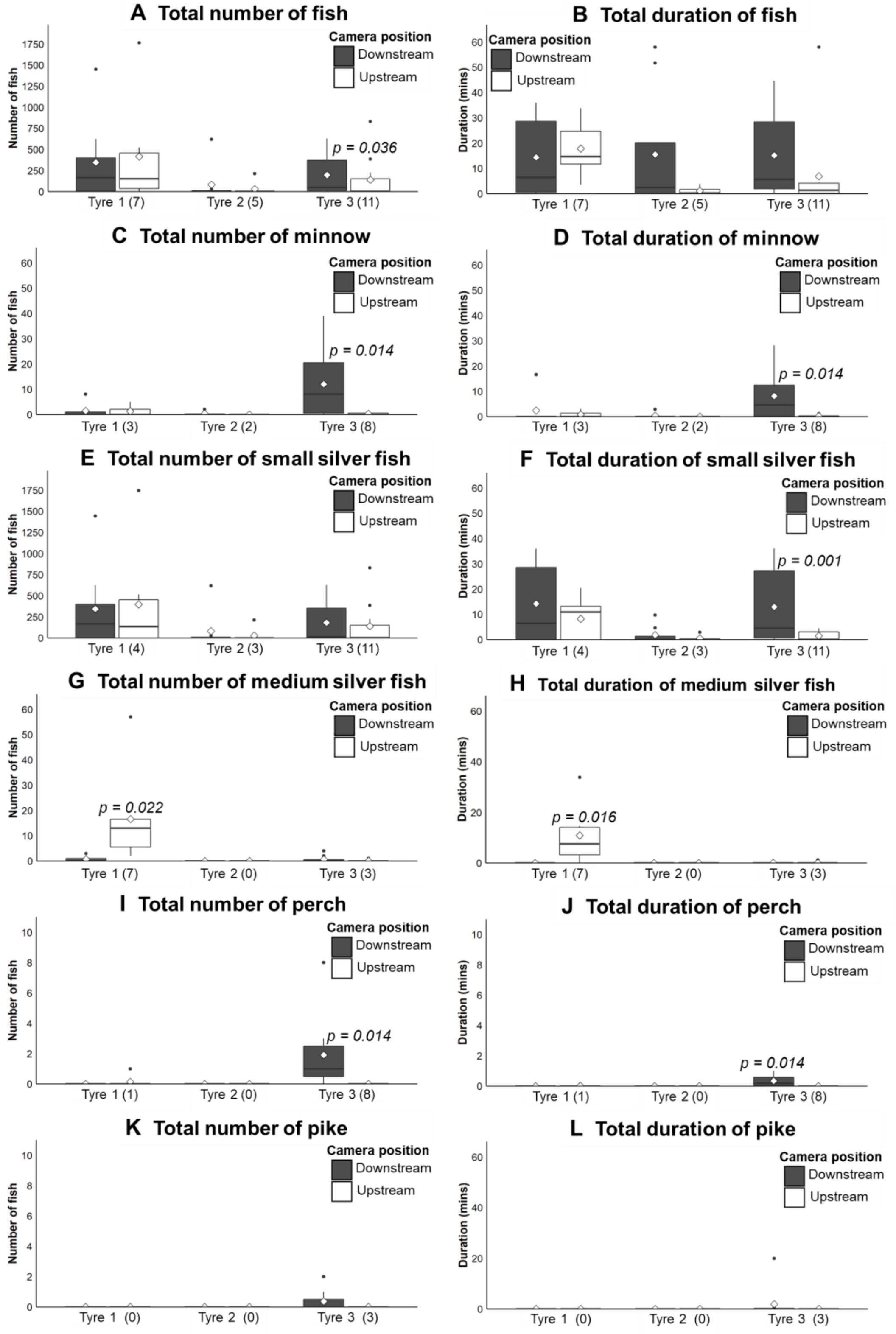

Figure 7.6: Box plots of the total number and duration (mins) of all fish (A, B) and individual fish classes (C to J). Box plots are based on the total number or duration of fish in each observation period: eight for tyres 1 and 3, and eleven for tyre 4. Numbers in brackets on *x*-axis show the number of observation periods each fish class was seen in. Mean values are shown as diamonds. The *t*-test *p* values are shown where there were significant differences between upstream and downstream abundance or duration.

The total number of fish, from all classes, was significantly greater downstream than upstream of tyre 3, but not for tyres 1 and 2. Downstream fish duration was also notably greater, though not significantly so, than that upstream for tyres 2 and 3. In fact, if the duration outlier downstream of tyre 3 (caused by a single bullhead which remained immobile for the whole 58 mins) is excluded from analysis, then the difference in duration at this tyre becomes significant ($V = 55$, $p = 0.002$).

As expected, given that they made up the majority of observed fish, the patterns for small silver fish were similar to that for all fish. Their abundance was similar between the two zones at all tyres, but they were present for longer durations downstream than upstream at all tyres, especially at tyre 3 where they were significantly so. At tyre 3, they spent an average (median and IQR) of 4.5 (0.6 to 27.3) mins downstream, compared to 0.1 (0 to 3.1) mins upstream. Several shoals and individuals travelled up the river as they were observed in both cameras one after the other. This explains why the total abundance was similar between zones, despite the differences in duration.

Minnow and perch were present in smaller numbers, and were generally limited to tyre 3, but they were also significantly more abundant and spent significantly more time downstream than upstream at this tyre. Small numbers of minnow were often included in mixed shoals with small silver fish, suggesting that small fish of multiple species preferred conditions downstream of tyres. Their mean duration per observation period downstream of tyre 3 was 8.1 ± 3.1 mins, compared to 0.2 ± 0.1 mins upstream. Perch made regular short visits to tyre 3 (their mean duration was 11 seconds) either as individuals or as pairs, perhaps looking for small prey fish. However, they were never observed alongside small silver fish or minnow at tyre 3, and did not exhibit any predatory behaviour. Pike were only found downstream of tyre 3, once spending around 20 minutes at the tyre and exhibiting hunting behaviour. During this time it was mostly motionless, but made several strikes at the shoal of small silver fish which were also present. Medium silver fish showed the opposite pattern to the other fish classes, and were almost exclusively observed upstream of tyre 1; only rarely were they observed downstream of any tyre. This difference explains why the mean total duration for all fish is similar between zones at tyre 1, as the upstream duration of medium silver fish balances out with the greater duration of small silver fish downstream.

