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Anthropogenic Litter and Microplastic in Urban Streams: Abundance, Source, and Fate

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ABSTRACT

The accumulation and ecological effects of anthropogenic litter (AL; trash) and microplastic (particles <5mm) are well-documented in marine ecosystems, but the role of rivers in transporting AL and microplastic is unknown. AL enters rivers from recreation, industry, runoff, and illegal dumping. Microplastic fibers (e.g., synthetic fabrics) and pellets (e.g., abrasives in personal care products) are abundant in wastewater treatment plant (WWTP) effluent that enters rivers. Our objectives were to: (1) quantify the abundance and composition of AL in urban streams, (2) measure AL flux in rivers by calculating input and output rates, and (3) measure the concentration and analyze bacterial community composition of microplastic in rivers. In summer 2014, we collected AL from 5 urban streams in Illinois, USA, which span a gradient of urban land use. We found higher AL density in riparian habitats and higher AL mass in benthic habitats. Overall, reach-scale metrics explained variation in AL abundance and composition, rather than watershed-scale characteristics. In our flux studies, we demonstrated that AL is a mobile substrate in rivers whose movement is mediated by material type and hydrology. Finally, we collected surface water samples upstream and downstream of 9 WWTPs in Illinois, USA and found higher microplastic concentration downstream in all but two streams. Using next generation sequencing of the 16S rRNA genes, we demonstrated that microplastic offers a uniquehabitat for microbial colonization, and it selects for bacteria associated with plastic degradation, biofilm formation, and human disease.

CHAPTER I

INTRODUCTION

Human alterations to global ecosystems

Increases in human population and technological innovation since the industrial revolution have altered global ecosystems through resource exploitation, manufacturing, urbanization, and agriculture (Zalasiewicz et al. 2008; Harden et al. 2014). The term 'Anthropocene' describes a newly emerging geologic era where the imprint of human activity and the associated environmental changes are now a permanent component of the Earth's stratigraphic signature (Zalasiewicz et al. 2008; Harden et al. 2014). Many physical and chemical environmental changes are associated with the Anthropocene, such as increasing erosion, denudation of the continents, increasing atmospheric carbon dioxide levels, decreased biodiversity, and sea level rise (Zalasiewicz et al. 2008).

The manufacturing and improper disposal of consumer products is a major human impact on ecosystems worldwide. For example, between 1950 and 2011, global plastic production increased from 1.7 to 280 million tons (Plastics Europe 2012) and a significant portion of man-made materials such as plastic accumulate in the environment (Cózar et al. 2014). Anthropogenic litter (AL; trash) refers to the assemblage of manufactured items that enter the environment (e.g., plastic, glass, metal, rubber, manufactured wood, paper), and is a visible, long-lasting manifestation of human activity. For instance, a recently discovered type of stone coined 'plastiglomerate,' which

forms from the combination of melted plastic, beach sediment, basaltic lava fragments, and organic debris, shows the persistent effect AL can have on the global geologic record (Corcoran et al. 2014). In addition, a growing field of research has documented the occurrence of microplastic (<5 mm particles) in oceans worldwide, with largely unknown ecological effects (Thompson et al. 2004; Browne et al. 2011; Eriksen et al. 2014). The high abundance of AL and microplastic in the environment is an emerging topic of ecological concern associated with the Anthropocene. In recognition of this issue, much research is focused on the abundance, composition, sources, and biological interactions of AL and microplastic in ecosystems worldwide.

AL distribution and abundance

AL has been documented globally, but a majority of AL research is focused on marine environments where AL has a broad geographic distribution and high density. For instance, floating plastic is estimated to account for over 5 trillion pieces of AL weighing over 250,000 tons in ocean surface waters (Eriksen et al. 2014). Many studies have documented AL abundance in benthic coastal zones (Moore and Allen 2000; Hess et al. 1999; Watters et al. 2010) and seafloors (Schlining et al. 2013; Pham et al. 2014; Abu-Hilal and Al-Najjar 2009; Debrot et al. 2014; Galil et al. 1995; Stefatos et al. 1999), as well as floating AL in coastal waters (Thiel et al. 2003) and the open ocean (Eriksen et al. 2014; Cózar et al. 2014). Additionally, several studies have recorded AL on coastal beaches (Smith and Markic 2013; Oigman-Pszczol and Creed 2007; Kusui and Noda 2003) and oceanic islands (Eriksson et al. 2013).

Several environmental factors influence the accumulation and retention of marine AL. Previous studies show that wind and surface currents contribute to AL accumulation

in subtropical gyres (Eriksen et al. 2014; Cózar et al. 2014). Additionally, benthic habitats characterized by high sediment accumulation and densely populated coasts are AL accumulation zones (Barnes et al. 2009). Sources of marine AL include offshore sources such as dumping by ships and land-based sources such as littering by beachgoers, trash dumping, runoff, and rivers (Ryan et al. 2009; Williams and Simmons 1997; Abu-Hilal and Al-Najjar 2009).

Although many studies consider rivers a major AL source to marine ecosystems (Corcoran et al. 2009; Galgani et al. 2000; Ivar do Sul et al. 2011; Araujo and Costa 2007), data on riverine AL is scarce. Few studies provide quantitative measurements on riverine AL abundance and composition (Williams and Simmons 1997; Williams and Simmons 1999; Rech et al. 2014; Hoellein et al. 2014). In the Taff River System, Wales, Williams and Simmons (1999) found that sewage-related material and 'fly-tipping' (illegal dumping) are significant sources of AL to riverbanks. Previous research by Williams and Simmons (1997) examined the influence of flooding on AL input rates, accumulation times, and movement patterns within river bank zones. Data from Rech et al. (2014) indicate that rivers are a source of AL to coastal beaches, as the composition of AL in riversides and beaches at the respective mouth of each river was similar. While these studies focused only on AL in riverbanks, Hoellein et al. (2014) compared AL density, abundance, and composition between 2 river habitats: the riparian benthic zones. Many questions remain regarding AL in rivers, including the exchange of AL between riparian and benthic habitats, the influence of watershed land use on AL abundance and composition, and AL export and accumulation rates at annual and seasonal scales.

Many studies from marine environments have focused on plastic AL exclusively (Ryan et al. 2009; Moore 2008; Eriksen et al. 2014; Cózar et al. 2014), which may underestimate the abundance, source, and ecological effects of AL in the environment. Plastic is inexpensive, versatile, and common in many single-use and disposal items (Andrady 2011; Barnes et al. 2009), which contributes to its high abundance in the environment. Furthermore, its buoyancy, durability, and ability to resist degradation makes it a problematic and persistent form of AL in the ocean (Eriksen et al. 2014; Cózar et al. 2014; Andrady 2011; Barnes et al. 2009; Derraik 2002). In marine environments, plastic frequently dominates AL assemblages and often comprises 60-80% of marine AL (Moore 2008; Table 1 in Derraik 2002). However, many other material types form the larger AL 'community,' including metal, glass, 1 rubber, cloth, and manufactured wood. Including the full spectrum of materials in AL research is important as these materials likely have distinct ecological effects, and categories other than plastic comprise a significant proportion of total AL in many studies, particularly those in benthic habitats (Whiting 1998; Pham et al. 2014; Schlining et al. 2013; Abu-Hilal and Al-Najjar 2009).

Ecological, social, and economic implications of AL

AL has several ecological implications such as entanglement, consumer ingestion, and enhanced dispersal of colonizing organisms. After becoming entangled in AL, organisms often die by drowning, strangulation, and/or a reduction in feeding efficiency (Moore 2008; Laist 1987). Derelict fishing nets and gear (i.e., ghost fishing) are a notable source of entanglement (Moore 2008; Laist 1987). Many organisms also ingest AL which can cause detrimental effects such as digestive system blockages, damage to stomach

linings, choking, a feeling of false satiation, or reduced feeding efficiency (Moore 2008; Ryan 1988; Laist 1987). Marine mammals (Jacobsen et al. 2010; Secchi and Zarzur 1999), seabirds (Robards et al. 1995; Ryan 1987; Moser and Lee 1992; Blight and Burger 1997), and turtles (Bjorndal et al. 1994; Tomás et al. 2002; Mascarenhas et al. 2004; Bugoni et al. 2001) are known to ingest AL. Finally, AL represents a new mode of invasive species dispersal in marine environments by serving as a raft for aquatic organisms (Barnes 2002). Barnes and Fraser (2003) reported that floating plastic transported nonnative gastropods and bryozoans to the Antarctic Peninsula. The increase of floating plastic debris is also linked to increased dispersal of harmful algal bloom (HAB) taxa (Masó et al. 2003).

In addition to ecological consequences, AL is also associated with potential human health problems and economic expenses (Moore 2008). AL contamination in recreation areas may result in physical injury or health hazards from sharp objects (e.g., metal, glass) or unhygienic items (e.g., used personal care and medical products). Additionally, a study in Orange County, CA, USA demonstrated that AL on coastal beaches causes economic losses in the tourism industry by dissuading visits to local beaches (Leggett et al. 2014). Furthermore, efforts to remove AL from beaches are expensive (Moore 2008). Rochman et al. (2013) estimate that $520 million annually is spent by taxpayers on the USA's west coast alone to remove AL.

Microplastic distribution and sources

Microplastic is a component of AL with a widespread distribution and significant ecological implications. Microplastic is commonly defined as particles <5 mm (Moore 2008; Yonkos et al. 2014; Arthur et al. 2009; Sadri and Thompson 2014), although some

studies define microplastic as particles <1 mm (Claessons et al. 2011; Browne et al. 2010), and others distinguish between large (1-5 mm) and small (<1 mm) microplastic (Wagner et al. 2014). Across all size ranges, microplastic particles are made of multiple types of plastic polymers. High-density polyethylene (HDPE), low-density polyethylene (LDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and polyamide fibers (nylon) are commonly found in the environment (Wagner et al. 2014).

Microplastic has several sources which are often classified into two categories: primary and secondary (Cole et al. 2011). Primary sources include microbeads, pellets, and spherules contained in personal care products, production pellets used to manufacture plastic items, and particles used in air-blasting technology (Cole et al. 2011; Gregory 1996; Fendall and Sewell 2009). Secondary microplastic forms through fragmentation of larger particulate plastic by biodegradation, photolysis, thermoxidation, and thermodegradation processes (Andrady 2011). Finally, washing synthetic textiles releases a high abundance of microplastic fibers into washing machine effluent (Browne et al. 2011). Microplastic pellets from personal care products and fibers enter the domestic wastewater infrastructure but may not be removed by wastewater treatment plants (WWTPs) due to their small size (Fendall and Sewell 2009; Browne et al. 2011). WWTP effluent can be a source of plastic fibers to marine sediment (Browne et al. 2011) and a source of pellets and fibers to river surface waters (McCormick et al. 2014).

Carpenter and Smith (1972) first reported microplastic in the Sargasso Sea, and subsequently, microplastic has been found in habitats worldwide including coastal surface water (Moore et al. 2002; Lattin et al. 2004; Gilfillan et al. 2009; Ng and Obbard

2006), the open ocean (Lusher et al. 2014; Doyle et al. 2011; Eriksen et al. 2013b; Goldstein et al. 2013; Law et al. 2010; Morét-Ferguson et al. 2010; Moore et al. 2001), beaches (Abu-Hilal and Al-Najjar 2009; Hildalgo-Ruz and Thiel 2013; Ivar do Sul et al. 2009; Liebezeit and Dubaish 2012; McDermid and McMullen 2004), and marine sediment (Browne et al. 2011; Thompson et al. 2004; Claessens et al. 2011; Van Cauwenberghe et al. 2013). It was recently estimated that microplastic accounts for 92% of plastic debris in the world's oceans (Eriksen et al. 2014) and >80% of intertidal plastic debris (Browne et al. 2007). Many areas with microplastic accumulation are near urban centers (Yonkos et al. 2014; Eriksen et al. 2013a; Browne et al. 2011) and oceanic gyres (Eriksen et al. 2013b; Law et al. 2010; Moore et al. 2001), but microplastic has also been found in remote habitats including alpine lakes (Free et al. 2014), isolated islands (Ivar do Sul et al. 2009), and Arctic sea ice (Obbard et al. 2014).

A majority of microplastic research has focused on marine environments, and research on microplastic in freshwaters and estuaries has only recently emerged (Wagner et al. 2014). Several studies have documented microplastic in lake shorelines (Zbyszewski and Corcoran 2011; Imhof et al. 2013), sediment (Corcoran et al. 2015), and surface waters (Free et al. 2014; Eriksen et al. 2013a; Faure et al. 2012). Distribution and abundance of microplastic in lakes is driven by proximity to areas of high population density and industrial centers, riverine inputs, and wind. Measurements of microplastic abundance in estuaries highlight the potential for rivers to transport microplastic to marine habitats (Yonkos et al. 2014; Dubaish and Liebezeit; Sadri and Thompson 2014; Lima et al. 2014), and estuarine microplastic concentration is controlled by precipitation, tides, proximity to urban areas, and inputs to rivers (i.e., WWTP and industrial effluents).

Finally, recent studies have measured microplastic concentration in riverine sediment (Castañeda et al. 2014) and surface waters (McCormick et al. 2014; Lechner et al. 2014; Moore et al. 2011). Moore et al. (2011) showed that rain events increase the concentration of riverine microplastic, and McCormick et al. (2014) demonstrated that WWTP effluent was a point source of microplastic in an urban river. A greater understanding of the sources, accumulation sites, and movement of microplastic in rivers is needed to quantify global microplastic distribution. Also, rivers are susceptible to the same sources of microplastic as marine environments and have relatively little water volume for microplastic dilution, so they are likely to have high concentrations.

Ecological effects of microplastic

Microplastic has several ecological effects on biota such as ingestion by consumers, transporting contaminants to organisms, and providing a novel habitat. Its small size makes microplastic accessible for ingestion by a wide range of organisms varying in size and trophic level (Wright et al. 2013; Barnes et al. 2009). Marine invertebrates including zooplankton, barnacles, amphipods, lugworms, mussels, lobsters, and sea cucumbers (Cole et al. 2013; Browne et al. 2013; Thompson et al. 2004; Browne et al. 2008; De Witte et al. 2014; Goldstein and Goodwin 2013; Murray and Cowie 2011; Van Cauwenberghe and Janssen 2014; Graham and Thompson 2009), fish (Boerger et al. 2010; Choy and Drazen 2013; Lusher et al. 2012; Davison and Asch 2011), and mammals (Rebolledo et al. 2013; Lusher et al. 2015) have all been documented to ingest microplastic. Once consumers ingest microplastic, the material can be retained in gut tissue, which can block digestion and suppress feeding due to false satiation (Wright et al. 2013). Organisms may egest microplastic (Thompson et al. 2004; Graham and Thompson

2009; Wright et al. 2013), but Browne et al. (2008) also demonstrated that microplastic can translocate from the digestive to circulatory system in mussels (*Mytilus edulis*) and remain within the body for 48 d. Microplastic ingestion by copepods (*Calanus helgolandicus*) decreased the consumed number of algal cells and algal carbon biomass by 11% and 40%, respectively (Cole et al. 2015). Furthermore, microplastic can bioaccumulate in food webs once it is ingested by lower trophic organisms. Several studies have documented the transfer of microplastic from prey to predator (Setälä et al. 2014; Murray and Cowie 2011; Farrell and Nelson 2013). Eriksson and Burton (2003) documented microplastic fragments in seal scat, and they proposed that trophic transfer of microplastic occurred by seals ingesting microplastic-containing fish.

Additional ecological effects of microplastic are associated with its role in the transport and release of contaminants (Teuten et al. 2009). During the manufacturing process, toxic compounds (i.e., nonylphenols (NP), phalates, alkylphenols, bisphenol A (BPA), and organotin compounds) are added to microplastic polymers (Teuten et al. 2009; Mato et al. 2001). Additives such as plasticizers ensure the function of plastic material, but these compounds can be harmful to organisms (Barnes 2009). These toxins have been associated with deterioration of immune function and endocrine disruption (Teuten et al. 2009; Mato et al. 2001). Additionally, seawater naturally contains low levels of contaminants, but microplastic can adsorb and concentrate these pollutants (Rios et al. 2010; Barnes et al. 2009; Mato et al. 2001). In marine surface waters, Rios et al. (2010) found high concentrations of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochloride pesticides, on microplastic surfaces. The levels of PCBs on plastic pellets

can be 1,000,000 times higher than ambient seawater concentrations (Teuten et al. 2009). Furthermore, Mato et al. (2001) demonstrated that the concentration of PCBs and dichlorodiphenyldichloroethylene (DDE) on polypropylene microplastic increased over time. There is concern that microplastic ingestion facilitates the transport of contaminants to organisms. For instance, when fed polyethylene with chemical pollutants adsorbed from the environment, fish displayed bioaccumulation of contaminants and hepatic stress (Rochman et al. 2013).

Microplastic provides a novel habitat for microorganisms in the environment. In an early documentation of marine microplastic, Carpenter and Smith (1972) reported hydroids and diatoms growing on microplastic. Using next-generation sequencing techniques, Zettler et al. (2013) coined the term 'plastisphere' to describe the diverse community of microorganisms living on microplastic. Additional studies demonstrate that microplastic selects for unique bacterial assemblages in surface water of an urban river (McCormick et al. 2014) and marine sediment (Harrison et al. 2014). Using scanning electron microscope (SEM) images, Carson et al. (2013) described bacterial and diatom communities on microplastic, and Reisser et al. (2014) demonstrated marine microplastic supports a community of diatoms, bacteria, cyanobacteria, fungi, and invertebrates. The use of microplastic as a habitat may expand an organism's geographic range. For instance, the high abundance of microplastic in the North Pacific Subtropical Gyre increased the oviposition habitat available to the pelagic insect *Halobates sericeus* (Goldstein et al. 2012). The presence of microplastic-attached organisms may also increase the likelihood of consumer ingestion (Reisser et al. 2014). Additionally, the

'fouling' caused by microplastic-colonizing organisms may contribute to microplastic sinking to benthic habitats (Barnes et al. 2009).