7.3.3. Differences in fish behaviour

Behavioural analysis focused on small silver fish as these were by far the most abundant group observed (97% of fish counted). Holding was the most common locomotion behaviour in both zones at tyre 1 and downstream of tyre 3, whereas fish spent similar proportions of time holding and swimming in both zones at tyre 2 and upstream of tyre 3 (Figure 7.7). At tyres 1 and 3 the proportion of time spent holding was noticeably greater in downstream zones than in upstream zones. On average at tyre 1, fish spent 85 ± 5% (mean ± SE) of their time downstream holding, compared to 69 ± 3% upstream. The variability in proportional time of locomotion behaviours at tyre 3 was considerably greater than at tyre 1 because there were a few observation periods where only a few fish were observed. These individuals or small shoals (five fish or less) were more likely to swim past the tyre rather than holding there. Nonetheless, there was still a clear difference between zones in the mean proportional time spent holding at tyre 3 (65 ± 12% downstream, and 48 ± 12% upstream). The interpretation of results for tyre 2 is limited by the relatively small number of observations at this tyre (small silver fish were only observed at tyre 2 during three observation periods), hence there was considerable variability in locomotion behaviour proportions at this tyre.

The proportion of time spent feeding was similar for upstream and downstream zones at tyre 1, but at tyres 2 and 3 feeding was only observed downstream (Figure 7.7). The proportional time spent holding and feeding downstream of tyre 3 were similar, as holding fish were often also feeding at this tyre.

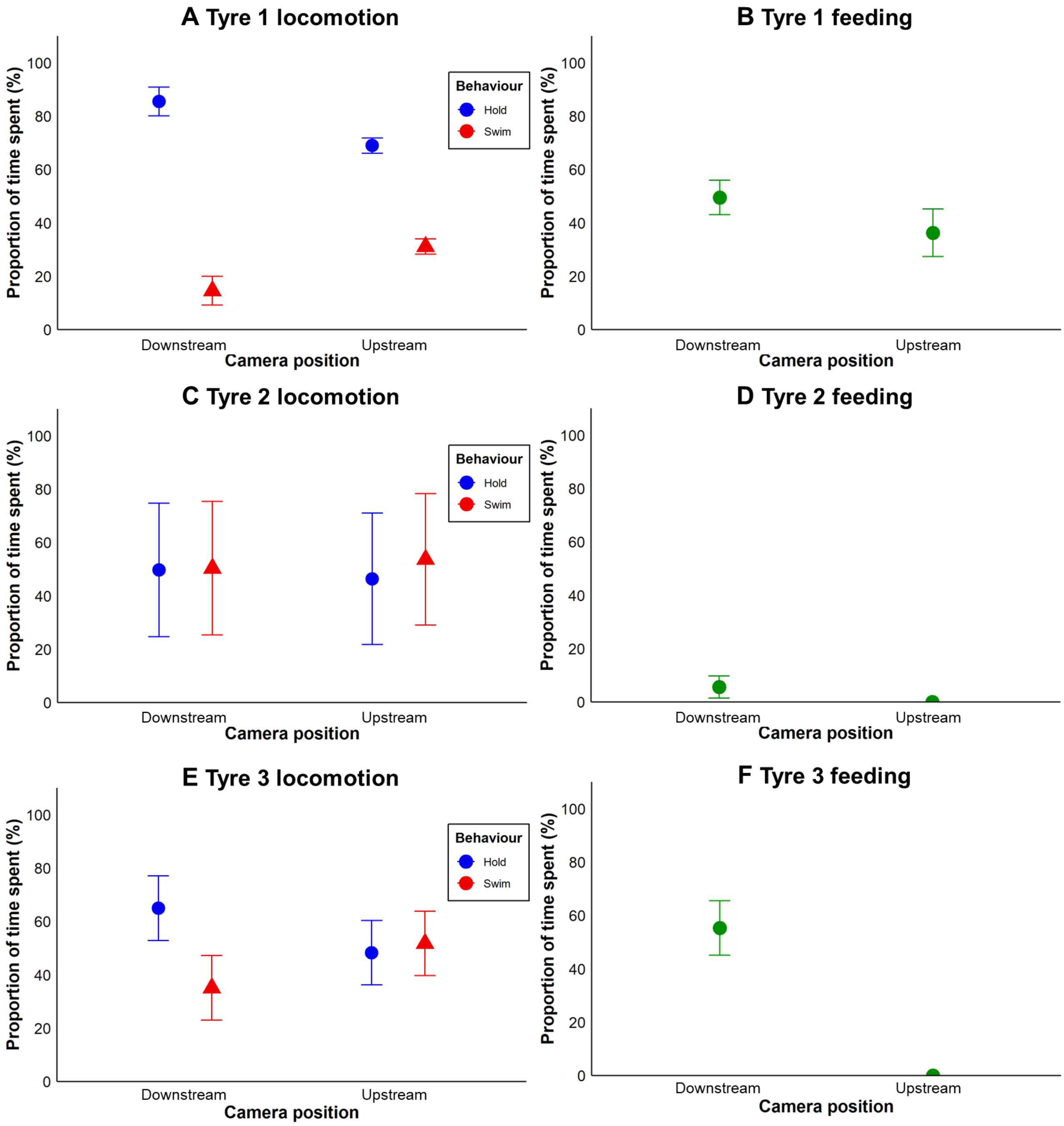

Figure 7.7: The proportion of the time (mean ± SE) juvenile silver fish were present that they spent doing certain behaviours: holding or travelling (A, C, E) and feeding (B, D, F). The proportional time fish spent holding or travelling adds up to 100% as fish were always in one of these behaviours (except when no fish were present which was included as 0%).

7.3.4. The middle of tyres

The only fish observed in the middle of tyre 2 was a stone loach, which mostly stayed immobile on the river bed (Figure 7.8). Although it was only visible for about 15 minutes, it was probably present, although hidden from view, for the whole of the observation period. In the middle of tyre 3, several individual and small shoals of silver fish were seen, but they were only seen during three observation periods. These fish did not stay long but were feeding on several occasions. However, there was one individual that seemed to spend nearly 27 minutes in the middle of the tyre, although it did swim in and out of view during this time. On one occasion there was one larger shoal (32 small silver fish) which entered the middle of tyre 3 at the same time as a pike approached the downstream camera. This suggests that the fish used the middle of the tyre to shelter from the predator. They then left when the danger had passed after approximately four minutes.

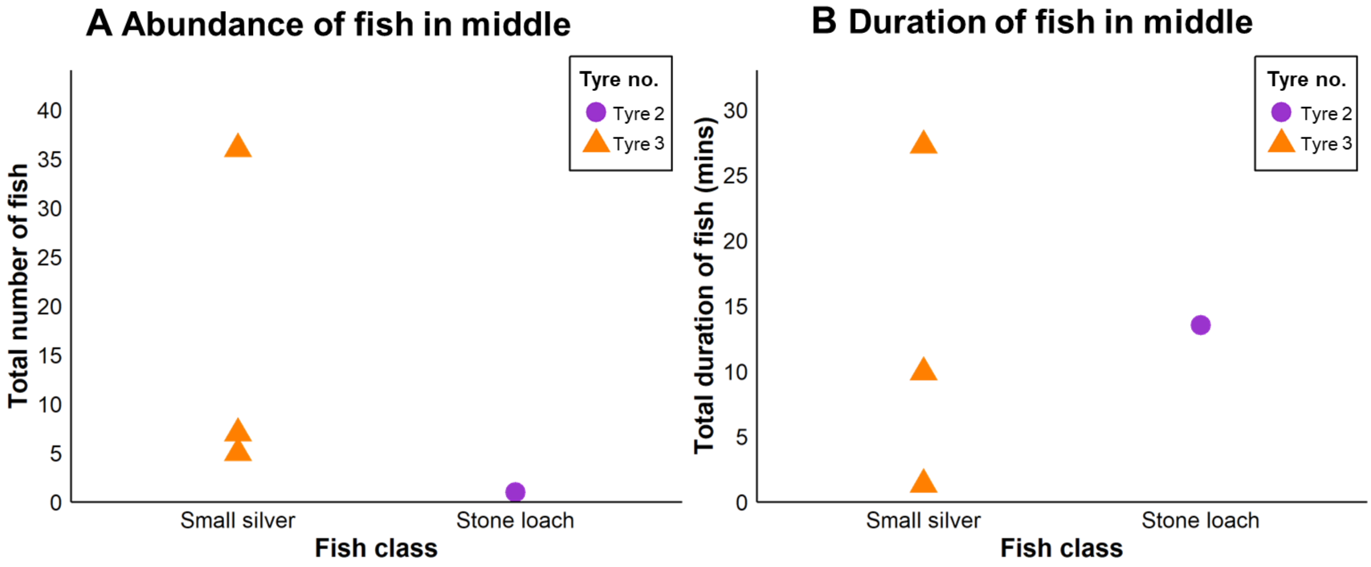

Figure 7.8: The abundance and duration of fish observed in the middle of tyres per observation period.

7.4. Discussion

Small silver fish, by far the most common fish observed in this study, tended to occupy the zone of slower flow downstream of tyres for longer periods of time than they did the upstream zone. However, there was little difference in their abundance between zones, probably because most fish passed both downstream and upstream cameras on their way through the river. Minnows, which often formed mixed shoals with the small silver fish, also spent more time downstream than upstream of tyres, although this was only seen at tyre 3 where they were most common. Measurements of flow velocity from Chapter 6 showed that flow speeds were considerably reduced downstream of tyres, and so it is likely that these small fish were occupying the area of slower flow to save energy and more easily hold station.

These results are similar to those of previous studies that show that even simple structures create hydraulic shelter by altering local flow characteristics (Shamloo *et al.*, 2001). Flow refuging behaviour, similar to that observed in this study, has been reported in laboratory experiments which used simple shapes like bricks or cylinders (Webb, 1998; Smith *et al.*, 2005; Liao *et al.*, 2003a; Przybilla *et al.*, 2010), sand ripples (Gerstner, 1998), or boulders (Kemp *et al.*, 2005) as structure. Similarly, investigations based in real rivers have shown that fish associate with structures that alter local flow velocity (Shirvell, 1990; Fausch, 1993; Shuler *et al.*, 1994; Tullos and Walter, 2015). Sheltering from fast flows reduces the energetic cost of holding station, exhibited as a reduction in muscle activity and body movements downstream of structures (McLaughlin and Noakes, 1998; Liao *et al.*, 2003b, 2006; Pryzbilla *et al.*, 2010).

Reduced flow velocity downstream of structures is not the only effect an obstacle has on local hydraulic conditions. Turbulence also increases in association with flow obstruction (Shamloo *et al.*, 2001; Smith and Foster, 2007; Lacey and Rennie, 2012; Zexing *et al.*, 2020). However, it is not fully understood how this turbulence affects different fish species and size classes (Smith *et al.*, 2014). Some researchers have found that turbulence increases swimming cost (Enders *et al.*, 2003; Lupandin, 2005; Tritico and Cotel, 2010), but others have shown that fish can utilise turbulent vortices to reduce an individual's energy use (Liao *et al.*, 2003a; Pryzbilla *et al.*, 2010). Without detailed measurement of flow patterns around the tyres under a range of different flow conditions, comment on the role of turbulence in this case is beyond the scope of this study. It would be a useful future extension to this work given that the turbulent properties of flow are increasingly considered of fundamental importance to fish and other river organisms (Smith *et al.*, 2014; Trinci *et al.*, 2017).