The ecological effects of microplastic in marine habitats are well-documented, but data for freshwater systems are lacking (Wagner et al. 2014). Previous research demonstrates microplastic may influence biofilm taxa in freshwater habitats (McCormick et al. 2014), which form the base of aquatic ecosystems. Studies that compare microplastic biofilms to natural microbial habitats are needed which span a broader geographic range. These analyses will show if microplastic selects for a particular community of microorganisms across a different environmental conditions.

Rivers are important in AL and microplastic research

Studying the pollution of rivers by AL and microplastic is critical because rivers are essential resources that provide many ecosystem services. Surface freshwaters provide drinking water, transportation, electricity generation, pollution disposal, and irrigation (Wilson and Carpenter 1999; Aylward et al. 2005). In addition, freshwater environments provide ecosystem services with intrinsic value such as habitat for plants and animals and supporting biodiversity (Wilson and Carpenter 1999). In urban areas, rivers also provide micro-climate regulation and recreational and cultural value (Bolund and Hunhammar 1999). However, growing water demands associated with rising human population and development have caused significant changes to freshwater ecosystems (Aylward et al. 2005). Despite our intense use of rivers, they represent only 0.49% of global surface freshwater (USGS, www.water.usgs.gov/edu/earthwherewater.html). Maintaining ecological integrity of rivers and mitigating effects of anthropogenic pollution from AL and microplastic is critical. As rivers are often considered important

sources of AL and microplastic to marine habitats, effective mitigation strategies may involve preventing AL and microplastic disposal into rivers and within watersheds. Rivers are often easier to access than many marine environments, so targeting clean-up efforts at rivers rather than oceans may be more efficient for reducing the amount of AL entering other habitats.

Thesis objectives

In this thesis, I examined the ecological dynamics of AL and microplastic in urban streams. In Chapter 2, I quantified the density, mass, and composition of AL in riparian and benthic zones of 5 rivers spanning a gradient of percent urban land use. I also measured the dynamic nature of AL by measuring AL accumulation and export rates in 2 flux studies at biweekly and seasonal temporal scales. I predicted that AL density and mass would be related to the relative proportion of urban land use in a watershed. I also hypothesized that AL density would be higher in riparian habitats but that AL mass would be higher in benthic habitats. For the flux studies, I predicted that an item's weight would influence its mobility so that lightweight items (e.g., plastic bags, wrappers) would be the most mobile items in riparian zones. I also predicted that stream hydrology and flooding would influence AL accumulation and export.

In Chapter 3, I quantified microplastic concentrations in 9 streams from sites located upstream and downstream of WWTP effluent outfalls to determine if treated wastewater is a point source of microplastic to rivers. Using next-generation sequencing, I also analyzed the bacterial assemblages on microplastic and compared these communities to those on 3 natural habitats: organic material, upstream water column, and downstream water column. These research questions were addressed in a recently

published study in a single river (McCormick et al. 2014). This chapter was designed to examine if the patterns from that study were consistent if measured over a larger geographic scale. I predicted that WWTP effluent would be a point source of microplastic to urban rivers and that microplastic concentrations would be significantly higher downstream of WWTP effluent outfalls than upstream. I also hypothesized that bacteria assemblages on microplastic would differ from those on natural substrates.

Results of both chapters will inform policies aimed at reducing AL and microplastic accumulation in urban streams. Additionally, research on the ecology of riverine AL and microplastic is newly emerging, and this thesis will provide a baseline for future studies on the ecological implications of both forms of pollution in the context of the global AL 'life cycle.'

CHAPTER II

ABUNDANCE, COMPOSITION, SOURCES, AND MOVEMENT OF ANTHROPOGENIC LITTER IN URBAN STREAMS

Introduction

Increases in human population and technological innovation since the industrial revolution have altered global ecosystems through resource exploitation, urbanization, and agriculture (Zalasiewicz et al. 2008; Harden et al. 2014). The manufacturing and improper disposal of consumer products is a major human impact on ecosystems worldwide. For example, between 1950 and 2011, global plastic production increased from 1.7 to 280 million tons (Plastics Europe 2012) and a significant portion of plastic accumulates in the environment (Cózar et al. 2014). Anthropogenic litter (AL; trash) refers to the assemblage of all manufactured items that enter the environment (i.e., plastic, glass, metal, rubber, manufactured wood, paper), and it is a visually-conspicuous, long-lasting impact of human activity with significant ecological implications.

Interactions between AL and biota in the environment include entanglement, consumer ingestion, and enhanced dispersal of colonizing organisms. After becoming entangled in AL, organisms often die by drowning, strangulation, and/or reducing feeding efficiency (Moore 2008; Laist 1987). Ingesting AL can cause detrimental effects such as digestive system blockages, damage to stomach linings, and reduced feeding efficiency (Moore 2008; Ryan 1988; Laist 1987). Finally, AL represents a new mode of invasive

species dispersal in marine environments by serving as a raft for aquatic organisms (Barnes 2002). In addition to ecological consequences, AL is also associated with economic expenses such as losses to the tourism industry (Leggett et al. 2014) and costs for AL removal (Moore 2008).

A majority of AL research is focused on marine environments where AL has a broad geographic distribution and high density (Eriksen et al. 2014; Pham et al. 2014; Eriksen et al. 2014). Sources of marine AL include offshore sources such as dumping by ships and land-based sources such as littering by beachgoers, trash dumping, runoff, and rivers (Ryan et al. 2009; Williams and Simmons 1997; Abu-Hilal and Al-Najjar 2009). While many studies consider rivers a major AL source to marine ecosystems (Corcoran et al. 2009; Galgani et al. 2000; Ivar do Sul et al. 2011; Araujo and Costa 2007), few studies provide quantitative measurements on riverine AL abundance and composition (Williams and Simmons 1997; Williams and Simmons 1999; Rech et al. 2014; Hoellein et al. 2014). Many questions remain regarding AL in rivers, including the exchange of AL between riparian and benthic habitats, the influence of watershed land use on riverine AL abundance and composition, and AL export and accumulation rates at annual and seasonal scales.

Studying the pollution of rivers by AL is critical because rivers are essential resources that provide many ecosystem services, including drinking water, transportation, and recreational value (Wilson and Carpenter 1999; Aylward et al. 2005). As rivers are often considered important sources of AL to marine habitats, effective mitigation strategies may involve preventing AL disposal into rivers and within watersheds. Since rivers are often easier to access than many marine environments, targeting clean-up

efforts at rivers rather than oceans may be more efficient for reducing the amount of AL entering other habitats.

The goal of our study was to quantify the density, mass, and composition of AL in riparian and benthic zones of 5 rivers spanning a gradient of urban land use. We also evaluated the dynamic nature of AL by measuring AL accumulation and export rates in 2 flux studies at biweekly and seasonal temporal scales. We predicted that AL density and mass would be related to the relative proportion of urban land use in a watershed, and we hypothesized that AL density would be higher in riparian habitats but that AL mass would be higher in benthic habitats. For the flux studies, we predicted that an item's weight would influence its mobility so that lightweight items (i.e., plastic bags, wrappers) would be the most mobile items in riparian zones. We also expected that stream hydrology and flooding would influence AL accumulation and export.

Materials and Methods

Study sites

We measured AL abundance and composition in 5 streams in the Chicago metropolitan region, which includes northeastern Illinois and northwestern Indiana. Study sites spanned an urban land use gradient and had similar watershed sizes (Fitzpatrick et al. 2005; Table 1). AL was collected from the benthic habitat and adjacent riparian zone in 3 reaches of each river (N=15). Reaches were located in publically accessible areas, including county parks and other recreational areas (Table 1). Permission and permits were obtained from county organizations before commencement of the study.

Collection and categorization of benthic and riparian AL

We collected AL in June-October 2014 (summer-autumn), except 3 reaches sampled in 2013 (Table 1). Reach lengths were 50-100 m. In each reach, AL was collected from the entire benthic habitat, and from the riparian zone on one bank. We defined the riparian zone for this study as 10 m from the water's edge. For consistency, the riparian bank chosen for AL collection was the one used to access the stream (except for Hickory Creek at Hillcrest Road). For collection, we slowly moved along the reach in teams of 2-3, picking up all AL encountered. We have confidence in our estimates given the consistency with previous measurements (Hoellein et al. 2014), but note that some items may have been overlooked on the surface of the benthic habitat. Also, this method does not account for buried AL. However, any underestimates are equal across sites and dates, and establish our results as conservative. AL was transported to the lab in garbage bags labeled by collection site.

In the laboratory, AL was laid in a single layer on plastic sheets to air dry (~2-3 d) prior to counting, weighing, and categorizing each item. Dried dirt and debris were removed manually, and each AL item was weighed. We adapted a protocol from Cheshire et al. (2009) to categorize AL by material type, function, and most probable source. We classified AL into 11 material categories: ceramic, cigarettes, cloth, glass, metal, paper and cardboard, plastic, rubber, Styrofoam, wood, and 'other' (Table A1). We used a code to classify the item's function (e.g., cutlery, clothing, cups; Table A1). Finally, we characterized the item's most probable source using 6 categories: consumables, construction/industrial, recreation, domestic, fishing, and 'unknown.' Consumable were those materials associated with smoking, eating, and drinking, and

likely discarded by a person visiting the stream. Construction and industrial materials included pipes, manufactured wood, pallet wrap, and bricks. Recreation items were golf balls, tennis balls, and Frisbees. Items were classified as domestic if they originated from a home (e.g., kitchenware, appliances, and personal hygiene). We acknowledge the uncertainty of this source estimate. For example, an item classified as consumable (i.e., plastic food wrapper), may have originated from a domestic source via wind or dumping of household trash. However, this approach has been used elsewhere to infer dominant AL sources (Hoellein et al. 2015; Ivar do Sul et al. 2011; Santos et al 2009).

At all 15 reaches, we assessed anthropogenic activity in 4 ways: the presence and distance of a walking trail, the intensity of human activity, the number of parking spaces present, and the distance to a road (Table 2). We collected these data on the same date the reach was sampled at 11 of 15 sites (Table 1). Human activity data were collected at a later date than AL at Bunker Hill (Sep 16, 2014), Miami Woods (Sep 16, 2014), 26th Street Woods (Aug 4, 2014), and Pilcher Park (Sep 26, 2014). Park trail presence and distance was classified as near (<50 m), far (>50 m), or none. We classified the intensity of human activity by the number of people observed at the reach or on a nearby trail during the sampling period (~3 h) as low (no people), medium (1-10 people), or high (>10 people). To quantify parking, we counted all parking spaces in the lot closest to the reach. Four reaches had no parking, 3 in residential areas and 1 at a road intersection. We used the distance measuring tool on GoogleMaps to measure the distance from the sampled reach to the nearest road.

Riverine AL compared to marine benthic habitats and beaches

We compared our AL density, mass, and composition results to published studies in rivers, marine benthic habitats, and beaches. Variation in methods, categories, and AL units complicates comparison across studies. For example, AL density is often reported as the number of items collected per unit area in benthic analyses (No. m^{-2}), but as number of items per length of transect (No. m^{-1}) in terrestrial and beach studies (Hoellein et al. 2014). Relative abundance of AL is reported by material type (i.e., glass, plastic, metal) (Rech et al. 2014; Abu-Hilal and Al-Najjar 2009) or function (i.e., food-related, dumping activities, medical/personal) (Hoellein et al. 2015). To compare AL density and mass in this study to published values, we included studies that reported results in number of items or mass per unit area and used similar material classifications. Finally, we note marine studies commonly use 'fishing' to classify AL by material type (Pham et al. 2014, Schlining et al. 2013, Abu-Hilal and Al-Najjar 2009), and we included these studies in our comparison. However, we considered 'fishing' to be a source of AL rather than type of AL (i.e., we classified collected monofilament line or fishing buoys as plastic). AL data from ecosystems around the world were included in our comparisons. Studies for comparison included marine benthic habitats in seas (Abu-Hilal and Al-Najjar 2009; Stefatos et al. 1999; Galgani et al. 2000), the open ocean (Pham et al. 2014; Pham et al. 2013), and near-shore habitats (Debrot et al. 2014; Donohue et al. 2001; Oigman-Pszczol and Creed 2007; Hess et al. 1999). Beach studies included ocean coastlines (Whiting 1998; Rosevelt et al. 2013; Madzena and Lasiak 1997; Smith and Markic 2013), estuaries (Rech et al. 2014), oceanic islands (Eriksson et al. 2013), and lakes (Hoellein et al. 2014).

AL flux: study sites

We examined the movement of riparian zone AL at two temporal scales, seasonal (i.e., multiple times over the course of 1 year) and biweekly (i.e., every 2 weeks during summer), in two different riparian reaches of the North Branch of the Chicago River. The seasonal study was conducted at Bunker Hill Forest Preserve in Niles, IL, and the biweekly study was conducted at Miami Woods, in Morton Grove, IL. These 2 reaches were also among the 15 sites used in our characterization of AL and are Cook County Forest Preserves (Table 1).

AL flux: seasonal measurement

Our seasonal flux study measured the accumulation of AL and export of marked AL items from the riparian zone over the course of 1 year. In November 2013, all AL was cleared from a riparian quadrat (40 m length x 10 m width) directly adjacent to the water's edge. This set a 'blank slate' so that any AL collected on subsequent dates represented new accumulation. We measured net accumulation for 3 periods: winter/spring (Nov 26, 2013-Apr 25, 2014), summer (May 28-Sep 16, 2014), and fall (Sep 16-Dec 18, 2014). We did so by carefully searching the riparian quadrat and collecting all unmarked (i.e., new) AL. We considered collected material to be the net accumulation of AL during the sampling interval. Accumulated AL was taken to the laboratory for quantification and classification as described above.

At the same time we measured net accumulation, we measured export of marked AL items. To measure export, we selected 4 common AL categories: glass bottles, metal cans, plastic food containers/wrappers, and plastic bags. We marked 10 items from each category with spray paint and an identification number (N=40) (sensu Bowman et al.

1998; Williams and Simmons 1997). This density of items was typical of measurements collected from riparian zone sites. On the start dates for the 3 seasonal sampling periods, the 40 marked AL items were haphazardly distributed throughout the riparian quadrat. The coordinates of each item's starting location within the quadrat were recorded. At the end of each sampling period, we carefully searched the quadrat for the marked and numbered AL items. In addition, we searched ~100 m downstream and 30 m inland from the quadrat for marked items. We recorded whether each item remained in its starting location, moved within the quadrat, or was no longer in the quadrat (i.e., export). We established a new map for the locations of all marked AL items at the end of each sampling interval. Because a different color spray paint was used for each time period, some AL was tracked for the entire year. We removed all marked AL items in the quadrat after the final date of the study (Dec 18, 2014). All marked AL used in this study was originally collected from the study site or areas downstream, so this project represents no addition of new AL to the environment.

We calculated net accumulation and export rates from the collected data. We expressed net AL accumulation as No. items d^{-1} and No. items m^{-2} d^{-1}. We calculated AL export as the proportion of items lost per day and proportion of mass lost per day (d^{-1}). We calculated the net accumulation and export rates for each season, and the mean annual export rate across the 4 AL categories. We used the initial standing stock of AL, the mean annual accumulation rate, and the mean annual export rate to calculate the net flux of AL at our study site over the entire year [Equation 1].

Eq. 1 $$Flux = Net\ accumulation - (Export\ rate \times Initial\ AL\ standing\ stock)$$

We multiplied the mean export rate (d^{-1}) by the initial AL standing stock (No. m^{-2}). By subtracting this value from the net accumulation rate (No. m^{-2} d^{-1}), we estimated net annual flux of AL (No. m^{-2} d^{-1}). Finally, we calculated turnover time (d) for each AL type as the inverse of its mean export rate (d^{-1}). Turnover time represents the average time an item spends in the riparian habitat before being exported.

AL flux: biweekly measurement

We conducted an additional study to measure AL net accumulation and export in a riparian habitat over shorter time intervals. This study lasted 18 weeks during summer 2014 (Jun 2 – Oct 2). We used the same quadrat dimensions, types of AL, and methods for measuring net accumulation and monitoring the movement of marked AL as described above. We visited the site every ~2 weeks (mean (±SE) = 15.1 (±1.3) d). The only difference in methods for the biweekly study compared to the seasonal analysis was that we characterized 2 types of export. We noted if the item was out of the quadrat, but in the adjacent area ~30 m inland or ~100 m downstream (export: adjacent), or was not found (export: lost). To examine patterns between AL movement and stream hydrology, we obtained discharge data from the US Geological Survey (USGS) for the North Branch of the Chicago River for the study period (USGS National Water Information System No. 05536000).

The effect of sampling interval on AL accumulation rates

In our seasonal and biweekly flux studies, we measured net AL accumulation rates in sampling periods ranging from 8 to 149 days. We combined our data with results from Smith and Markic (2013, Figure 2 in that study) which showed that the temporal scale of AL sampling affects AL accumulation rates.

Data analysis

We used 2-way analysis of variance (ANOVA) to compare differences in total AL density and mass among streams and between habitats (riparian and benthic). We conducted additional 2-way ANOVAs for each of the 11 material categories individually. Significant ANOVA results ($p<0.05$) were followed by Tukey's multiple comparison test. When data did not meet the assumptions of ANOVA, we applied a natural log transformation, or $\ln(x+0.5)$ when appropriate. However, several variables could not be transformed to meet the homoscedasticity and normality assumptions of ANOVA, which appears to be common in AL datasets (Hoellein et al. 2015). For these variables, we used a nonparametric statistical approach and performed two Kruskal Wallis tests. One tested for differences among streams and the other between habitats. This nonparametric approach limited our ability to test for an interaction effect, however, we found no significant interactions between stream and habitat for variables analyzed with ANOVA. All ANOVAs, Tukey's tests, and Kruskal Wallis tests were completed in SYSTAT 13.0 (Systat, Inc. Chicago, IL).

We used a nonmetric multidimensional scaling (nMDS) approach to analyze differences in AL composition among streams and between habitats (sensu Rech et al. 2014, Pham et al. 2014). We calculated Bray-Curtis similarity indices on $\log(x+1)$ transformed AL relative composition data for abundance and mass. The distance matrix was visualized with nMDS ordinations. We determined whether there were differences in AL composition by site and habitat using analysis of similarities (ANOSIM). We calculated Bray-Curtis indices, nMDS coordinates, and ANOSIM analyses in Primer V.5 (Primer-E Ltd., Plymouth, United Kingdom). Finally, principal component analysis

(PCA) was used to analyze relationships between variables associated with the anthropogenic activity at each reach and AL density by material type. We performed 2 PCA analyses (benthic and riparian). PCA was performed in PC-ORD V.6 (McCune and Mefford 2011) using a correlation matrix because our data included both environmental and AL density variables with varying units of measurement (Clarke and Warwick 2001).

Table 1. Location and land use characteristics for the 15 study reaches in streams used for anthropogenic litter (AL) characterization.

Stream	Urban Land Use (%)	Pop. Density (No. km^{-2})	Water-shed area (km^2)	Reach	Function	Date Sampled	City	County (State)	Latitude, Longitude
Salt Cr.	73*	1236*	128*	26th St Woods	For. Pres.	28-Oct-13	Berwyn	Cook (IL)	41.84263, -87.85952
				Sleepy Hollow Park	Resid.	31-Jul-14	Elmhurst	DuPage (IL)	41.88092, -87.95849
				Bemis Woods	For. Pres.	4-Aug-14	W. Springs	Cook (IL)	41.82662, -87.91062
Turkey Cr.	53+	333+	105+	Hidden Lake	Co.Park	6-Jun-14	Merrillville	Lake (IN)	41.50357, -87.32773
				Broadway St	Commer.	6-Jun-14	Merrillville	Lake (IN)	41.50315, -87.33676
				Hidden Lake	Co. Park	7-Jun-14	Merrillville	Lake (IN)	41.50417, -87.33054
N. Br. Chi.R.	48*	572*	110*	Bunker Hill	For. Pres.	23-Sep-13	Niles	Cook (IL)	42.00044, -87.78357
				Miami Woods	For. Pres.	2-Jun-14	Morton Grove	Cook (IL)	42.02745, -87.79372
				LaBagh Woods	For. Pres.	30-Jul-14	Chicago	Cook (IL)	41.97802, -87.74271
Hickory Cr.	21*	352*	127*	Pilcher Park	Nat. Cent.	28-Oct-13	Joliet	Will (IL)	41.52624, -88.00703
				Hillcrest Rd	Resid.	26-Sep-14	Joliet	Will (IL)	41.52511, -88.04092
				Schoolhouse Rd	Intersect.	26-Sep-14	Joliet	Will (IL)	41.51699, -87.93331

Plum Cr.	8*	88*	85*	Plum Cr For. Pres.	For. Pres.	14-Aug-14	Beecher	Will (IL)	41.39317, -87.62436
				Goodenow Nat. Pres.	For. Pres.	14-Aug-14	Beecher	Will (IL)	41.40366, -87.60918
				Ridgeland Ave	Resid.	28-Sep-14	Chicago Heights	Cook (IL)	41.48271, -87.53194

* indicates data were obtained from Fitzpatrick et al. 2005. + indicates data were obtained from Northwestern Indiana Regional Planning Commission 2012. Abbreviations: Cr=creek, N=north, Br=branch, Chi=Chicago, R=river, Pop=population, St=street, Rd=road, For=forest, Pres=preserve, Resid=residential, Co=county, Commer=commercial, Nat=nature, Cent=center, Intersect=intersection.

Table 2. Anthropogenic activity for the 15 study reaches in streams used for anthropogenic litter (AL) characterization.

Reach	Distance to trail (m)	Parking spaces	Distance to road (m)	Activity Observed
26th St Woods	43	40	93	Frequent walkers, cyclists
Sleepy Hollow	7	na	32	Moderate walkers
Bemis Woods	45	140	134	Little observed
Hidden Lake	na	105	27	Fishing, walking, vehicle traffic
Broadway St	na	130	140	Industrial employees
Hidden Lake	33	100	211	Recreational (sports fields)
Bunker Hill	121	250	229	Frequent walkers, cyclists
Miami Woods	20	180	230	Frequent walkers, cyclists
LaBagh Woods	30	200	154	Little observed
Pilcher Park	na	40	44	Little observed
Hillcrest Rd	na	na	20	Little observed
Schoolhouse Rd	na	na	5	Vehicle Traffic
Plum Cr Forest Pres	62	137	823	Little observed
Goodenow Nat. Pres	133	100	237	Little observed
Ridgeland Ave	na	na	20	Vehicle Traffic

na indicates that no trail or parking lot was present. Abbreviations: Cr=creek, St=street, Rd=road, Pres=preserve, Nat=nature.

Results

AL abundance across streams and between habitats

Total AL density (No. m^{-2}) was significantly different among sites (2-way ANOVA, p=0.006; Figure 1A; Table 3), where the 3 most urbanized watersheds had the highest AL densities (Figure 1A), and the two less urbanized watersheds had lower AL densities. There was no significant difference in AL density between riparian and benthic zones (2-way ANOVA, p=0.120; Figure 1A; Table 3), but there was a pattern of higher AL density in the riparian zone compared to the benthic zone at all sites except Plum Creek (Figure 1A). Total AL mass (g m^{-2}) was highest at Turkey Creek and similar among the other 4 sites (2-way ANOVA, p=0.005; Figure 1B; Table 3). Benthic habitats had significantly greater AL mass than riparian zones (2-way ANOVA, p<0.001; Figure 1B; Table 3). Additionally, there was no correlation between land use and riparian AL density (r=0.48, p=0.072) or mass (r=0.20, p=0.479). There was also no relationship between land use and benthic AL density (r=0.33, p=0.232) or mass (r=0.15, p=0.599).

When considered by material type, AL density was variable among streams and between habitats. Plastic density was significantly greater in the riparian zone (2-way ANOVA, p=0.002; Table 3) and different among sites (2-way ANOVA p=0.002; Table 3). Styrofoam and paper were also more abundant in the riparian zone, but there were no differences among sites (Table 3). Ceramic density was higher in the stream benthic habitats, but did not differ among sites (Table 3). In contrast, rubber and cloth densities were similar between habitats, but variable among sites (Table 3). Finally, metal, glass, wood, cigarette, and 'other' AL densities did not differ between habitats or among sites (Table 3).

Patterns for the mass of each AL category were variable among streams and between habitats. In general, the heaviest AL types had higher mass in benthic habitats, including rubber (Kruskal Wallis, p=0.039), metal (2-way ANOVA, p=0.003), and ceramic (2-way ANOVA, p=0.005; Table 3). In contrast, the mass of paper was greater in the riparian zone (Kruskal Wallis, p=0.010; Table 3). Rubber and cloth mass were the only types that differed among sites (Table 3). Finally, the mass of plastic, Styrofoam, glass, wood, cigarettes, and 'other' did not differ between habitats or among sites (Table 3).

Relative AL composition among streams and between habitats

While riparian zones showed a trend of greater AL density (Figure 1A) than benthic zones, a significant proportion of the AL assemblage consisted of light-weight materials such as plastic and Styrofoam (Table 4). For example, the relative abundance of plastic, which consisted largely of food packaging and plastic bags, was higher in the riparian zone (48-65%) than in the river benthic zones (21-46%; Table 4). Benthic habitats had generally lower AL density (Figure 1A), but heavier items such as metal, wood, and ceramic had greater relative abundance than in riparian zones (Table 4). For example, metal and ceramic accounted for an average of 28% and 21% of the mass in benthic habitats, respectively, but 14% and 6% of the mass in riparian habitats (Table 4).

We calculated Bray-Curtis similarity indices for AL assemblages based on relative composition of AL density and mass. There was substantial overlap of AL assemblages based on composition by density (Figure 2A), with no significant dissimilarity among streams (ANOSIM, R=0.084, p=0.140; Figure 2A) or between habitats (ANOSIM, R=0.133, p=0.139; Figure 2A). One riparian reach at Plum Creek

(coordinates 2.03, -1.81; Figure 2A) strongly influenced the comparison. This site had a very low AL density, and half of the items were manufactured wood, a generally uncommon material in riparian sites. The site also lacked many of the AL types typical of other riparian zones such as glass, metal, paper, and Styrofoam (Table 4).

When comparing relative AL composition by mass, there was marginal dissimilarity between habitats (ANOSIM, R=0.267, p=0.027), but no differences among streams (ANOSIM, R=0.036, p=0.321; Figure 2B). One riparian reach in Turkey Creek (coordinates -0.03, -2.39) and one in Plum Creek (coordinates -2.52, 0.71) are distinct on the nMDS ordination (Figure 2B). This Plum Creek reach is also distinct in the density nMDS ordination (Figure 2A). At the Turkey Creek reach, 3 tires accounted for >96% of the AL mass, so the relative contribution of rubber at this site was much higher than the other sites (Table 4).

Comparing AL by most probable source showed differences between benthic and riparian habitats. A higher proportion of AL in stream benthic habitats came from construction and industrial sources than in riparian zones (Figure 3). This category included manufactured wood, metal, ceramic (i.e., bricks and cinderblocks), and other building materials. In contrast, riparian habitats consisted of a higher relative abundance of AL from consumable goods associated with on-site littering (Figure 3). All recreation materials collected for this study were golf balls, and they were more abundant in the benthic than riparian zones (Figure 3). AL items associated with fishing were uncommon at all of the study sites (Figure 3).

Anthropogenic factors influencing the AL density

We examined relationships between 4 variables related to anthropogenic activity and the density of our 11 AL categories using principal components analysis (PCA), with a separate PCA for benthic and riparian AL densities. For benthic density, the first component of the PCA (PC1) explained 27.22% of the variation (Table 5) and was positively related with 3 measures of anthropogenic activity (number of parking spaces, intensity of activity, and proximity of a trail) as well as all AL types (except ceramic, cigarettes, and 'other'; Table 6; Figure 4A). The second component (PC2) explained 19.45% of the variation, and had a negative relationship with 3 measures of anthropogenic activity (the number of parking spaces, distance to a road, and proximity of a trail) (Table 5; Table 6). PC2 was negatively related to Styrofoam density, and positively related to densities of ceramic, glass, metal, rubber, and wood (Table 6; Figure 4A). Finally, PC3 showed no significant relationship with any human activity variables (Table 6). We note the heaviest items such as metal, rubber, and wood were clustered on the PCA diagram, and ceramic density was uncorrelated with any anthropogenic activities (Figure 4A).

In riparian habitats, PC1 explained 34.62% of the variation in the data (Table 5) and was negatively related to human activity (number of parking spaces and intensity of activity) and AL densities for all categories (except for ceramic, cigarettes, and wood; Table 6; Figure 4B). In contrast, all 4 human activity characteristics showed a significant positive relationship with PC2, which explained 16.65% of the variation in the data (Table 5; Table 6). However, few AL categories were related to PC2 (ceramics and metal had a negative relationship and wood a positive relationship; Table 6; Figure 4B).

Finally, PC3 showed a negative relationship with the number of parking spaces and the distance to a road, and a positive relationship with paper and wood density in the riparian zone (Table 6). Vectors for plastic, rubber, glass, cloth, metal, and 'other' were clustered in the PCA diagram (Figure 4B). Like for benthic density, vectors for Styrofoam and paper densities were related to the number of parking lots and intensity of human activity, while ceramic had a distinct negative relationship with all 4 anthropogenic variables (Figure 4B).

AL density, mass, and composition across ecosystem types

The density of AL at our riparian sites was within the range reported in the literature for marine beaches, however, AL density in the stream benthic zone was higher than most data from marine benthic environments (Table 7). Mean (±SE) riparian AL density was 0.293 (±0.076) items m^{-2}, approximately the median of results assembled from other aquatic-terrestrial transitional habitats (Table 7). In contrast, mean (±SE) benthic AL density of 0.117 (±0.021) items m^{-2} was at least 1 order of magnitude higher than measurements in the marine benthic habitats (Table 7). The only exception was marine AL density in the Gulf of Aqaba (Red Sea) which showed a mean (±range) of 2.8 (±0.9-5.9) items m^{-2} (Table 7; Abu-Hilal and Al-Najjar 2009). Far fewer studies report AL in units of mass, yet our results for benthic and riparian habitats were consistent with the range reported in the literature from ocean sites (Table 8).

While AL density is variable within sites, among locations in the same region, and among sites in different parts of the world, several trends emerge when comparing the relative abundance of AL among published studies. For example, the abundance of metal in our benthic and riparian habitats (18% and 9%, respectively) was comparable to

the proportion of metal in marine benthic studies (range=3-27%) but higher than metal abundance in all but one beach (range=0-35%; Figure 5). The relative abundance of glass at our study sites was higher than all other studies except 2 beaches (Figure 5). While plastic was a major component of AL assemblages in rivers (range=30-55%) and marine benthic sites (range=19-64%), beaches were more likely to be dominated by plastic (range=32-95%; Figure 5). Styrofoam was uncommon in marine benthic sites (range=0-1%; Figure 5), relatively rare in benthic riverine habitats (range=3-8%; Table 4) and riparian zones (range =3-15%; Table 4) at our study sites, and more likely to be common on beaches (range=0-41%; Figure 5). Finally, an important difference in AL composition between the marine benthic zones and other habitats was the prevalence of fishing items (Figure 5).

We calculated Bray-Curtis similarity indices for AL assemblages in marine benthic habitats, rivers, and beaches worldwide and visualized the results in an nMDS ordination. There were differences in AL composition among ecosystems (ANOSIM, R=0.351, p=0.002; Figure 6). AL assemblages from marine benthic habitats clustered together on the nMDS ordination, while beaches showed variation in AL composition (Figure 6). Most riverine benthic habitats and riparian assemblages clustered together and within the marine beach sites, except for the Taff River (Williams and Simmons 1999) which was similar to marine benthic habitats (Figure 6).

Seasonal flux: net accumulation and export rates

Patterns of net accumulation and export revealed that AL was highly mobile. Across the 3 seasonal intervals, mean (±SE) net accumulation of AL was 1.1140 (±0.2193) items d^{-1} or 0.0028 (±0.0005) items m^{-2} d^{-1}. Mean (±SE) export rate for the AL

types combined was 0.3794 (±0.0230) % d^{-1}, and was higher in spring and summer relative to fall (Table 9). Across AL types, there were no significant differences in export rates (1-way ANOVA, p=0.061). The mean (±SE) turnover time among the 4 AL types was 264 (±41) d, where aluminum cans had the shortest (197 d), and glass and plastic wrappers the longest (330 and 368 d, respectively) turnover times (Table 10). This suggests all 4 AL types are likely to leave the study reach within 1 year.

Using the original density of AL in the reach (0.9883 items m^{-2}), we calculated annual AL flux from this riparian zone site with the following calculation:

$$Flux = Net\ accumulation\ rate - Export\ rate$$

$$= 0.002785\ No.m^{-2}d^{-1} - (0.9883\ No.m^{-2})(0.003793\ d^{-1})$$

$$= -0.000964\ No.m^{-2}d^{-1}$$

Thus, the AL net flux from the study quadrat was -0.000964 items m^{-2} d^{-1}. This is consistent with a mean turnover time of 264 d (i.e., <1 y). Scaled to the quadrat dimensions (400 m^2) over the course of the year, the total export was 547 items y^{-1}, net accumulation was 407 items y^{-1}, and the flux was a net loss of -141 items y^{-1}.

Biweekly flux: net accumulation and export rates

To complement our annual flux assessment, we measured net accumulation and export over shorter time scales. At a biweekly scale, net AL accumulation rates in the riparian zone was 0.8 - 9 items d^{-1} (mean (±SE) = 3.435 (±1.050) items d^{-1} and 0.009 (±0.003) items m^{-2} d^{-1}). The biweekly accumulation rates were higher than those from the seasonal study (Table 9). Plastic and glass dominated AL input (Figure 7), and glass was typically in the form of broken bottles. There was no clear relationship between the river discharge and changes in input rates or relative AL composition (Figure 7).

Export of AL was related to material type, river discharge, and proximity of each item to the river's edge. After 15 d, 100% of glass bottles, 60% of metal cans, 80% of plastic wrappers, and 70% of plastic bags remained in their original location (Figure 8). After 36 d, however, 30% of glass bottles, 20% of metal cans, 50% of plastic wrappers, and 50% of plastic bags were in their original locations (Figure 8). From that date onwards, the number of stationary items was relatively constant (Figure 8). Overall, glass and metal were more frequently exported from the quadrat than plastic wrappers and bags (Figure 8). Exported plastic wrappers and bags that moved were commonly exported nearby (i.e., export: adjacent; Figure 8C, D), while glass and metal were lost. Many of the plastic items accumulated in a debris dam ~20 m inland from the study quadrat. The highest river discharge occurred at the end of June, corresponding to the period of greatest AL movement (Figure 8).

Movement of AL revealed the influence of flooding on export. The third of the quadrat closest to the water's edge had the lowest proportion of stationary AL items (Figure 9). By the second sampling date (36 d), only 7% of items were in their original location for this area, and all AL was gone by the end of the study (Figure 9A). In contrast, for the AL items in the middle (3.3-6.7 m) and inland (6.7-10 m) sections of the quadrat, 50% and 54% of items, respectively, remained in their original locations after 36 d (Figure 9B, C). In addition, items in the middle and inland sections were more likely to remain in the vicinity of the quadrat when exported (export: adjacent), while items exported in the section near the water's edge were much less likely to be recovered nearby (export: lost; Figure 9).