As well as minimising energetic costs of holding station, the altered flow conditions around tyres could have also affected feeding rates. Small silver fish, which primarily feed on zooplankton, phytoplankton, small invertebrates and plant materials (Maitland, 2004), spent more time feeding downstream of tyres (especially at tyres 2 and 3 where fish only fed in the downstream zone). Therefore, tyres may have increased the quantity or availability of food organisms, or their ease of capture. Macroinvertebrate monitoring six months after tyre installation and one month after filming was completed (as documented in Chapter 6) showed that macroinvertebrates were more abundant downstream and to the side of tyres, than upstream. Other researchers have also found enhanced production of food organisms on and around structures (Benke and Wallace, 2003; Pilotto *et al.*, 2014, 2016). However, as the small silver fish primarily fed on drifting particles, rather than from the river bed or water's surface, it seems likely that their feeding is a response to patterns of drift rather than production at the tyres. Drift feeding fish preferentially occupy slow flowing areas adjacent to fast flows where they can maximise their energy intake by feeding on drifting prey in the fast flows, whilst preserving energy by holding position in the slower flows (Bachman, 1984; Fausch, 1984). This means that structures which provide hydraulic refuge within the vicinity of areas of accelerated flow, caused by flow splitting around the obstacle, provide opportunity for drift feeding fish to maximise their energy intake (Crook and Robertson, 1999; Allouche, 2002; Hafs *et al.*, 2014). For example, in an experimental stream study with installed boulders and deflectors, Mitchell *et al.* (1998) found greater abundance of drifting invertebrates per unit time in fast flowing areas created beside structures. However, if turbulence or velocity is too extreme, foraging efficiency decreases due to an increase in prey flux and decreased prey capture success (Rosenfeld *et al.*, 2014).

Medium silver fish did not show the same preference for downstream zones as small silver fish. Instead, they were significantly more abundant and spent significantly more time upstream of tyres rather than downstream, although this was only at tyre 1 where nearly all (93%) of the medium silver fish were observed. Different size classes of fish have different habitat requirements (Aadland, 1993; Lamouroux *et al.*, 2001; Nislow and Armstrong, 2011; Huang *et al.*, 2019). Smaller fish are known to prefer the shallow and slow flowing areas along river margins (Bain *et al.*, 1988; Lamouroux *et al.*, 2001), with larger fish of the same species occupying deeper areas of river (Harvey and Stewart, 1991; Santos *et al.*, 2018). This relates to the stronger swimming ability of larger individuals (Tudorache *et al.*, 2007), the greater vulnerability of large fish to terrestrial predators that tend to hunt in shallow water (Harvey and Stewart, 1991; Steinmetz *et al.*, 2008), and because larger fish can outcompete and predate on smaller fish thus forcing them into shallower areas (Schlosser, 1987). Because of my agreement with the EA, tyres were placed into relatively shallow marginal zones. During filming, water levels were even lower (mean water depth at tyres during filming was 0.24 m) as monitoring was conducted in August when discharge is typically low, therefore the shallow water depth around tyres may have made these areas less attractive to medium silver fish. Additionally, the stronger swimming ability of medium silver fish may mean they had no need to shelter from what were already relatively slow flows. Researchers have shown that under slow flow velocity fewer fish choose to associate with structures and remain at structures for less time than they do under faster flows (Mitchell *et al.*, 1998; Webb, 1998; Smith *et al.*, 2005). Similarly, larger fish persist in faster flows for longer than small fish before choosing to take shelter (Smith *et al.*, 2005). When flows are high, such as in winter, and fish have more need to shelter and preserve energy, it is possible that tyres may become more important for medium silver fish, and indeed for other fish classes. Fish behaviour and use of structures is known to change diurnally, seasonally and with flow conditions. For example, during seasonally high flow conditions fish are more likely to select sheltered positions (Vehanen *et al.*, 2000; Santos *et al.*, 2018). Future research should investigate whether the ways in which fish respond to tyres are different at other times of day, during different seasons, or under different environmental conditions (e.g. temperature, flow conditions).

Structures are also important for predator avoidance. Small silver fish and minnows may have been congregating downstream of tyres for this purpose, as structures can visually isolate prey from predators (Crook and Robertson, 1999; Schneider and Winemiller, 2008). Habitat choice is affected by perceived predation risk (Magoulick, 2004; Schneider and Winemiller, 2008). Therefore, prey fish will spend more time in risky areas if shelter is nearby (Fraser and Cerri, 1982), and they may delay their flight response when structures are closer (McLean and Godin, 1989; Dill, 1990). However, the complexity of a structure has been found to be an important factor in how much protection from predation it provides (Warfe and Barmuta, 2004; Sass *et al.*, 2006, 2012; Miyamoto *et al.,* 2021). The ratio of inter-structural space to predator size is thought to be particularly important (Bartholomew *et al.*, 2000), with more complex structures providing more protection for prey. As tyres are simple shapes and only the middle of tyres provides shelter from visually orientated predators, it is likely that fish using the downstream region were primarily benefitting from its effects on flow rather than predation. However, the middle of tyres was used by fish, and on one occasion this was a direct response to the approach of a predator. It would be very difficult for a pike to successfully enter the tyre and capture prey given its relative size. Nonetheless, only a small number of the total number of small silver fish that were downstream of the tyre as the pike approached, used the tyre as shelter; most simply swam elsewhere, suggesting that the inside of tyres is not an ideal habitat.

The area downstream of tyre 3 was also regularly visited by piscivorous fish (perch and pike). It may be that these fish had learnt that large numbers of small silver fish regularly congregated at this location and so revisited these productive sites to hunt (Odling-Smee *et al.*, 2011). Some piscivorous predators have greater foraging success at structures, which can negate any benefit of the structure for prey species (DeBoom and Wahl, 2013). Perch were never seen to actively predate downstream of tyres, despite making numerous, short duration visits here. They use an active searching strategy (Eklöv and Diehl, 1994), which may be why they repeatedly searched the downstream zone for short durations (mean duration 11 seconds). In contrast, pike use a sit-and-wait hunting strategy, often using structures like macrophyte stands to hide in whilst waiting for prey to approach (Bean and Winfield, 1995; Craig, 2008). Only four pike were seen, but one of these held just downstream of tyre 3 for approximately 20 minutes, occasionally striking at the small silver fish which were also present. The tyre would not have hidden the pike from view but could have created the impression of shelter from which it could ambush.

Benthic fish (bullhead, gudgeon, and stone loach) are relatively weak swimmers (Tudorache *et al.*, 2007), so might have been expected to benefit from sheltered flow conditions downstream. However, despite their small size, bullhead abundance and duration was no different between zones, suggesting that tyres had no effect on their distribution. Bullhead are specially adapted to holding in fast flows by using their lack of a swim bladder and pectoral fins to anchor themselves on the river bed (Egger *et al.*, 2020), and have been shown to resist even fast flow speeds (Tudorache *et al.*, 2007). Rather than swimming to hold station they can occupy positions on the substratum in between or behind gravels (Webb *et al.*, 1996; Egger *et al.*, 2020). As such, they had no need to use tyres to shelter from flows, in fact, in this study one individual remained upstream of tyre 3, and therefore exposed to fast flows, for the full 22.1 observation period.

Gudgeon and stone loach are also benthic species, which have the same ability to occupy positions on the substratum, but in this study gudgeon was more common downstream of tyres, and the only stone loach I observed was spotted in the middle of tyre 2. These fish may have been using the tyre to shelter from flows, but given the low abundance of these species (only one stone loach) there is not enough evidence to conclude this. Gudgeon do tend to spend more time continuously swimming, instead of holding onto the substrate than other benthic species (Egger *et al.*, 2020), so it is possible that they chose to occupy downstream zones for shelter because their behaviour causes them to be exposed to higher flows than the bullhead. Stone loach tend to hide under stones or in thick vegetation during the day (Maitland and Campbell, 1992), which might explain why this individual was present in the middle of a tyre.

7.4.1. Conclusions and implications

This chapter considers the small-scale patterns in habitat choices and behaviours of fish related to the presence of car tyres in a river where other structures are relatively uncommon. The results suggest that tyres affect small silver fish, and perhaps minnows too, by creating a sheltered zone downstream of the tyre which protects fish from fast flows and provides an opportunity for feeding. It is also possible that the middle of tyres may present a refuge from predators, although this finding is more tentative, and there is some evidence predators were visiting tyres for the purpose of hunting on the small fish. Larger fish and benthic species were infrequently observed and did not show the same response to tyres.

If small silver fish downstream of tyres can reduce their energetic costs and increase their food intake by taking advantage of the sheltered flow conditions, this suggests that artificial structures could affect their health and abundance. As such, large litter is unlikely to support populations of fish which would otherwise be unviable, but it could support the recruitment and survival of juvenile and small fish.

Chapter 8: Discussion

8.1. Achievement of aims

This thesis investigated the characteristics and ecological impacts of anthropogenic litter in rivers through the following aims:

1) To improve current understanding of the amount and types of litter present in UK rivers, and to investigate whether there is a relationship between litter density and macroinvertebrate communities (Chapter 4).
2) To assess whether there are differences in the macroinvertebrate communities inhabiting anthropogenic litter and natural mineral substrates (i.e. rocks) (Chapter 5).
3) To measure the impacts of large anthropogenic litter items on local habitats, macroinvertebrates and fish (Chapters 6 and 7).

8.1.1. Aim 1: anthropogenic litter characteristics and relationship to macroinvertebrates

Chapter 4 addressed Aim 1 by surveying benthic anthropogenic litter densities and characteristics in four different rivers over a range of litter densities, and then comparing these results to the findings of other aquatic litter surveys. I also sampled the macroinvertebrates living at these sites in order to assess whether there was a relationship between macroinvertebrates and the quantity of anthropogenic litter.

This study found similar anthropogenic litter densities in the sampled river beds to those reported by other studies, including marine litter surveys. This supports arguments that rivers are not simply conduits for anthropogenic litter but are stores of it, meaning that river organisms probably regularly encounter litter, and demonstrating the need for increased litter research within rivers. I also found that litter density and composition was highly variable within and between rivers. This high variability could relate to spatial patterns in litter sources, river geomorphology, infrastructure and vegetation, and the properties of the litter itself; for example, large immovable litter can accumulate additional litter items. The results also suggested that plastic is less dominant in river beds than it is in marine environments or the riparian zone of rivers. This is likely to be because of the properties of plastic; it is more mobile than other litter materials like glass or metal, but is also more easily entangled on vegetation or deposited on the banks during high flows. This means we should be cautious applying what is known about marine and riparian anthropogenic litter to the benthic zone of rivers.

The results of the chapter also showed that anthropogenic litter density was significantly positively associated with macroinvertebrate diversity (^{1}D and ^{2}D). The most likely explanation for this is that an increase in litter density increases the diversity of available habitat for macroinvertebrates because it provides an assortment of substrates with a huge variety of shapes, sizes, colours, textures, and other characteristics. This was considered further in Chapters 5 and 6.

8.1.2. Aim 2: the differences between macroinvertebrate communities on litter and rocks

In Chapter 5, I investigated differences in the macroinvertebrate communities inhabiting anthropogenic litter and natural mineral substrates (rocks) to see whether the novel properties of litter meant that there were differences in the communities colonising the different substrates (Aim 2).

I found that the macroinvertebrate communities on anthropogenic litter were consistently more diverse than those on rocks. This is likely to be because of the greater surface complexity of the litter. The lowest macroinvertebrate diversity was thus found on the smooth surfaces of rocks and glass. The macroinvertebrate community composition was also distinctly different between anthropogenic litter materials and rocks. Plastic and fabric litter communities were especially different to those on rocks, which, given the types of taxa found in greater numbers on these materials, suggested that the flexibility of these litter items meant they mimicked macrophytes.

8.1.3. Aim 3: the effects of large litter

Chapter 6 detailed an experiment that looked at how car tyres installed into two rivers with different bed substrates (one sand-bed and one gravel-bed) changed the habitats and macroinvertebrate communities in the surrounding river bed six and twelve months after installation.