We calculated AL flux for the summer using biweekly data, the original AL density in the reach (0.037 items m^{-2}), and Eq 1. :

$$Flux = 0.008588\ No.m^{-2}d^{-1} - (0.037\ No.m^{-2})(0.005328\ d^{-1})$$

$$Flux = 0.008391\ No.m^{-2}d^{-1}$$

The effect of sampling interval on AL accumulation rates

Previous research suggests there is a relationship between accumulation rate (No. m^{-2} d^{-1}) and sampling interval (Smith and Markic 2013). We used a power function to quantify this relationship for our seasonal and bi-weekly flux results (R^2=0.559, p=0.005; Figure 10). In addition, we combined these data with similar measurements for a beach in Australia (Figure 2 from Smith and Markic 2013). The relationship was significant with all data combined (R^2=0.888, p<0.001; Figure 10).

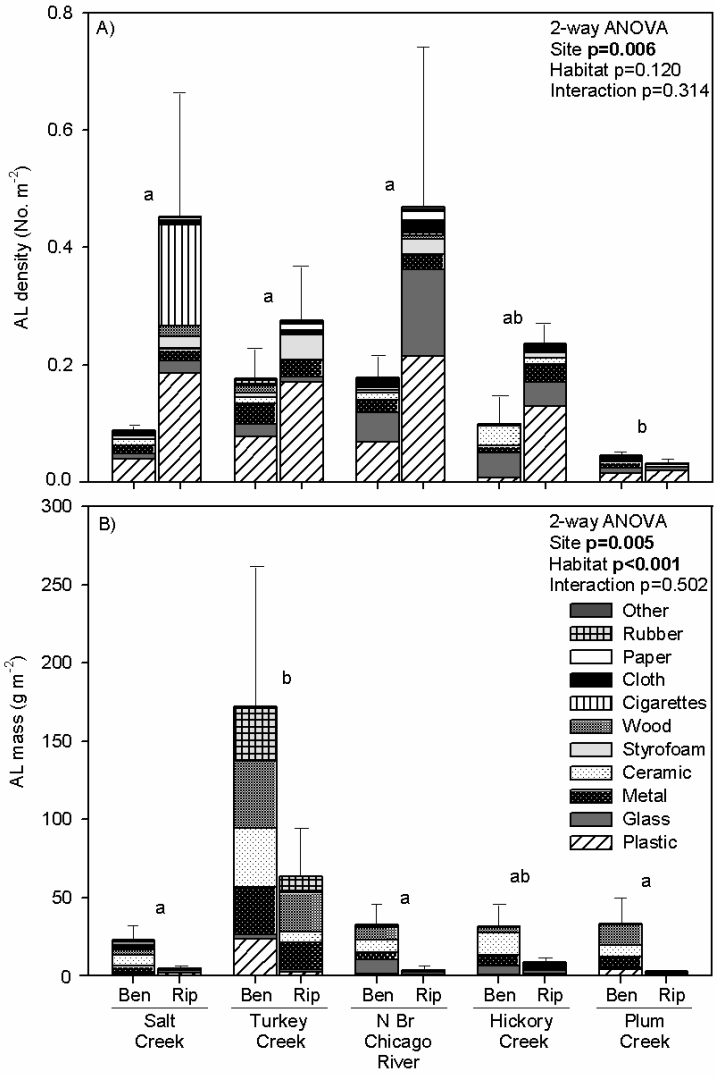

Figure 1. Mean (±SE) density (A) and mass (B) of all anthropogenic litter (AL) in 5 streams and 2 habitats (river benthic and riparian zones). Sites are arranged from high to low proportion of urban land use. Each bar section represents the mean density or mass of that category (N=3). Letters indicate a difference among sites using Tukey's test following a significant 2-way ANOVA ($p \leq 0.05$).

Table 3. Statistical results comparing anthropogenic litter (AL) density and mass by habitat (riparian and benthic) at 5 streams (site).

		Density		Mass				Density		Mass	
AL type	Factor	Test Stat.	p-value	Test Stat.	p-value	AL type	Factor	Test Stat.	p-value	Test Stat.	p-value
Total*	Habitat	2.64	0.120	17.43	**<0.001**	Paper	Habitat	6.85#	**0.009**	6.60#	**0.010**
	Site	4.94	**0.006**	5.19	**0.005**		Site	5.56#	0.235	5.88#	0.208
	Interaction	1.27	0.314	0.87	0.502		Interaction	-	-	-	-
Plastic*	Habitat	12.25	**0.002**	3.33	0.083	Cloth	Habitat	2.80#	0.095	0.74#	0.391
	Site	6.35	**0.002**	1.71	0.188		Site	13.81#	**0.008**	10.05#	**0.040**
	Interaction	2.50	0.075	1.54	0.228		Interaction	-	-		
Rubber	Habitat	1.71#	0.191	4.25#	**0.039**	Glass†	Habitat	0.01#	0.917	3.54	0.075
	Site	14.84#	**0.005**	12.03#	**0.017**		Site	8.38#	0.079	1.42	0.262
	Interaction	-	-	-	-		Interaction	-	-	0.76	0.564
Metal	Habitat	0.39	0.538	11.46+	**0.003**	Wood†	Habitat	0.19#	0.660	2.76	0.112
	Site	2.14	0.113	2.09+	0.120		Site	3.56#	0.468	0.22	0.925
	Interaction	0.83	0.524	0.121+	0.973		Interaction	-	-	0.23	0.920
Ceramic†	Habitat	6.54#	**0.011**	10.17	**0.005**	Cig.	Habitat	2.43#	0.119	2.28#	0.131
	Site	5.02#	0.285	0.89	0.487		Site	2.83#	0.587	2.45#	0.654
	Interaction	-	-	0.09	0.986		Interaction	-	-	-	-
Styro.	Habitat	6.97	**0.016**	2.44#	0.118	Other	Habitat	1.47#	0.226	3.71#	0.054
	Site	1.91	0.148	4.59#	0.332		Site	3.57#	0.467	2.12#	0.715
	Interaction	1.11	0.379	-	-		Interaction	-	-	-	-

Test statistics represent ANOVA F-ratio unless denoted with # which denotes the KW Test Statistic from the non-parametric Kruskal-Wallis test. *ln(x) transformation; †ln(x+0.5) transformation.

Table 4. Anthropogenic litter (AL) composition by density and mass for benthic and riparian habitats for the 5 study streams (n=3 reaches per stream).

	Benthic					Riparian				
	Salt Cr	Turkey Cr	N Br Chi R	Hickory Cr	Plum Cr	Salt Cr	Turkey Cr	N Br Chi R	Hickory Cr	Plum Cr
Density										
Ceramic	12.5	8.3	5.1	28.3	7.5	0.1	0.4	0.3	5.2	0.7
Cigarettes	0.0	0.0	0.2	0.5	2.2	19.9	0.3	0.7	0.0	0.7
Cloth	1.3	0.9	4.5	0.5	0.0	1.3	0.8	9.7	3.4	0.0
Glass	10.4	10.0	27.0	27.7	18.2	5.4	3.4	21.7	16.6	12.0
Metal	14.3	22.2	11.1	16.0	27.1	7.0	7.8	6.7	12.7	9.9
Other	0.9	2.4	0.4	1.4	4.5	0.2	0.4	0.6	1.3	0.0
Paper	0.0	0.3	1.6	0.0	0.0	1.5	3.7	3.9	1.3	0.0
Plastic	45.9	42.9	44.0	20.8	30.8	47.8	65.2	48.7	55.2	57.2
Rubber	3.2	3.7	1.0	0.5	0.0	0.2	2.3	0.2	0.0	0.0
Styrofoam	8.4	4.0	2.6	3.2	3.7	6.8	14.5	5.6	3.5	2.8
Wood	3.1	5.2	2.4	1.1	6.0	9.7	1.3	1.9	0.8	16.7
Mass										
Ceramic	19.5	19.7	20.0	32.0	12.1	0.0	18.4	0.3	10.8	0.3
Cigarettes	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0
Cloth	7.9	0.1	2.7	0.1	0.0	9.3	3.2	21.6	17.2	0.0
Glass	9.7	2.2	33.7	11.2	6.6	31.9	3.8	31.2	21.0	12.5
Metal	23.3	40.8	10.8	31.5	32.7	6.7	16.4	16.2	11.9	19.4
Other	4.2	1.0	0.2	2.0	6.0	0.7	0.1	1.4	10.5	0.0
Paper	0.0	0.0	0.1	0.0	0.0	1.3	0.1	6.9	0.2	0.0
Plastic	8.1	12.6	13.1	4.0	19.6	36.1	5.6	20.6	23.5	34.4

Rubber	11.4	10.3	0.6	0.5	0.0	0.2	32.2	0.3	0.0	0.0
Styrofoam	0.1	0.0	0.0	0.2	0.0	0.5	0.3	0.6	0.3	0.1
Wood	15.9	13.3	18.8	18.5	23.0	12.6	19.8	0.9	4.7	33.3

Abbreviations: Cr=creek, R=river

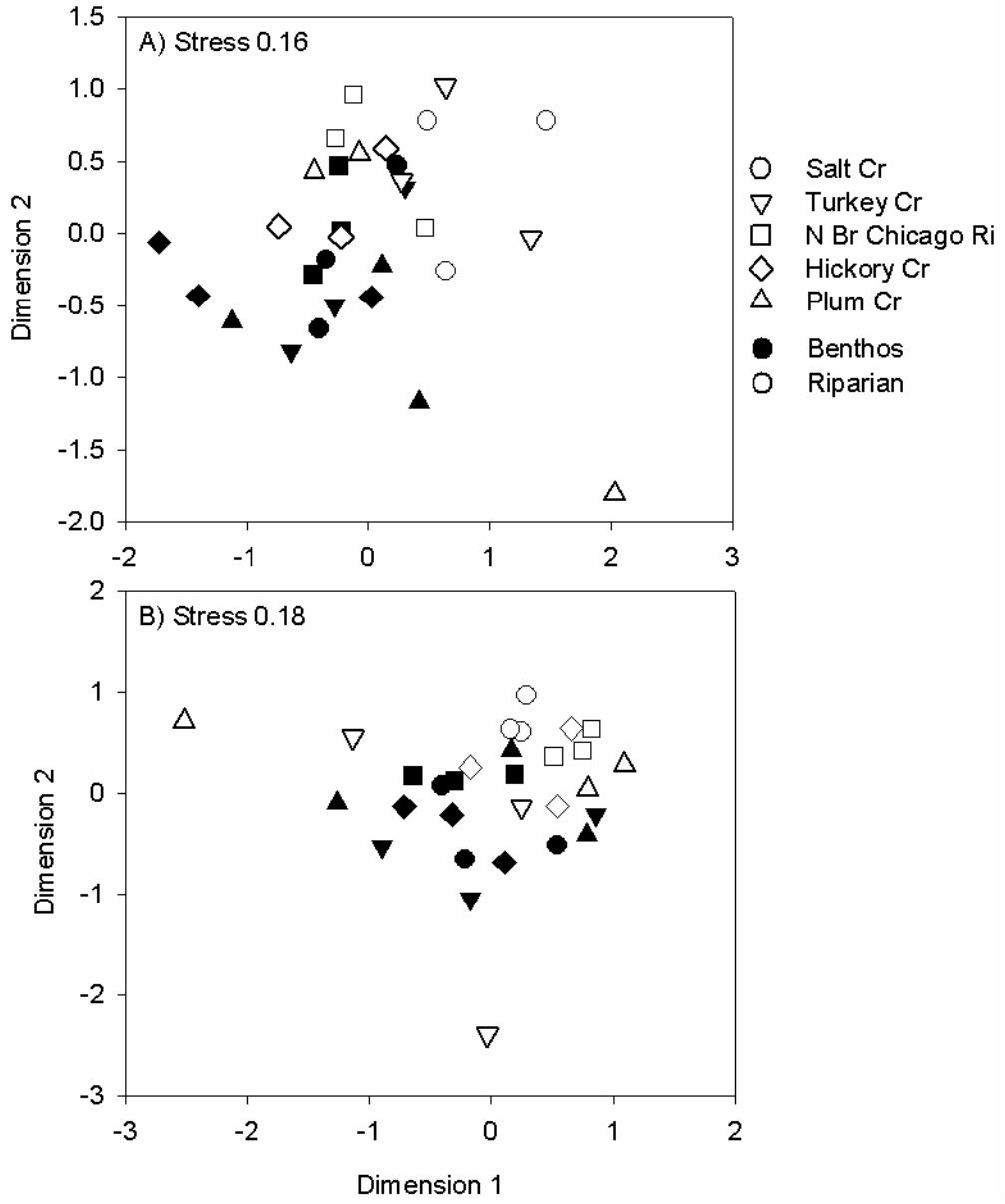

Figure 2. Non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarity index of anthropogenic litter (AL) composition in 5 streams and 2 habitats based on relative abundance (A) and relative mass (B) of 11 AL categories. Relative composition results for AL abundance and mass were log(x+1) transformed.

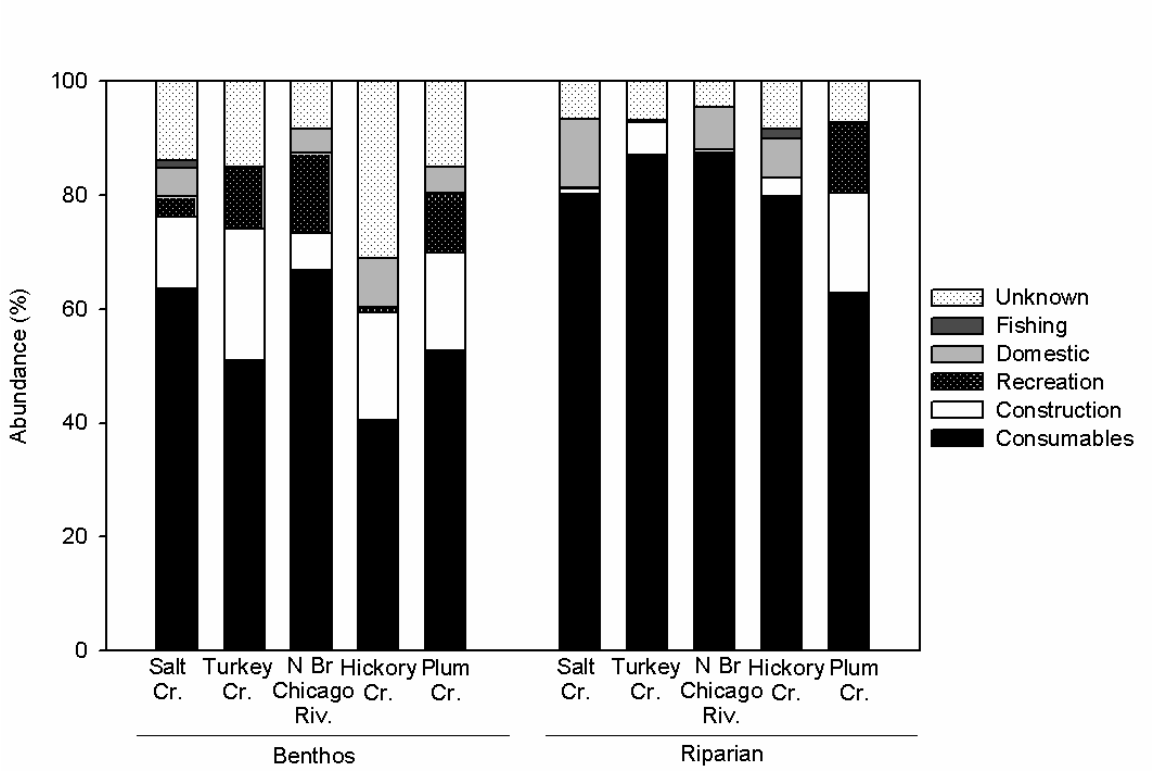

Figure 3. Relative proportion of probable sources contributing to anthropogenic litter (AL) at 5 study sites, separated by habitat.

Table 5. Contribution of first 3 principal components for explaining variation in anthropogenic litter (AL) density by habitat type.

Density, benthic			*Density, riparian*		
Axis	Variation (%)	Cumulative variation (%)	Axis	Variation (%)	Cumulative variation (%)
1	27.22	27.22	1	34.62	34.62
2	19.45	46.66	2	16.65	51.27
3	13.81	60.48	3	12.13	63.40

Table 6. Correlation coefficients for anthropogenic litter (AL) abundance and site characteristics for principal components (PCs) 1, 2, and 3. Correlation coefficients are considered significant at ≥0.3 and ≤-0.3, which are marked in bold.