This chapter found that the tyres themselves were inhabited by distinct macroinvertebrate communities that were different to those found anywhere else in the river. However, whereas in the gravel-bed macroinvertebrate communities on tyres were less diverse than those in the surrounding river bed, the communities on the surface of tyres were significantly more diverse than those elsewhere in the sand-bed river. This is probably because tyres in the sand-bed river provided a solid surface that could be colonised by macroinvertebrates that are sensitive to fine sediments and prefer fast flows. These taxa also score highly in biotic indices, so the presence of tyres could have consequences for reach-scale environmental quality assessment.

Tyres also changed habitat conditions in the surrounding river bed. The habitat preferences of the different macroinvertebrate communities found in different positions around the tyre, suggested that tyres had similar effects to natural structures like large wood and boulders. Nevertheless, in the gravel-bed river, the tyres did not create habitat conditions that were outside of the range of those found elsewhere in the river. In the sand-bed river, however, I found that the diversity of macroinvertebrates in the river bed surrounding the tyres was significantly greater than that found anywhere else, suggesting that tyres acted as hot spots for diversity. This is probably because the substrate in the sand-bed river is naturally highly mobile and the river lacks natural habitat structures, so the tyres changed the surrounding river bed into a relatively unique and otherwise scarce habitat by stabilising the sediment and accumulating organic matter and plant material.

In Chapter 7, fish were monitored around three of the tyres in the gravel-bed river to see if they were affected by the structure created by the tyres; in particular this study investigated whether fish would shelter in the slower flow zone downstream of tyres. This was assessed by comparing fish abundance and behaviour upstream and downstream of tyres using video cameras.

Small silver fish (chub, roach, and dace < 15 cm long) were by far the most common fish observed (>97% of fish). They spent more time downstream of tyres than upstream, and spent a greater proportion of their time holding station and feeding when in the downstream zone. This suggests that tyres provided structure that enabled small silver fish to conserve energy and feed by taking refuge from fast flows. Additionally, small silver fish were observed using the middle of a tyre to hide from an approaching pike, suggesting that the middle of tyres could provide shelter from predators. Other fish, including larger silver fish, seemed to be less effected by the presence of tyres, although pike did hunt downstream of tyres, and perch made a greater number of visits to the downstream zone.

8.2. Discussion and implications of the thesis

In meeting the thesis aims this research has highlighted that anthropogenic litter is a significant component of many rivers, which can provide (Figure 8.1) and create (Figure 8.2) habitat for macroinvertebrates and fish. I have shown that riverine anthropogenic litter is widespread and extensively colonised. For instance, all of the 300 litter items sampled in Chapter 5 were inhabited by macroinvertebrates. Litter is diverse and unusual in physical structure, and is often more complex than natural substrates, meaning it can increase local macroinvertebrate diversity and support distinct communities. Large items of anthropogenic litter also changed habitat conditions within their vicinity, altering patterns of flow, and accumulating fine sediment, organic matter, and additional litter items. This affected the distribution of macroinvertebrates and provided shelter for fish.

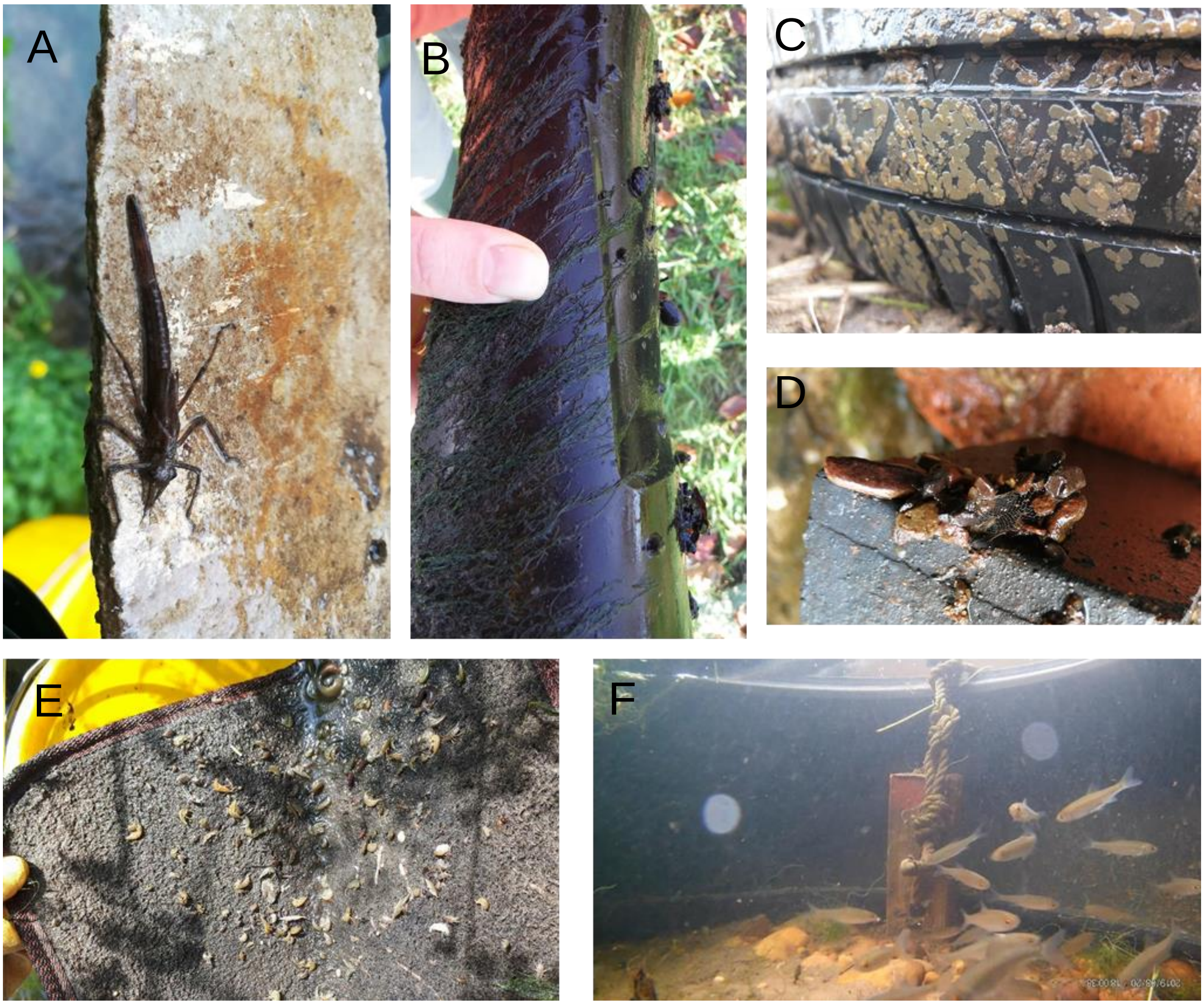

Figure 8.1: Examples of macroinvertebrates (and their eggs: C) and fish (F) colonising or interacting with anthropogenic litter from fieldwork conducted during the PhD.