	Benthic			Riparian		
	PC1	PC2	PC3	PC1	PC2	PC3
Site characteristics						
Parking	**0.597**	**-0.495**	0.183	**-0.554**	**0.563**	**-0.426**
Road	-0.048	**-0.569**	-0.088	0.093	**0.490**	**-0.602**
Activity	**0.519**	-0.153	-0.296	**-0.491**	**0.441**	0.118
Trail	**0.314**	**-0.727**	0.195	-0.018	**0.855**	0.298
AL abundance						
Ceramic	-0.125	**0.711**	**0.490**	-0.051	**-0.763**	0.193
Cigarettes	-0.234	0.280	0.068	-0.046	0.079	0.457
Cloth	**0.554**	0.065	**0.465**	**-0.896**	-0.065	-0.086
Glass	**0.335**	**0.485**	**0.712**	**-0.847**	-0.069	-0.192
Metal	**0.826**	**0.393**	-0.076	**-0.771**	**-0.352**	0.336
Other	0.247	-0.108	**-0.418**	**-0.856**	-0.168	-0.274
Paper	**0.375**	-0.251	**0.631**	**-0.368**	0.124	**0.497**
Plastic	**0.940**	-0.098	-0.075	**-0.891**	0.012	0.121
Rubber	**0.721**	**0.373**	**-0.441**	**-0.773**	-0.089	-0.095
Styrofoam	**0.376**	**-0.551**	0.240	**-0.506**	0.212	0.238
Wood	**0.663**	**0.540**	**-0.320**	0.059	**0.444**	**0.607**

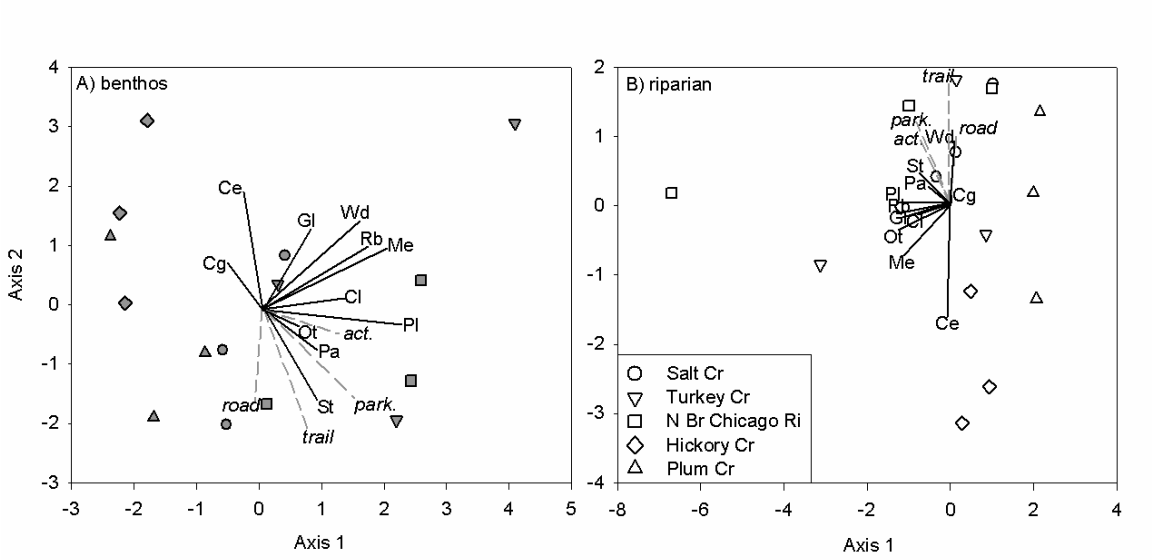

Figure 4. Principal component analysis (PCA) of site characteristics (presence and distance of a trail, number of parking spaces, distance to a road, and level of human activity) (gray, dashed lines) and anthropogenic litter (AL) abundance at the 15 sampling sites. Abbreviations: park.=parking, act.=activity, Ce=ceramic, Cg=cigarettes, Cl=cloth, Gl=glass, Me=metal, Pa=paper and cardboard, Pl=plastic, Rb=rubber, St=Styrofoam, Wd=wood, Ot=other.

Table 7. Published anthropogenic litter (AL) densities for worldwide benthic and aquatic-land transitional habitats.

Location	Ecosystem	Habitat	N	Measurement	AL Density (No. m^{-2})	Source
Benthic habitats						
Combined study sites	River	Benthic	15	Mean (±SE)	0.117 (0.021)	*This study*
N. Br. Chicago R., USA	River	Benthic	3	Mean (±SE)	0.076 (0.018)	Hoellein et al. 2014
Gulf of Aqaba, Red Sea	Marine	Benthic	6	Mean (Range)	2.8 (0.9-5.9)	Abu-Hilal and Al-Najjar 2009
Mediterranean Sea	Marine	Benthic	2	Mean	0.000165	Stefatos et al. 1999
Caribbean Islands	Marine	Benthic	24	Mean (Max)	0.0027 (0.0046)	Debrot et al. 2014
Condor Seamount, PT	Marine	Benthic	NR	Mean	0.00098	Pham et al. 2013
NW Hawaiian Islands	Marine	Benthic	2	Mean (Range)	0.000033	Donohue et al. 2001
European Seas	Marine	Benthic	18	Range	0-0.101	Galgani et al. 2000
Atlantic Ocean	Marine	Benthic	21	Range	0.0003-0.0032	Pham et al. 2014
Mediterranean Sea	Marine	Benthic	10	Range	0.0004-0.0032	Pham et al. 2014
Arctic Ocean	Marine	Benthic	1	Mean	0.00136	Pham et al. 2014
Armacao dos Buzios, BR	Marine	Subtidal	10	Mean (Range)	0.029 (0.003-0.065)	Oigman-Pszczol and Creed 2007
Aquatic-terrestrial transitional zones						
Combined study sites	River	Riparian	15	Mean (±SE)	0.293 (0.076)	*This study*
N. Br. Chicago R., USA	River	Riparian	3	Mean(±SE)	0.095 (0.017)	Hoellein et al. 2014
Lake Michigan, USA	Lake	Beach	3	Mean(±SE)	0.007(0.002)	Hoellein et al. 2014
Lake Michigan, USA	Lake	Beach	5	Mean(±SE)	0.009 (0.005)	Hoellein et al. 2015
Sea of Japan, Japan	Marine	Beach	18	Mean (Range)	3.41 (0.46-12.72)	Kusui and Noda 2003
Sea of Japan, Russia	Marine	Beach	8	Mean	0.21	Kusui and Noda 2003
Gulf of Aqaba, Red Sea	Marine	Beach	3	Mean (Range)	4.51 (1.64-7.38)	Abu-Hilal and Al-Najjar 2004
Israel	Marine	Beach	6	Range	0.03-0.88	Bowman et al. 1998
Monterey Bay, USA	Marine	Beach	12	Mean (Range)	1 (0.03-17.1)	Rosevelt et al. 2013

Charlesworth Bay, AU	Marine	Beach	1	Standing stock	0.24	Smith & Markic 2013
Armacao dos Buzios, BR	Marine	Beach	10	Mean (Range)	0.138 (0.233-0.034)	Oigman-Pszczol and Creed 2007
Curacao, West Indies	Marine	Beach	5	Mean (±SD)	0.365 (0.410)	Nagelkerken et al. 2001

USA=United States, PT=Portugal, BR=Brazil, AU=Australia, NR = not reported.

Table 8. Published anthropogenic litter (AL) mass for worldwide benthic and aquatic-land transitional habitats.

Location	Ecosystem	Habitat	N	Measurement	AL Mass (g m^{-2})	Source
Benthic habitats						
Combined study sites	River	Benthic	15	Mean(±SE)	58.40 (16.74)	*This study*
N. Br. Chicago R., USA	River	Benthic	3	Mean(±SE)	13.43 (0.65)	Hoellein et al. 2014
Gulf of Aqaba, Red Sea	Marine	Benthic	6	Mean (Range)	310 (60-1060)	Abu-Hilal and Al-Najjar 2009
Aquatic-terrestrial transitional zones						
Combined study sites	River	Riparian	15	Mean(±SE)	16.74 (8.20)	This study
N. Br. Chicago R., USA	River	Riparian	3	Mean(±SE)	18.04 (5.10)	Hoellein et al. 2014
Lake Michigan, USA	Lake	Beach	3	Mean(±SE)	0.20 (0.12)	Hoellein et al. 2014
Curacao, West Indies	Marine	Beach	5	Mean(±SD)	187 (532)	Nagelkerken et al. 2001
Sea of Japan, Japan	Marine	Beach	18	Mean (Range)	21.4 (1.4-73.3)	Kusui and Noda 2003
Sea of Japan, Russia	Marine	Beach	8	Mean (Max)	13.4 (46.9)	Kusui and Noda 2003

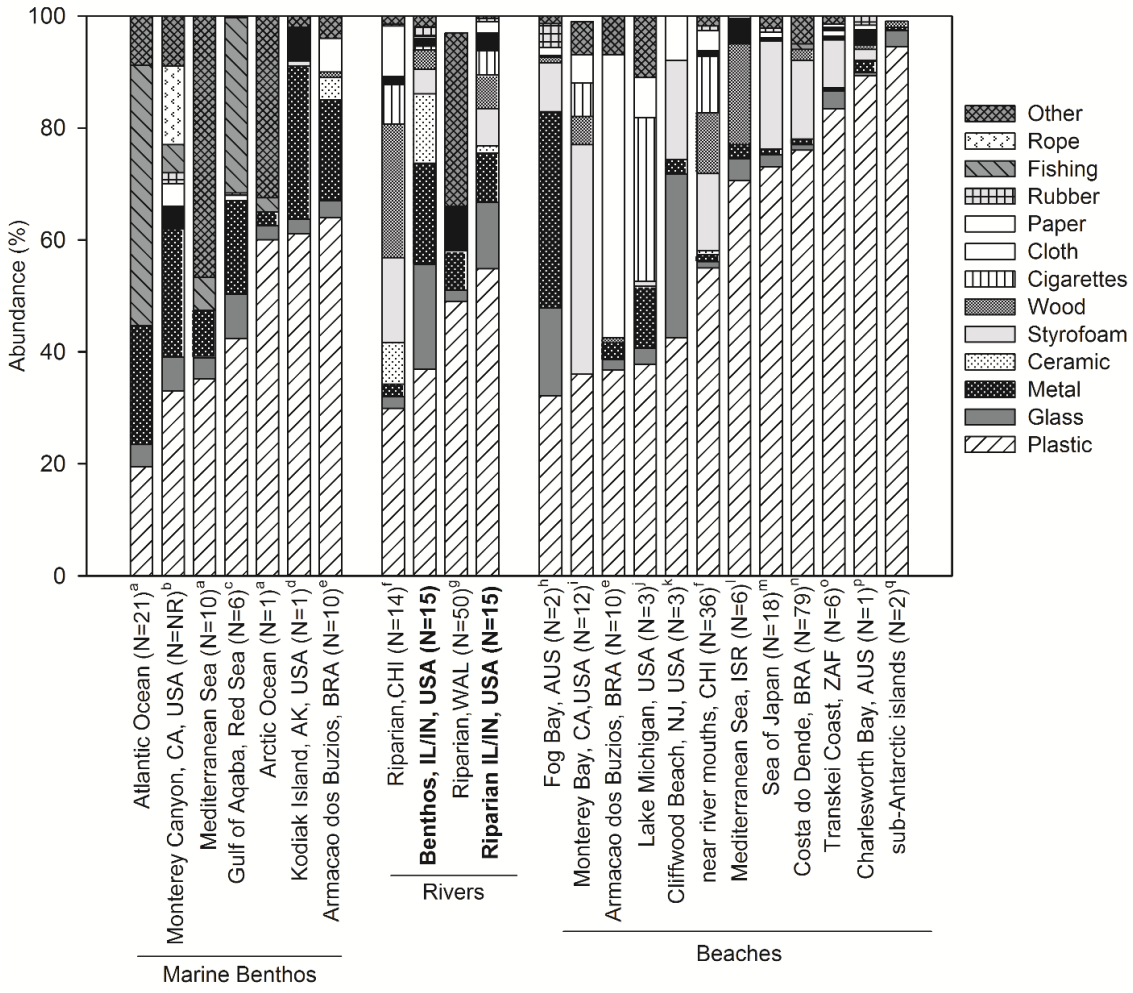

Figure 5. Relative abundance of anthropogenic litter (AL) categories in marine benthic, river, and beach habitats. Bars from this study represent the overall mean relative abundances for all riparian data combined and all benthic data combined**.** Abbreviations: USA=United States, BRA=Brazil, WAL=Wales, CHI=Chile, AUS=Australia, ZAF=South Africa, ISR=Israel. Letters refer to the following sources: (a) Pham et al. 2014; (b) Schlining et al. 2013; (c) Abu-Hilal and Al-Najjar 2009; (d) Hess et al. 1999; (e) Oigman-Pszczol and Creed 2007; (f) Rech et al. 2014; (g) Williams and Simmons 1999; (h) Whiting 1998; (i) Rosevelt et al. 2013; (j) Hoellein et al. 2014; (k) Thornton and Jackson 1998; (l) Bowman et al. 1998; (m) Kusui and Noda 2003; (n) Santos et al. 2009; (o) Madzena and Lasiak 1997; (p) Smith and Markic 2013; (q) Eriksson et al. 2013.

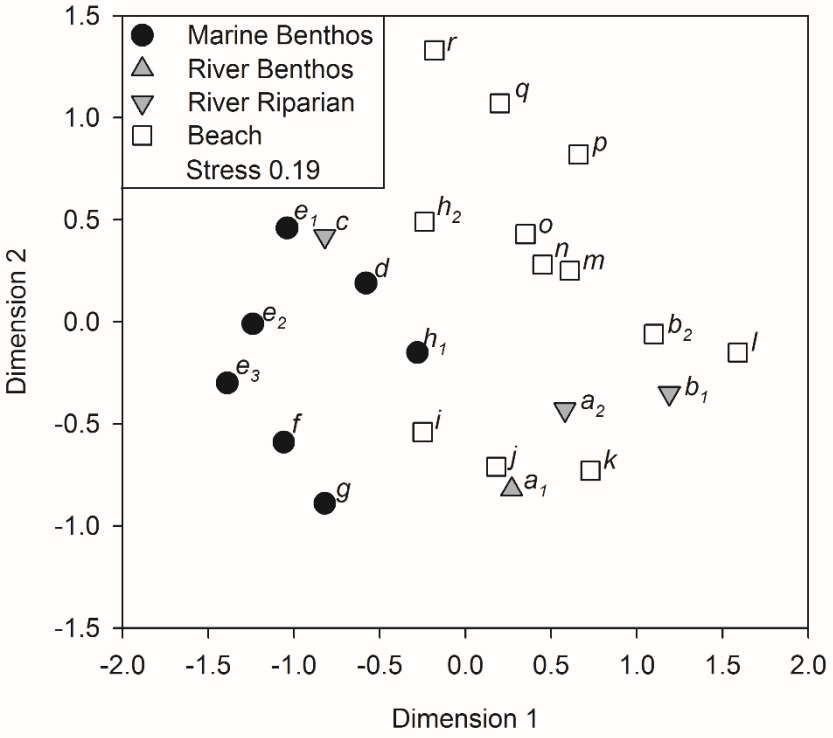

Figure 6. Non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarity index of anthropogenic litter (AL) composition based on relative abundance in marine benthic, river, and beach habitats. Relative AL abundance data were log(x+1) transformed. Letters refer to the following measurements: (a) Mean river benthic habitat (1) and riparian zone (2), this study; (b) river riparian zone (1) and beaches near river mouths (2) Chile, Rech et al. 2014; (c) Taff River riparian zone, Wales, Williams and Simmons 1999; (d) Kodiak Island, AK, USA, Hess et al. 1999; (e) Arctic Ocean (1), Mediterranean Sea (2), Atlantic Ocean (3), Pham et al. 2014; (f) Gulf of Aqaba, Red Sea, Abu-Hilal and Al-Najjar 2009; (g) Monterey Canyon, CA, USA, Schlining et al. 2013; (h) subtidal zone (1), beach (2), Armacao dos Buzios, Brazil, Oigman-Pszczol and Creed 2007; (i) Lake Michigan, Hoellein et al. 2014; (j) Fog Bay, AUS, Whiting 1998; (k) Cliffwood Beach, NJ, USA, Thornton and Jackson 1998; (l) Monterey Bay, CA, USA, Rosevelt et al. 2013; (m) Sea of Japan, Kusui and Noda 2003; (n) Costa do Dende, Brazil, Santos et al. 2009; (o) Transkei Coast, South Africa, Madzena and Lasiak 1997; (p) Charlesworth Bay, AUS, Smith and Markic 2013; (q) Mediterranean Sea, Israel, Bowman et al. 1998; (r) sub-Antarctic islands, Eriksson et al. 2013.

Table 9. Net accumulation and export of anthropogenic litter (AL) by season.

Sampling Interval			Net Accumulation		Export rate (% d^{-1})					(% mass d^{-1})	Export	
Start	End	d	No. d^{-1}	No. m^{-2} d^{-1}	Glass	Metal	Wrapper	Bag	Total	Total	No. m^{-2} d^{-1}	g m^{-2} d^{-1}
26-Nov-13	25-Apr-14	149	0.8121	0.0020	0.2685	0.5369	0.3356	0.4698	0.4027	0.2894	0.0040	0.0221
28-May-14	16-Sep-14	111	1.5405	0.0039	0.2815	0.4505	0.4204	0.4851	0.4022	0.2936	0.0040	0.0224
16-Sep-14	18-Dec-14	93	0.9892	0.0025	0.3584	0.5376	0.0597	0.4032	0.3332	0.3665	0.0033	0.0280

Table 10. Export and turnover time for 4 anthropogenic litter (AL) types.