This is the first known research to show that anthropogenic litter can provide a habitat in rivers; however, colonisation of litter by a range of organisms has been recognised in other aquatic systems. For example, abundant epibenthic communities have been found to inhabit anthropogenic litter in tidal estuaries (Chapman and Clynick, 2006), shallow seas (Katsanevakis *et al.*, 2007), continental shelves (Watters *et al.*, 2010; Schlining *et al.*, 2013; Crocetta *et al.*, 2020), submarine canyons (Mordecai *et al.*, 2011; Schlining *et al.*, 2013; Song *et al.*, 2021), and coastal beaches (García-Vazquez *et al.*, 2018; De-la-Torre *et al.*, 2021). In reservoirs in Poland, Jatulewicz (2007) and Czarnecka *et al.* (2009) found extensive macroinvertebrate colonisation of litter items, including bottles, textiles, string, polythene, and rubber items. Mobile organisms have also been observed interacting with litter. For instance, octopus use beer bottles as dens in Puget Sound (USA) (Anderson *et al.*, 1999), small cryptic fish and juveniles were seen sheltering around litter in Sydney Harbour (Australia) (Chapman and Clynick, 2006), and juvenile fish, gastropods, and hermit crabs sheltered in and around litter in the shallow seafloor of the Saronikos Gulf (Greece) (Katsanevakis *et al.*, 2007).

As anthropogenic litter provides habitat, its removal through litter clean-ups represents a loss of habitat. The significance of this loss depends on the quality and diversity of natural habitat available to organisms. For instance, the tyres in the gravel-bed River Idle did not change habitat conditions outside of the range found elsewhere in the river, but in the sand-bed River Maun, tyres created otherwise scarce, novel and stable habitat (Chapter 7). Rivers with the highest litter density seem to be those in the most urbanised areas with high anthropogenic activity (Chapter 4; Williams and Deakin, 2007; McCormick and Hoellein, 2016), where rivers are also likely to have been heavily modified to make space for urban development, manage flood risk, or enable navigation (Walsh *et al.*, 2005). These types of rivers are consequently lacking in habitat structure and diversity as their channels have been simplified and habitat structures removed (Walsh *et al.*, 2005; Bernhardt and Palmer, 2007). Under these conditions, the presence of anthropogenic litter could be an important form of habitat for river organisms. Therefore, the justification for removing the litter should be carefully considered. Removing anthropogenic litter without replacing the habitat is unlikely to lead to biodiversity improvements and may in fact reduce biodiversity (Wilson *et al.*, 2021). Many traditional restoration techniques are not feasible in urban rivers, but there may be opportunity to utilise artificial structures to improve habitats (Francis and Hoggart, 2008; Nakano *et al.*, 2018).

Furthermore, the process of clearing anthropogenic litter could itself be damaging to river organisms. There are a variety of ways to collect floating litter (Helinski *et al.*, 2021), but nearly all litter I encountered whilst conducting this research was in the river bed. Manual clearance is generally the only option for removing benthic litter (Helinski *et al.*, 2021), but this involves considerable disturbance to the river bed through the pulling up of partially or completely buried litter items. It could also resuspend substantial amounts of fine and potentially anaerobic sediments (Backhurst and Cole, 2000). Excessive transport and deposition of fine sediment is hazardous for fish and macroinvertebrates (Jones *et al.*, 2011; Kemp *et al.*, 2011). Similarly, disturbance of the river bed can lead to mortality of fish eggs and pre-emergent fry (Roberts and White, 1992; Lapointe *et al.*, 2000), and removing litter kills any attached organisms. Whilst the ecological impacts of benthic litter removal have yet to be measured, it is likely that there are at least short-term negative impacts on biota. These risks could be mitigated somewhat by conducting clean-ups outside of the fish spawning season and by washing organisms off anthropogenic litter items before disposal.

The properties of anthropogenic litter are extremely diverse; so too are its impacts. For instance, different litter materials have been shown to support different communities (Chapter 5; Chapman and Clynick, 2006; Pace *et al.*, 2007; Czarnecka *et al.*, 2009; Rech *et al.*, 2018). Flexible litter materials may mimic macrophytes (Chapter 5), and beverage bottles or cans can shelter organisms in their interior (Katsanevakis *et al.*, 2007; Czarnecka *et al.*, 2009). Similarly, not all anthropogenic litter materials release toxic compounds as they degrade. The different characteristics of litter also regulate its mobility and where it is stored within the river (see review in Chapter 2); factors which influence the availability of anthropogenic litter to organisms. Further research is needed to understand the properties of different types of anthropogenic litter, and which of these characteristics provide beneficial ecological functions so that these functions can be replaced when litter is removed. Crucially, the current focus on plastic litter, risks ignoring the effects of the sizable proportion of anthropogenic litter which is made of other materials (Chapter 4).