AL	Export (% d^{-1})	Turnover time (d)
Glass	0.3028	330
Metal	0.5083	197
Wrapper	0.2719	368
Bag	0.4527	221
Mean	0.3794	264

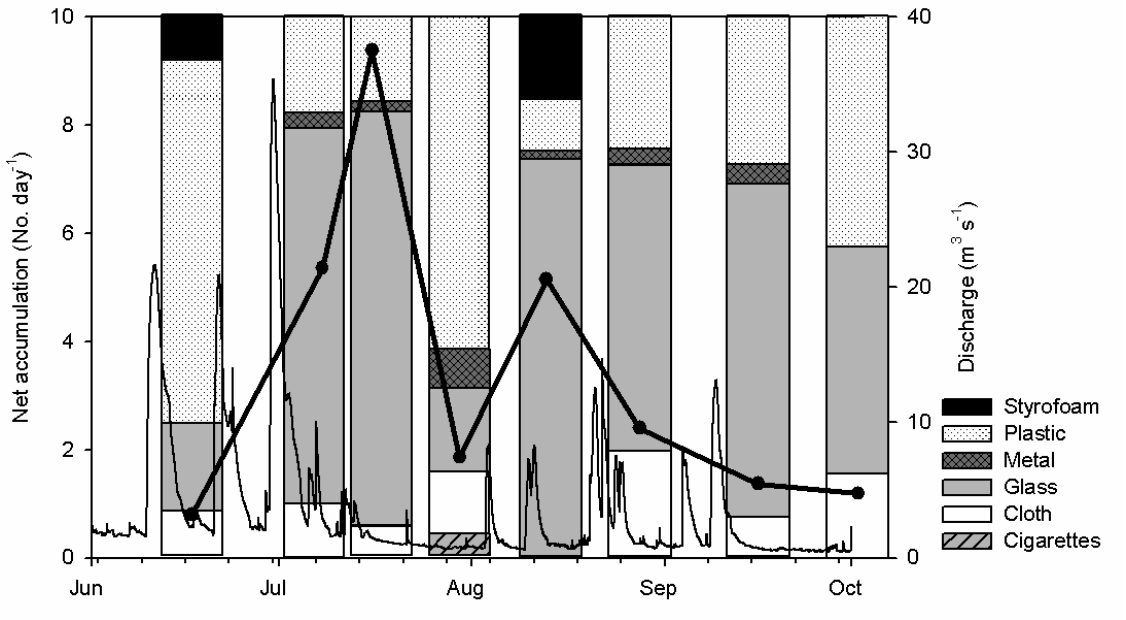

Figure 7. Abundance and composition of anthropogenic litter (AL) net accumulation in summer 2014 in the riparian zone of the North Branch of the Chicago River in Miami Woods. Bolded line represents net accumulation of unmarked litter items during the sampling period. Stacked bars represent the composition of AL net accumulation that was collected for each sampling interval. The thin line shows the discharge data for the North Branch of the Chicago River (USGS) during the study duration.

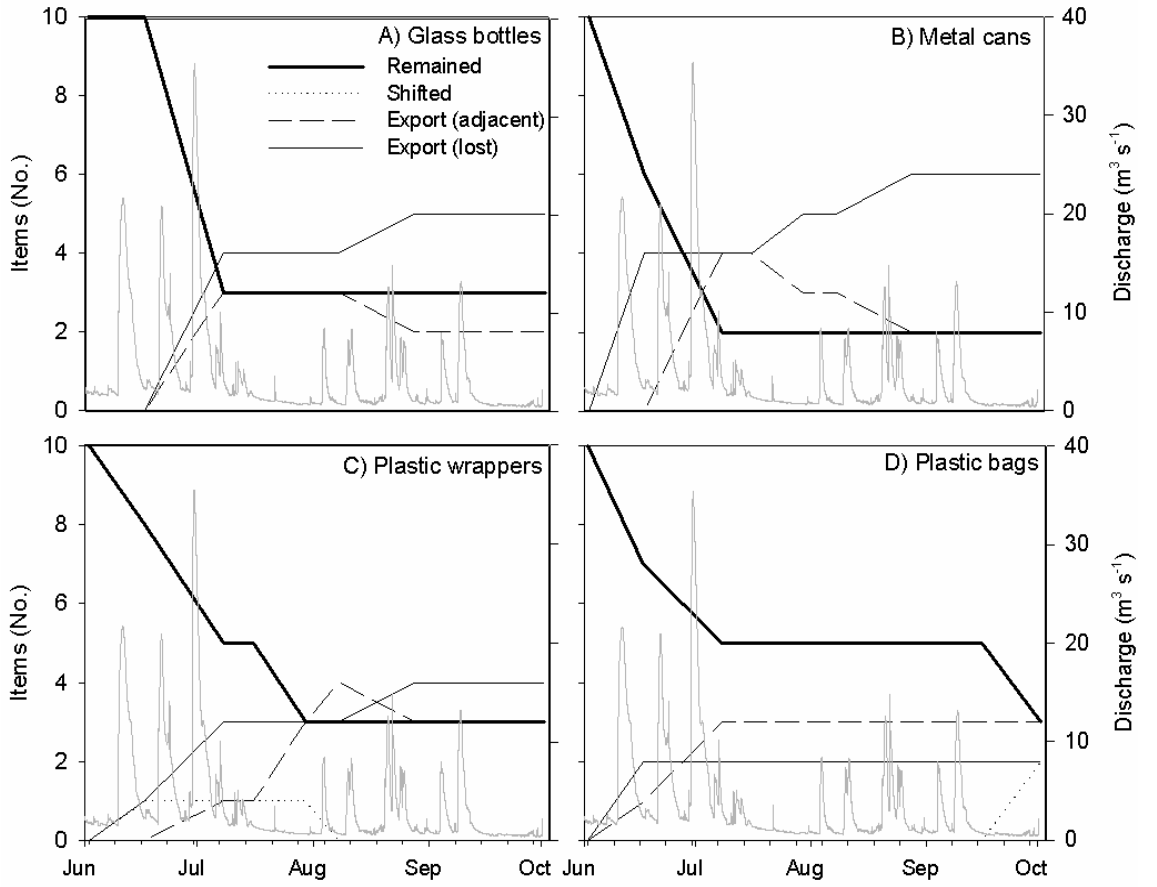

Figure 8. Movement patterns of marked glass bottles (A), metal cans (B), plastic wrappers (C), and plastic bags (D) during the biweekly flux study conducted in the riparian zone of the North Branch of the Chicago River in Miami Woods. 'Remained' indicates the item was stationary. 'Shifted' indicates the item moved within the study quadrat. 'Exported (adjacent)' indicates the item left the quadrat but remained within 100 m downstream and 40 m inland. 'Exported (lost)' indicates the item was exported from the quadrat and not found. The gray line represents discharge data for the North Branch of the Chicago River (USGS) during the study duration.

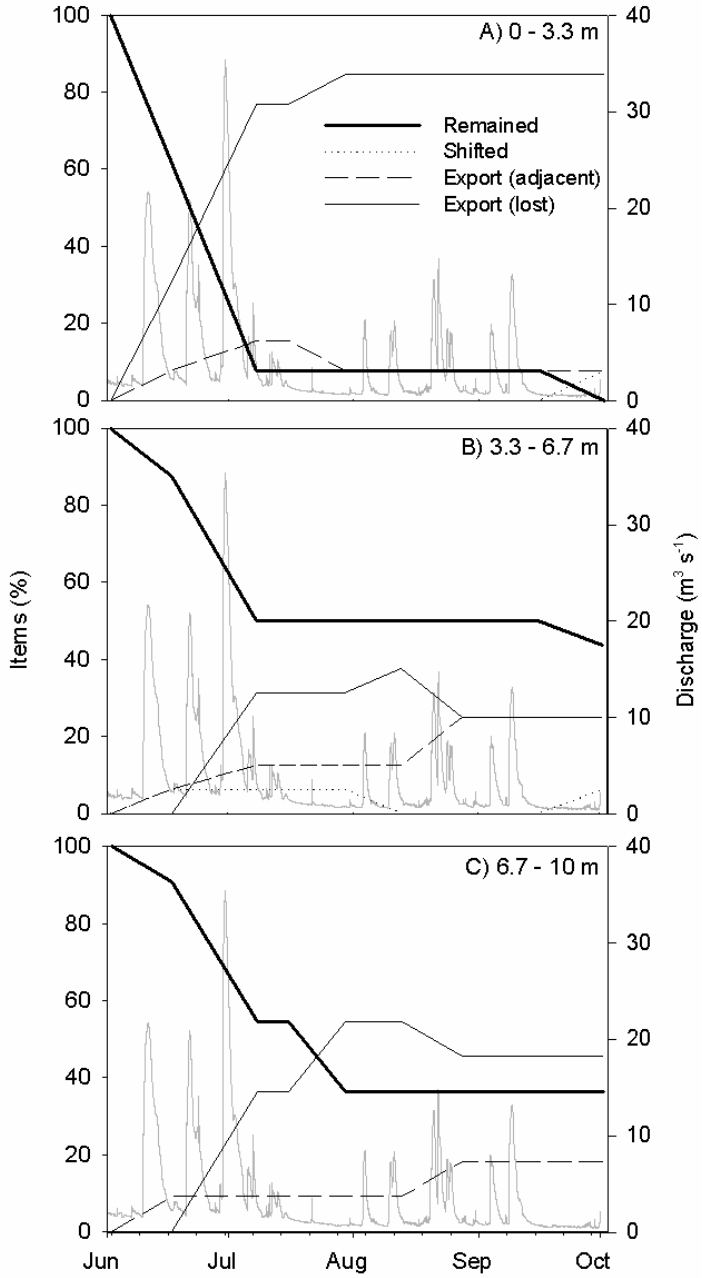

Figure 9. Movement of marked anthropogenic litter (AL) near the water's edge (0-3.3 m inland) (A), the middle (3.3-6.7 m inland) (B), and farthest inland (6.7-10 m) (C) in a riparian quadrat during summer 2014 in North Branch of the Chicago River at Miami Woods. 'Remained' indicates the item was stationary. 'Shifted' indicates the item moved within the study quadrat. 'Exported (adjacent)' indicates the item left the quadrat but remained within 100 m downstream and 40 m inland. 'Exported (lost)' indicates the item was exported from the quadrat and not found. The gray line represents discharge data for the North Branch of the Chicago River (USGS) during the study duration.

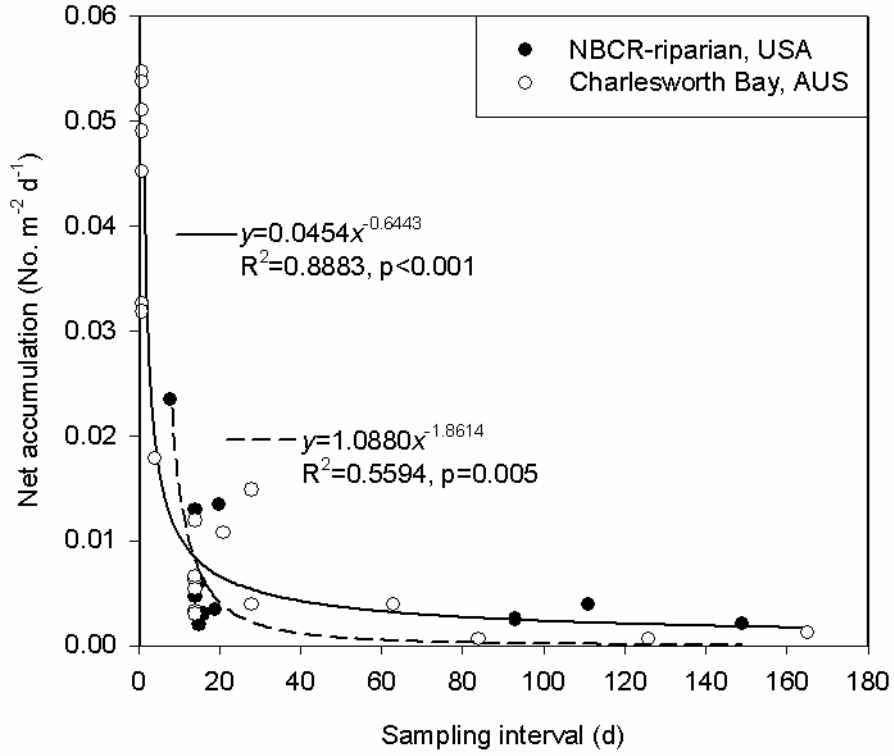

Figure 10. Plot of estimated daily accumulation rate of anthropogenic litter (AL) compared to time between sampling periods. The graph displays data from this study in the North Branch of the Chicago River (NBCR) as well as data from Figure 2 of Smith and Markic 2013. The solid line indicates the line of best fit for the two data sets combined and the dashed line indicates the line of best fit for NBCR data.

Discussion

AL abundance and composition differed between riparian and benthic habitats

Results were generally consistent with our predictions of (1) higher AL density in the riparian zone, (2) higher AL mass in benthic habitats, and (3) different AL assemblages between habitats. Hydrology and buoyancy most likely control differences in AL between the riparian and benthic zones. Heavier materials accumulate in the benthic zone as they take more energy to move, and the AL community in benthic habitats was dominated by recalcitrant materials (e.g., manufactured wood, glass, ceramic, and metal). Lighter AL such as paper and plastic remained in the riparian zone or was deposited onto the riparian zone during floods. Within the river, the buoyant material is transported downstream, deposited in riparian sites, or entrained in debris dams. These patterns are reflected in the spatial distribution of AL within each reach. We observed heavy types of benthic AL were randomly distributed in the reach, while lighter benthic AL was concentrated in debris accumulations. There are few published datasets on riverine AL abundance to compare our results, but these data are consistent with a previous study in the North Branch of the Chicago River which showed higher AL density in riparian than benthic habitats (Hoellein et al. 2014).

Reach-scale, not watershed-scale factors explained AL abundance and composition

We predicted that urban land use would be positively correlated with AL metrics among the 5 rivers, however, AL density, mass, and composition were unrelated to watershed-scale characteristics. There were no significant correlations between land use and riparian or benthic AL density, but we note the 3 streams with the most urbanized watersheds had higher AL densities. There was also no correlation between land use and

riparian or benthic AL mass, which was uniform among sites except Turkey Creek. These results could be attributed to the range of urban land use among the 5 rivers, or that reach-scale factors are more important than watershed-scale factors in determining AL abundance. All streams were located in developed, urban or suburban areas, where less urban sites had greater proportion of agriculture. A wider land use gradient with more rural, non-agricultural sites may have revealed a stronger association between watershed characteristics and AL abundance. For example, Williams and Simmons (1999) found AL density was lower and composition was different in rural streams relative to heavily urbanized tributaries. In addition, delineation of separate watershed land use types (e.g., industrial, residential, parkland) within watersheds may generate insight into watershed-scale factors that determine AL composition and abundance.

Reach-scale measurements of human activity were strongly related to AL composition, suggesting riparian activities were more important than watershed land use in driving AL patterns. Plastic, paper, and Styrofoam densities were clustered with the intensity of human activity, parking spaces, and trail proximity on the PCA diagram (Figure 4). Unsurprisingly, these patterns suggest people visiting the sites to eat, drink, and smoke are sources of AL to riparian and benthic habitats. However, the data also suggest visitors conducting illicit AL disposal are sources of non-consumable AL types. Material associated with trash dumping (e.g., metal, wood, and rubber) had PCA vectors of similar direction and magnitudes, and ceramic had strong negative correlation with all 4 measurements of human activity. Ceramic materials originate from construction (i.e., bricks, cinder blocks, pipes) or domestic waste (i.e., dishware). We suspect that people engaging in illegal disposal seek secluded reaches away from trails to avoid witnesses.

Illegal dumping or ‘fly tipping’ contributed to AL accumulation in other rivers (Williams and Simmons 1999).

In addition to park visitors and illicit dumping, we suspect prohibited recreation at secluded sites was a driver of AL composition. For example, Bunker Hill is part of the Cook County Forest Preserve system which is popular for recreation, and it had the highest riparian AL density of all 15 sites (0.992 No. m^{-2}). The park has many visitors and is easily accessible within Chicago, but the study reach at Bunker Hill is not visible from the main park trail. We repeatedly found evidence of alcohol consumption (i.e., cans and bottles) and vandalism (i.e., graffiti on trees) at this site. We concluded that because the reach is hidden from view but is located within a popular, easily accessible park, it is used for surreptitious activities. Miami Woods is also part of the Cook County Forest Preserve and used for similar activities as Bunker Hill, including running and cycling. However, the reach at Miami Woods was adjacent to the main trail, and it had relatively low AL density (0.037 No. m^{-2}). Reach visibility may reduce AL density if visitors are less likely to engage in illegal activity. Future studies on AL may consider the capacity to engage in prohibited recreational activity via reach visibility and accessibility as a control on stream AL composition.

Reach function and human activity also impacted AL abundance and composition at sites dominated by commercial activity. For example, the Turkey Creek reach at Broadway Street had a high riparian AL density (0.443 No. m^{-2}) and high human activity. Unlike other study sites, all individuals we observed were not engaged in recreation, but were from a company with a parking lot adjacent to the stream. We observed eating, drinking, and smoking at the riparian zone, where the AL composition was dominated by

consumable materials such as Styrofoam, metal cans, wrappers, bags, and beverage containers. At sites like these, commercial land use may reduce the intrinsic 'value' of the stream site to visitors, who may be more likely to litter if the habitat is deemed less important than streams in residential or park areas. In addition, Williams and Simmons (1999) note littering shows positive feedback, where "waste attracts more waste."

Hydrology and AL material type influence accumulation and export

The influence of hydrology on AL accumulation and export was dependent upon material type, physical structure, and river proximity. We hypothesized that AL export would be controlled by weight, where lightweight plastic wrappers and bags would move most often, metal cans would be intermediate, and glass bottles would move least. Unexpectedly, metal cans had the highest levels of export in both the biweekly and seasonal flux studies, and glass bottles had a higher export rate than plastic wrappers and bags. Additionally, metal and glass were more likely to be exported and lost rather than exported to an area adjacent to the study quadrat. Thus, complexity of the AL physical structure was more important than weight in controlling AL export. Though plastic wrappers and bags are lightweight and moved by wind and water, they were more likely to be retained on woody debris or vegetation due to their pliability (Williams and Simmons 1999; Hoellein et al. 2014). Conversely, metal cans and glass bottles lack the physical complexity to be entrained by debris and were more easily moved. We did not measure total transport distance of AL, but expect it can be long. For example, we coincidentally recovered a marked can while collecting AL at a reach ~7 km downstream from its initial placement.

Flooding can redistribute riparian AL by transporting material downstream or by moving it further into the riparian zone. For example, AL was more likely to be exported if it was placed within 3 m of the water's edge. The time period with the highest discharge was associated with the greatest AL movement during the bi-weekly study. Urban rivers typically have 'flashy' hydrographs (Walsh et al. 2005), suggesting that AL is a mobile substrate that may be frequently redistributed within the riparian zone or move between riparian and stream-channel habitats. Previous research on riverine AL has also linked floods with large-scale AL redistribution within the riparian zone (Williams and Simmons 1997).