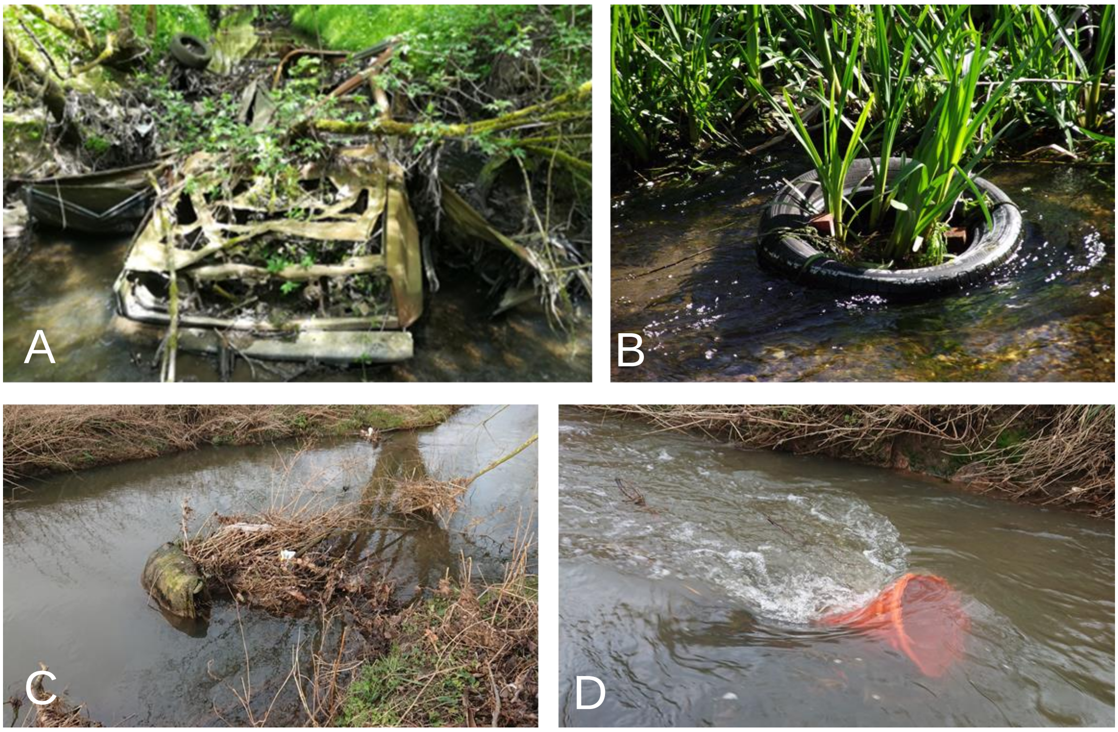

Figure 8.2: Photos from my fieldwork which show anthropogenic litter affecting river habitats. A) A car blocks the entire river channel in Alfreton Brook. B) A car tyre (tyre 5 in Chapter 6) supports vegetation establishment in its centre in the River Idle. C) A shopping trolley creates small vegetated island in Black Brook by accumulating fine sediment, organic matter and additional litter. D) A traffic cone alters the patterns of flow in Black Brook.

In considering how best to manage anthropogenic litter it is important to remember that litter is not truly a replacement for natural habitats. Both my research, and the work of other researchers have found differences in the communities inhabiting litter and those occupying natural substrates like sand, rocks, or vegetation (Chapters 5 and 6; Katsanevakis *et al.*, 2007; Czarnecka *et al.*, 2009; García-Vazquez *et al.*, 2018). This means that high densities of anthropogenic litter could alter local communities, potentially with undesirable implications for ecosystem functions. For example, Katsanevakis *et al.* (2007) found that litter introduced into a shallow sandy sea, substantially increased the abundance of predatory species, changing the relationships between species and introducing new predator-prey interactions. Rare or endemic species have been found on litter (Song *et al.*, 2021), but so have invasive species (Tyrrell and Byers, 2007; García-Vazquez *et al.*, 2018; Rech *et al.*, 2018). It is thought that anthropogenic litter could increase the competitive advantage of non-native species and provide a means for species invasions (Katsanevakis *et al.*, 2007;

Holdredge and Bertness, 2011). Further study is therefore needed to understand the differences between the habitat provided by anthropogenic litter and natural habitats, and the reasons for these differences. It should also consider the potentially complex and subtle consequences of any changes to communities, such as the introduction of invasive species.

It is also important to consider that anthropogenic litter has numerous other negative effects on rivers and other aquatic systems. Anthropogenic litter can harm organisms through accidental entanglement or ingestion (Gall and Thompson, 2015), or through chemicals released during break down or ingestion (Teuton *et al.,* 2009; Gunaalan *et al.,* 2020). It can have adverse human health consequences as some of its sources (especially sewage infrastructure) are associated with harmful pathogens (McCormick *et al.*, 2014), and people may be injured through contact with litter (Campbell *et al.*, 2016). There is also a significant cost involved in removing litter and dealing with blockages of culverts or trash screens (Mouat *et al.*, 2010). High anthropogenic litter density also affects the aesthetics of an area, with consequences for recreation, tourism, crime, and property values (Schultz *et al.*, 2011; Wyles *et al.*, 2016).

Given the known negative impacts of anthropogenic litter and our relatively limited understanding of its implications for ecological communities, this research should not be used to justify continued littering or deliberate dumping of litter in rivers. However, river managers often have to make difficult and nuanced decisions to prioritise finite resources effectively. This might mean focusing litter clean-up efforts at sites where efforts will be the most effective at reducing anthropogenic litter density (Carpenter and Wolverton, 2017). Alternatively, certain hazardous litter materials could be prioritised for removal; already many river clean up groups focus on plastic items, tyres and batteries. Recognising that anthropogenic litter can provide and create valuable habitat in heavily managed rivers that with poor natural habitat, can help justify habitat restoration when litter is removed. More evidence on the impacts of litter are needed to inform and justify these kinds of decisions. As anthropogenic litter is persistent in the environment, and given that projections of waste and litter generation are expected to continue to increase (Vannela, 2012; Kaza *et al.*, 2018), the question of how to manage litter in rivers will only become more pressing over time. There has already been a dramatic recent increase in published studies on the topic (Blettler *et al.*, 2018; Nielsen *et al.*, 2019). Further investigation into the consequences of anthropogenic litter is therefore imperative to understand its impacts and improve river ecosystems, where there are some of the highest litter concentrations of any aquatic environment.

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