Other factors that influence AL movement and accumulation include temperature, burial, legacy land-use, and reach complexity. In the seasonal flux study, we observed that ~13% of glass bottles shattered. We expect the bottles broke as they moved during flooding, but we also observed some glass breaking from freeze-thaw cycles. Many items collected on the riverbank or benthic habitat were partially buried. Burial and/or exposure to sunlight could also affect AL breakage, decomposition, and movement. Benthic bottles and cans frequently contained sediment, which likely promoted their sinking and entrainment. Similarly, many AL materials associated with dumping such as ceramic, glass, rubber tires, and metal were heavy and partially buried. Some of these items appeared relatively old and indicated a legacy of past land use. For instance, at one reach of Turkey Creek we found many metal car parts, and were informed that a car dealership used to be at the site. Additionally, at Hillcrest Road in Hickory Creek, we found many old glass bottles manufactured by local companies long out of business such as Webb & Riley and Flint Sanitary Milk Co. Finally, woody debris or increased habitat complexity

contributed to AL retention. In the biweekly flux study, almost all items that were exported to the area adjacent to the quadrat were captured in a single woody debris accumulation. Plastic items were often entangled in vegetation overhanging the river, creating what has been called a “Christmas tree” effect (Williams and Simmons 1999; Hoellein et al. 2014).

AL is a dynamic, mobile substrate

Our results show accumulation rates increased with more frequent sampling, which has implications for estimating total AL density and movement in rivers. This has also been documented on marine beaches. For example, Smith and Markic (2013) found that AL accumulation was 10 times higher when sampled daily rather than monthly, and Eriksson et al. (2013) documented similar patterns for daily plastic accumulation rates on a sub-Antarctic island. Similarly, Ryan et al. (2014) found that daily sampling resulted in ~2.5 times greater AL accumulation than weekly sampling. These data suggest that individual ‘snapshots’ of AL density at a beach or river on one date do not accurately reflect its mobile nature. While it may appear the amount of AL is not changing over repeated visits at a site, some amount of AL has likely accumulated and been exported between visits, so its total abundance and ecosystem effects may be easily underestimated.

The mobile nature of AL was also apparent from its relatively rapid turnover time. All 4 types of AL were estimated to leave the riparian zone in approximately 1 year or less. Previous research suggests rivers are likely effective at transporting buoyant AL downstream (i.e., Styrofoam, wood, plastic, cigarettes), but retain non-buoyant AL such as ceramic, metal, and glass (Rech et al. 2014). However, our data showed rivers readily

mobilize and transport heavier items such as glass bottles and metal cans, at least following their initial placement in the riparian zone.

Scaling up AL density, mass, and flux to the watershed

We scaled up our values for AL density, mass, and export from the reach to the watershed scale at each site. Scaling up required first estimating the total riparian area of each river within 10 m of the river bank and the total benthic area of the river (Table 11). Results indicate that the study sites contained 7,800-158,100 items in the benthic zone (5,700-29,900 kg), and 20,200-554,900 items in the riparian zone (1,800-24,900 kg) (Table 11). We compared the AL density in the benthic and riparian zone (within 10 m of the stream only) to population size in each watershed. Results showed AL abundance was 3.7-11.3 times higher than population size, with 1.32 kg of riverine AL per watershed resident.

We also scaled up our riparian export data to the watershed scale to estimate the total AL exported from riparian zones over a year. We multiplied the total riparian AL density and mass in the entire river (Table 11) by the mean export rates for item abundance (0.003794 items d^{-1}) and mass (0.003165 g d^{-1}), respectively. The data showed that that the riparian zones of our 5 study streams exported up to 28,000-768,500 items y^{-1}, weighing 2,000-28,800 kg y^{-1} (Table 11). Among the 5 sites, the average riparian export was 10,000 kg y^{-1} (11 metric tons y^{-1}). These figures are lower than the few published values which estimate riverine AL transport. Gasperi et al. (2014) measured the abundance of floating plastic debris captured by a network of debris-retention booms in the Seine River at 27 tons y^{-1}. Lechner et al. (2014) report that 1,533 tons y^{-1} of plastic debris enter the Black Sea via the Danube River. However, the variation in AL transport

rates among sites are likely driven by human population and watershed size (Gasperi et al. 2014), and we suggest future studies would benefit from calculations that take into account watershed size (i.e., kg km^{-2} y^{-1}). In addition, we measured AL export from the riparian zone rather than the water column, and we considered movement of several AL categories, not plastic alone.

AL abundance and composition across ecosystems

There are relatively few studies that have examined AL assemblages in rivers, but comparing riverine AL across a broad geographic range illuminates some patterns in AL sources. The high relative abundance of plastic at rivers in Illinois, Chile (Rech et al. 2014) and Wales (Williams and Simmons et al. 1999) suggests that onsite littering is an important source of AL to rivers. A key difference among the 3 river systems, was the high abundance of sewage in the Taff River (Williams and Simmons 1999). Sewage material, a majority of which were feminine hygiene products, comprised 23% of the total AL (Williams and Simmons 1999). We collected a very small amount of sewage-related material which we categorized as 'other,' which only comprised <1% of our riparian AL.

Most research on AL is conducted in marine habitats, so we compared our data to sites worldwide. The comparison indicates worldwide AL abundance and composition are influenced by the physical characteristics and human activities across ecosystem types. We predicted that AL assemblages in the benthic zone of rivers would be similar to those in the benthic zone of marine habitats, while riparian zones and marine beaches would be similar. Overall, the density of AL in our stream benthic habitats was higher than a majority of studies from marine benthic habitats (Table 7). In both benthic

ecosystems, however, materials such as metal and glass had much a higher relative abundance than beach habitats, likely due to the material's relatively high mass. Conversely, other material types such as Styrofoam, paper, and cigarettes were more common in beaches and riparian zones than benthic habitats. Rech et al. (2014) refer to these items as "short-term buoyant" (i.e., paper and cigarettes) and "persistent buoyant" (i.e., Styrofoam), which do not persist in aquatic environments or lack the capacity to sink and reach the benthic zone.

Differences in AL assemblages among ecosystems are also driven by variations in AL classification. Marine benthic assemblages clustered in the nMDS ordination, and the riparian AL composition from a river in Wales was similar to marine benthic sites (Figure 6). However, several studies in this group had a large proportion of AL categorized as 'other,' which contributed to some of the similarity. Authors either reported items as 'other,' or we used 'other' to classify miscellaneous AL categories not used in our study (e.g., 'clinker' in Pham et al. 2014; 'sewage-related material' in Simmons and Williams 1998). Some authors classified a large proportion of AL as 'other' because they grouped several material types. For instance, some studies used 'other' to include cloth, ceramic, paper/cardboard, and/or wood (Pham et al. 2014; Williams and Simmons 1999; Abu Hilal and Al-Najjar 2009; Oigman-Pszczol and Creed 2007). Another difference in AL classification across studies was inconsistencies in separating Styrofoam (polystyrene) from plastic. Several studies either do not mention Styrofoam or polystyrene or categorize it as plastic (Williams and Simmons 1999, Pham et al. 2014, Abu-Hilal and Al-Najjar 2009, Schlining et al. 2013, Oigman-Pszczol and Creed 2007, Bowman et al. 1998, Eriksson et al. 2013), while other studies distinguish the two (Rech et al. 2014,

Hess et al. 1999, Thornton and Jackson 1998, Whiting 1998, Hoellein et al. 2014, Kusui and Noda 2003, Santos et al. 2009, Madzena and Lasiak 1997). We suggest that future research on AL abundance and composition adopt a standard protocol such as the one designed by the United Nations Environment Programme (UNEP) (Cheshire et al. 2009) for AL classification, that we present in the appendix (Table A1).

The classification of AL into 'mixed media' categories drove some patterns in similarities of AL composition across ecosystems. For instance, 'fishing' was a category in all but 1 marine benthic study. However, only 2 marine beach studies considered 'fishing' a category, and it accounted for ≤1% of material these studies (Figure 5). Since fishing is an AL category considered almost exclusively in marine benthic studies, some of the similarities in marine benthic AL assemblages are likely driven by that category. Fishing gear is ecologically detrimental and important to quantify (Gregory 2009), but its inclusion as an AL material category complicates cross–ecosystem comparisons because fishing gear is a 'mixed' material type (i.e., plastic monofilament and nets, metal traps and lures). Other AL types consist of multiple material types, so we recommend future studies classify items both by their material composition (i.e., plastic, glass, metal) and function or source (i.e., fishing, commercial, recreational).

Comparing AL accumulation rates across different ecosystems is complicated by variation in sampling area (Ryan et al. 2014) and AL units. For example, beach data are often reported in No. items m^{-1} d^{-1} (Ryan et al. 2014, Smith and Markic 2013, Eriksson et al. 2013, Bowman 1998), which is difficult to compare to areal data (No. items m^{-2} d^{-1}). This difference in methodologies inhibited comparison of our AL accumulation rates to many literature values. We recommend that future studies report AL accumulations in

No. items per unit reach length over time (No. m^{-1} d^{-1}) as well as area (No. m^{-2} d^{-1}) to facilitate syntheses.

AL is abundant and mobile in rivers, with unknown biological effects

This study shows that rivers store and transport AL, and the results will support further research on AL movement, degradation, biological interactions, and mitigation strategies in rivers. The source, retention device, retention time, and transport distance of AL in rivers is driven by material type, habitat complexity, hydrology, and human activity at the reach scale. Contrary to common perception, riverine AL is mobile, and selective retention drives the contribution of AL from in-stream and riparian habitats to downstream rivers and marine ecosystems. While our initial research showed AL can select for some unique biofilm communities (Hoellein et al. 2014; McCormick et al. 2014), interactions between AL and other freshwater organisms are unknown. Finally, rivers may represent the location where mitigation strategies for reduction of downstream accumulation are most efficient, thus, they should be a priority for research on AL ecology.

Table 11. Total abundance of anthropogenic litter (AL) in benthic and riparian zones of each site.

	Salt Creek	Turkey Creek	N Br Chicago Riv	Hickory Creek	Plum Creek
Length (m)*	61355	19553	58874	39938	31182
Width (m)	18.6	8.9	15.1	14.9	5.5
Riparian Density (No. m^{-2})	0.452	0.275	0.470	0.236	0.032
Benthic Density (No. m^{-2})	0.088	0.176	0.178	0.099	0.045
Riparian River (No. items)	554,934	107,502	553,215	188,783	20,212
Benthic River (No. items)	100,596	30,545	158,090	58,609	7,795
Total River (No. items)	655,530	138,047	711,304	247,392	28,007
Riparian Mass (g m^{-2})	4.923	63.673	3.379	8.830	2.892
Benthic Mass (g m^{-2})	22.694	172.166	32.453	31.637	33.066
Riparian River (kg)	6,041	24,900	3,979	7,053	1,803
Benthic River (kg)	25,954	29,860	28,889	18,776	5,712
Total River (kg)	31,996	54,760	32,867	25,829	7,515
Total River (metric ton)	32	55	33	26	8
Riparian Export (No. d^{-1})	2,105	408	2,099	716	77
Riparian Export (No. y^{-1})	768,478	148,870	766,097	261,429	27,989
Riparian Export (kg d^{-1})	19	79	13	22	6
Riparian Export (kg y^{-1})	6,979	28,765	4,596	8,147	2,083

*United States Geological Survey National Hydrography Dataset high-resolution flowline data from the National Map (accessed Mar 10, 2015).

CHAPTER III

MICROPLASTIC IN URBAN STREAMS: SOURCE, ABUNDANCE, AND SELECTION OF UNIQUE BACTERIAL ASSEMBLAGES

Introduction

A growing field of research has documented microplastic (<5 mm particles) abundance, sources, movement, and biological interactions in the environment (Thompson et al. 2004; Eriksen et al. 2014; Browne et al. 2011). Many areas with microplastic accumulation are near urban centers (Yonkos et al. 2014; Eriksen et al. 2013a; Browne et al. 2011) and oceanic gyres (Eriksen et al. 2013b; Law et al. 2010; Moore et al. 2001). However, microplastic has also been found in remote habitats (Free et al. 2014; Ivar do Sul et al. 2009; Obbard et al. 2014).

Microplastic has several sources such as microbeads contained in personal care products and production pellets used to manufacture plastic items (Cole et al. 2011; Gregory 1996; Fendall and Sewell 2009). Microplastic also forms through fragmentation of larger particulate plastic by biodegradation, photolysis, thermoxidation and thermodegradation processes (Andrady 2011). Finally, washing synthetic textiles releases a high abundance of microplastic fibers into washing machine effluent (Browne et al. 2011). Microplastic pellets from personal care products and fibers enter the domestic wastewater infrastructure but may not be filtered by wastewater treatment plants (WWTPs) due to their small size (Fendall and Sewell 2009; Browne et al. 2011). WWTP

effluent can be a source of plastic fibers to marine sediment (Browne et al. 2011) and a source of pellets and fibers to river surface waters (McCormick et al. 2014).

A majority of microplastic research has focused on marine environments, and studies on microplastic in freshwaters and estuaries have only recently emerged (Wagner et al. 2014). Measurements of microplastic abundance in estuaries highlight the potential for rivers to transport microplastic to marine habitats (Yonkos et al. 2014; Dubaish and Liebezeit; Sadri and Thompson 2014; Lima et al. 2014). Rivers are also susceptible to the same sources of microplastic as marine environments and have relatively little water volume for microplastic dilution, so they are likely to have high concentrations. A few recent studies have found high microplastic concentrations in riverine sediment (Castañeda et al. 2014) and surface waters (McCormick et al. 2014; Lechner et al. 2014; Moore et al. 2011). A greater understanding of the sources, accumulation sites, and movement of microplastic in rivers is needed to quantify global microplastic distribution.

Microplastic has several ecological effects on biota such as ingestion by consumers and transporting contaminants to organisms (Wright et al. 2013; Rochman et al. 2013). Once consumers ingest microplastic, the material can be retained in gut tissue, which can block digestion and suppress feeding (Wright et al. 2013). Previous research has also shown that microplastic can translocate from the digestive to circulatory system in mussels (Browne et al. 2008). Furthermore, microplastic can bioaccumulate in predators when it is ingested by lower trophic organisms. (Setälä et al. 2014; Murray and Cowie 2011; Farrell and Nelson 2013). During the manufacturing process, toxic compounds are often added to plastic, and these compounds can be harmful to organisms by deteriorating immune function and disrupting endocrine processes (Barnes 2009;

Teuten et al. 2009; Mato et al. 2001). Additionally, seawater naturally contains low levels of persistent organic pollutants (POPs), but microplastic can adsorb and concentrate these contaminants (Rios et al. 2010; Barnes et al. 2009; Mato et al. 2001). There is concern that microplastic ingestion facilitates the transport of these contaminants to organisms (Rochman et al. 2013).

Microplastic also provides a novel habitat for microorganisms in the environment. Zettler et al. (2013) coined the term 'plastisphere' to describe the diverse community of microorganisms living on microplastic in the open ocean. Additional studies demonstrate that microplastic selects for unique bacterial assemblages in surface water of an urban river (McCormick et al. 2014) and marine sediment (Harrison et al. 2014). Biofilm formation on microplastic may increase the likelihood of consumer ingestion (Reisser et al. 2014) and contribute to microplastic sinking to benthic habitats (Barnes et al. 2009). However, studies which compare microplastic biofilms to natural microbial habitats across a broader geographic range are needed. These analyses will show if microplastic selects for a particular community of microorganisms across different environmental conditions.

In this study, we quantified microplastic concentrations in 9 streams from sites located upstream and downstream of WWTP effluent outfalls to determine if treated wastewater is a point source of microplastic to rivers. Using next-generation sequencing, we also analyzed the bacterial assemblages on microplastic and compared these communities to those on 3 natural habitats: organic material, upstream water column, and downstream water column. These research questions were addressed in a recently published study in a single river (McCormick et al. 2014). This study was designed to

examine these patterns across a larger geographic scale. We predicted that microplastic concentrations would be significantly higher downstream of WWTP effluent outfalls than upstream. We also hypothesized that bacterial assemblages on microplastic would differ from those on natural substrates.

Materials and Methods

Study sites

Our study streams were in the Chicago metropolitan area (N=8) and central Illinois (IL) (N=2) and receive treated WWTP effluent (Table 12). Streams spanned a gradient of discharge and relative contribution of WWTP effluent to stream flow (Table 12). The WWTPs that discharge effluent into the study sites spanned a range of municipality size, volume of effluent released per day, and treatment methods for filtration and effluent disinfection (Table 12).

Sample collection and microplastic quantification

We collected microplastic from surface water with neuston nets (0.52 × 0.36 m) of 333 µm mesh during July-October 2014 (McCormick et al. 2014; Eriksen et al. 2013). In the North Shore Channel, we deployed nets behind a stationary boat. All other streams were shallower, so we waded in and held the nets in place manually at the water's surface. Each researcher held a net in front of them, perpendicular to the water flow, taking care not to disturb the net tail. We measured deployment time (15-20 min), water depth in the net, and water velocity at the center of each net (Marsh-McBirney Flo-Mate Model 2000 Portable Flowmeter, Loveland, CO). We collected 4 separate net samples upstream and 4 downstream of the WWTP effluent site, selecting sites that represented well-mixed waters. Material was rinsed from the net into 1 L containers with unfiltered

site water, and then placed into a cooler on ice for transport to the laboratory where they were stored at 4ºC until processing for microplastic counts. At Schererville Ditch, very low water velocity upstream of the WWTP effluent site precluded analysis of microplastic concentrations.

Downstream of WWTPs, we collected additional samples to measure bacterial community composition on microplastic, seston, and in the water column. We also measured water column bacteria upstream of WWTPs. For microplastic and seston, we conducted additional net deployments. Material from the nets was rinsed onto a white tray, which had been sterilized with ethanol. Individual microplastic particles were removed by hand using sterilized forceps, and placed in a 160 mL sterile specimen container with ~20 mL of site water. Organic material from the sample was also removed by hand and placed in separate containers. At 3 sites (Goose Creek, Little Kickapoo Creek, and East Branch of the DuPage River) we found no visible microplastic in the samples, so we did not have microplastic-associated bacteria from those sites. To measure water column bacteria, we collected 2 L of unfiltered site water from the water column (depth = ~10 cm) at the upstream and downstream sites using acid-washed containers. The specimen containers and 2 L water column samples were transported on ice to the laboratory where they were stored at 4ºC until processing (within 24 h). We also recorded temperature and conductivity (YSI Model 30, YSI Incorporated, Yellow Springs, OH) and dissolved oxygen (DO) (HQ40d portable meter with LDO101 DO probe, Hach Company, Loveland, CO) at all upstream and downstream sampling locations. Finally, we collected triplicate 20 mL filtered water samples (glass microfiber filter; GF/F; Sigma-Aldrich Co., St. Louis, MO) to measure dissolved nutrients at the

upstream and downstream sites. Filtered water samples were frozen at -20ºC until solute analyses.

We adapted a protocol for the quantification of microplastic from marine water column samples to measure microplastic (Baker et al. 2011; McCormick et al. 2014). Samples were first run through 4.75 mm and 330 µm stacked sieves. The 0.330-4.75 mm fraction was stored in glass beakers in a drying oven at 75ºC. Organic material was degraded through wet peroxide oxidation (0.05 M Fe(II) and 30% hydrogen peroxide) at approximately 75ºC. Plastic is resistant to wet peroxide oxidation, while organic matter is degraded (Baker et al. 2011; Eriksen et al. 2013). We added sodium chloride (final concentration = 6M) for a salinity-based density separation in which the sample was placed in a glass funnel, microplastic floated, and heavier material was drained from the sample (Baker et al. 2011). Microplastic was filtered (Whatman glass microfiber filters) and counted under a dissecting microscope. We recorded the microplastic type (i.e., fragment, pellet, foam, film, or fiber) for each particle and counted all particles of fragments, pellets, foam, and film individually on the filter. For fibers, which were very abundant and tended to stick to the filter, we used a sub-sample approach (McCormick et al. 2014). For each sample, we counted 3 random subsamples for each quadrat of the filter (each subsample was 3% of the filter area). The mean value from 12 subsamples was scaled up in proportion to the whole filter to determine microplastic fiber abundance for the sample. We calculated microplastic concentration by dividing the number of particles by water volume (No. items m^{-3}) and surface area (No. items km^{-2}). All reagents were checked for microplastic contamination, and none was found. Control samples were processed identically to environmental samples to measure procedural contamination

(N=5). We found no microplastic contamination of fragments, pellets, film, or foam. Average procedural contamination by microplastic fibers was 4.67 per sample, which we subtracted from each environmental sample.

DNA extraction and sequencing

DNA was extracted from microplastic, suspended organic matter, downstream water column, and upstream water column samples using MoBio Powersoil DNA extraction kits (MoBio Laboratories, Carlsbad, CA). For the microplastic and seston, we collected material manually from the specimen containers and placed it into 2 mL microcentrifuge tubes for DNA extraction. We separated the 2 L water column samples into 4, 500 mL portions, each filtered with Millipore Sterivex 0.22 µm filter cartridges (N= 4 downstream and 4 upstream). The filters were removed from cartridges, cut with a sterilized razorblade, and placed into 2 mL microcentrifuge tubes for DNA extraction (Crump et al. 2003; McCormick et al. 2014).

Bacterial assemblages were profiled via next-generation amplicon sequencing of 16S rRNA genes. PCR amplification was performed using primers 515F and 806R, which amplify the V4 hypervariable region of bacterial and archaeal 16S rRNA genes (Caporaso et al. 2011). For all samples, we confirmed successful DNA amplification by agarose gel electrophoresis. Amplicons were sequenced in a 2×250 paired end format using the Illumina MiSeq platform (Caporaso et al. 2012) by the DNA Services Facility, University of Illinois at Chicago. Sequences were processed by using MOTHUR v.1.33.0 as described by Schloss et al. (2011) and Kozich et al. (2013). Briefly, paired reads were assembled and demultiplexed, and any sequences with ambiguities or homopolymers longer than 8 bases were removed from the data set. Sequences were aligned using the

SILVA-compatible alignment database available within MOTHUR. Sequences were trimmed to a uniform length of 293 base pairs, and chimeric sequences were removed using Uchime (Edgar et al. 2011). Sequences were classified using the MOTHUR-formatted version of the RDP training set (v.9) and any unknown (i.e., not identified as bacterial), chloroplast, mitochondrial, archaeal and eukaryotic sequences were removed. Sequences were clustered into operational taxonomic units (OTUs) based on 97% sequence identity. In order to avoid biases associated with uneven numbers of sequences across samples, the entire dataset was randomly subsampled to 14,541 sequences per sample.

Water chemistry

Water samples were analyzed for soluble reactive phosphorus (SRP), ammonium (NH_4^+), and nitrate (NO_3^-) using an AutoAnalyzer 3 (Seal Analytical, Inc., Mequon, WI, USA). SRP was measured using the antimonyl tartrate technique (Murphy and Riley 1962), NH_4^+ with the phenol hypochlorite technique (Solorzano 1969), and NO_3^- was measured with the cadmium reduction technique (APHA 1998).

Data analysis

We used 2-way analysis of variance (ANOVA) to compare total microplastic concentration among sites and relative to WWTP effluent input (i.e., upstream versus downstream). We applied a natural log transformation to ensure concentration data met the homoscedasticity and normality assumptions of ANOVA. Following a significant interaction in the 2-way ANOVA, we compared upstream and downstream concentrations at each site individually, using a Bonferroni correction ($\alpha=0.05/9=0.006$) for multiple pairwise comparisons. After applying the $\ln(x+0.5)$ transformation, we also

used 2-way ANOVA to compare concentrations of each microplastic category (fragments, pellets, foam, film, and fibers). We also calculated the ratio of downstream to upstream microplastic concentration to examine the WWTP effect among sites. One replicate each from downstream and upstream were randomly paired to calculate the ratio, and we used a 1-way ANOVA on the natural log of the concentration ratio to detect differences among streams, followed by Tukey's multiple comparison test.

The bacterial assemblages on microplastic, organic matter, upstream water column, and downstream water column samples were compared by calculating the Bray-Curtis similarity index for each pair of samples and visualizing the resulting distance matrix using non-metric multidimensional scaling (nMDS) run within MOTHUR. The statistical significance of differences in assemblages between sample types based on the Bray-Curtis index was assessed by the analysis of molecular variance (AMOVA) run within MOTHUR. Microbial diversity, based on the observed numbers of OTUs and Shannon-Weiner (H') and Shannon Evenness (E_H) indices, was also calculated for each sample using MOTHUR. We used 1-way ANOVA to assess the effects of sample type on microbial diversity metrics followed by Tukey's multiple comparison test. Bacterial genera making the largest contributions to the dissimilarities between sample types (based on the Bray-Curtis index) were identified by a SIMPER analysis run in Primer 6 (Primer-E Ltd., Plymouth, United Kingdom). Two analyses were completed with SIMPER: comparing upstream to downstream water column communities and comparing communities on plastic to those on organic matter. For all genera identified as contributing to dissimilarities between sample types, a t-test was completed to determine whether there were statistically significant differences in the relative abundances of the

genera between sample types. All ANOVAs, Tukey's tests, and t-tests were completed in SYSTAT 13.0 (Systat, Inc. Chicago, IL).

Table 12. Sampling locations in study streams receiving wastewater treatment plant (WWTP) effluent.

Plant	Receiving Water Body	Location	2013 Mean Effluent (MGD)	Effluent date sampled (MGD)	Contrib. of effluent to downstream flow (%)	Tertiary sand bed (Y/N)
James C. Kirie WRP	Higgen's Cr	Des Plaines, IL	38.72	22.38	110.82	N
Wheaton WWTP	Springbrook Cr	Wheaton, IL	7.39	7.83	86.18	Y
Bloomington SE	L Kickapoo Cr	Bloomington, IL	4.24	4.03	78.93	Y
Schererville WWTP	Schererville Ditch	Schererville, IN	4.32	3.88	70.22	N
Terrence J. O'Brien WRP	N Shore Ch.	Chicago, IL	225.00	132.28	70.00*	N
Bloomington W Oakton	Goose Cr	Bloomington, IL	15.93	10.41	46.51	Y
Springbrook WRP	DuPage R	Naperville, IL	19.68	18.84	20.82	Y
Bartlett WWTP	W Br DuPage R	Bartlett, IL	2.16	3.10	15.99	N
Elmhurst WRP	Salt Cr	Elmhurst, IL	7.03	3.41	13.17	N
Woodridge Gr.Val. WRP	E Br DuPage R	Woodridge, IL	10.00	7.70	13.24	Y

*Estimate came from Illinois Coastal Management Program (2011). Abbreviations: MGD=millions of gallons per day, Contrib=contribution, WRP=water reclamation plant, Cr=creek, L=little, N=north, Ch=channel, R=river, W=west, Br=branch, E=east, Gr=Greene, Val.=valley

Results

Physical and chemical characteristics of study streams

Nutrients and conductivity were variable among study streams, but generally higher values downstream from WWTPs illustrated the influence of effluent on water chemistry. For example, NO_3^- concentrations were higher downstream than upstream at all sites, and at 1 site NO_3^- concentration was 58 times higher downstream (Goose Creek, Table 13). SRP concentration was higher downstream at all but 1 site (West Branch of the DuPage River, Table 13). Conductivity was higher downstream than upstream at 7 sites (Table 13). Finally, there were no patterns for DO concentration upstream and downstream of WWTPs across sites (Table 13).

Microplastic concentration

Microplastic was found in every net sample, and microplastic concentration was higher downstream of the WWTP effluent outfall than upstream at all but 2 sites (Figure 11; Table 14). The 2-way ANOVA showed a significant interaction between site and effluent effects ($p<0.001$, Table 15), so we conducted t-tests at each site with a Bonferroni correction. This approach indicated 2 streams had significant differences in downstream and upstream microplastic concentrations (Higgen's Creek and Salt Creek; Figure 11A). Given the high variation in microplastic among sites, we examined the ratio of downstream to upstream concentrations, which was significantly different among sites (Figure 11B). The ratio was >0 at 7 of 9 sites, and significantly higher at Higgen's Creek, Springbrook Creek, the West Branch of the DuPage River, and Salt Creek, relative to Goose Creek, which was lowest (Figure 11B).

We also examined patterns in concentration for the 5 microplastic categories. Across all sites, pellets, fibers, and fragments were the most common microplastic types, while film and foam were uncommon (Table 14; Figure 12). All categories showed significant interactions between site and effluent input effects (Table 15). After performing multiple comparison tests with a Bonferroni correction for each microplastic category at each site, we documented significantly higher concentrations of fragments and pellets downstream of the WWTP at Higgen's Creek and a higher concentration of pellets downstream at the West Branch of the DuPage River (Table 14). Unexpectedly, foam concentration was higher upstream than downstream in the DuPage River (Table 14). On average, pellets made up a larger proportion of total microplastic downstream of WWTPs than upstream, and fibers and fragments had higher relative abundances at upstream locations (Figure 12).

Overall, the proportion of WWTP effluent in stream discharge and sand filtration had no significant effect on microplastic concentrations. There was no relationship between the proportion of WWTP effluent in stream discharge and the mean ratio of downstream to upstream microplastic concentration ($r=0.19$, $p=0.617$) or the mean difference between downstream and upstream microplastic concentration ($r=0.29$, $p=0.443$). We also found that sand filtration (n=5 WWTPs with sand filters and 4 without) had no effect on the mean ratio of downstream to upstream microplastic concentration (t-test, $p=0.084$) or the mean difference between downstream and upstream microplastic concentrations (t-test, $p=0.356$).

Bacterial assemblages across habitats

We found diverse bacterial assemblages on all 4 habitats: upstream water column, downstream water column, downstream organic material, and microplastic, which had mean (±SE) numbers of observed OTUs of 2902 (±105), 2989 (±74), 2979 (±81), 1748 (±103), respectively. Mean Good's coverage of sampling, calculated in MOTHUR, for the upstream water column, downstream water column, organic material, and microplastic was 86.4%, 86.5%, 87.9%, 92.5%, respectively. Microplastic bacterial assemblages had significantly lower taxa richness (ANOVA, $p<0.001$), community diversity (H' index, ANOVA, $p<0.001$), and community evenness (E_H index, ANOVA, $p<0.001$) than the other habitats (Figure 13). Downstream organic material had significantly higher community diversity and evenness measured by the Shannon-Weiner (H') index and Shannon Evenness (E_H) indices than other habitats (Figure 13B, 13C).

The composition of bacterial assemblages was significantly different among habitats (Figure 14). Bray-Curtis indices were significantly different when comparing all habitats (AMOVA, p-value <0.001) and when comparing any one category to another (AMOVA, all $p<0.001$; Table 16). When all sites were combined, there were clear differences among the 4 habitats in the relative abundance of bacterial OTUs at the phylum level (Figure 15). The relative abundance for Bacteriodetes decreased from the upstream water column (44.1%), downstream water column (31.8%), organic material (23.6%), and plastic (9.5%). In contrast, the relative abundance of Proteobacteria increased across the upstream water column (33.7%), downstream water column (46.8%), organic material (56.9%), and plastic (74.9%). Within Proteobacteria, Betaproteobacteria had a higher relative abundance on plastic (32.1%), than in the upstream water column

(23.2%), downstream water column (25.1%), and organic material (25.0%). The relative abundance of Gammaproteobacteria was also higher on plastic (32.5%) than the upstream water column (5.0%), downstream water column (12.3%), and organic material (15.0%). Finally, the phylum Actinobacteria was more abundant in the water column samples than organic material and plastic, and Firmicutes had a higher relative abundance on plastic than other habitats (Figure 15).

Family-level resolution of bacterial OTUs also showed differences among the 4 habitats. The 3 most common families were different in each habitat. The most common in the upstream water column were Flavobacteriaceae, unclassified Actinomycetales, and Cytophagaceae, and in the downstream water column the most common were Flavobacteriaceae, unclassified Betaproteobacteria, and unclassified bacteria (Figure 16; Table 17). The most common families in the organic material included unclassified bacteria, Comamonadaceae, and Flavobacteriaceae, and on plastic the most common were Pseudomonadaceae, unclassified Gammaproteobacteria, and Comamonadaceae (Figure 16; Table 17).

Several families were more abundant on microplastic compared to the other habitats. Pseudomonadaceae was significantly more abundant on plastic, and it accounted for 12.2% of total sequences on the plastic but only 0.8% of the total sequences from the upstream water column and 2.0% and 2.5% of total sequences from the downstream water column and organic matter respectively (Table 17; Figure 16). Similarly, unclassified Gammaproteobacteria represented 9.3% of sequences on plastic, but <2% of the total sequences on all other habitats (Table 17; Figure 16). On plastic, Burkholderiales_incertae_sedis was 5.5% of sequences, but only 1.2% on organic

material and <1% in the upstream and downstream water columns (Table 17; Figure 16). Finally, Veillonellaceae and Campylobacteraceae accounted for 4.2% and 1.7% of total sequences on the plastic, respectively but <1% of the total sequences in the other 3 habitats, but we note that this increased abundance on plastic was not statistically significant for these 2 families (Table 17; Figure 16).

There were 60 OTUs that accounted for 60.7% of the variation between plastic and downstream organic material (Table 18). The taxa contributing most to this variation were unclassified Gammaproteobacteria (6.9%), which was 5.3 times more abundant on plastic than organic material, and unclassified bacteria (6.2%), which was 2.9 times more abundant on organic material than plastic (Table 18). *Pseudomonas* and *Aquabacterium* were 8.7 and 14.5 times more abundant on plastic than organic material, respectively. Other groups that were significantly more abundant on plastic than organic material were unclassified Pseudomonadaceae, unclassified Betaproteobacteria, *Rheinheimera*, *Acinetobacter*, *Arcobacter*, and *Azospira*. *Flavobacterium* and unclassified genera from Bacteroidetes, Sphingobacteriales, Rhodobacteraceae, Rhizobiales, Chitinophagaceae, and Alphaproteobacteria were significantly higher on the organic material than plastic (Table 18).

The data also revealed differences between bacterial assemblages in the water column upstream and downstream of WWTP effluent, with 41 OTUs contributing to 63.4% of the variation between habitats (Table 19). The taxa contributing most to this variation were unclassified Actinomycetales (6.6%), which was more abundant in the upstream water column, and unclassified Betaproteobacteria (5.9%), which was more abundant downstream (Table 19). Other taxa that were more abundant upstream were

Sediminibacterium, *Polynucleobacter*, *Algoriphagus*, *Fluviicola*, and unclassified taxa of Cytophagaceae, Bacteroidetes, Opitutae, Cryomorphaceae, Microbacteriaceae, and Sphingobacteriales (Table 19). Taxa that were more abundant in downstream water column included unclassified bacteria, *Rheinheimera*, unclassified Proteobacteria, *Undibacterium*, and *Acinetobacter* (Table 19).

Microplastic bacterial assemblages

The relative abundance of microplastic-associated taxa showed variation among study streams. For instance, unclassified Gammaproteobacteria was the most dominant bacteria taxa on plastic from Schererville Ditch (28.7%) and the DuPage River (13.8%), but its relative abundance at other sites ranged from 0.9-11.2% (Figure 17). *Pseudomonas* was present on plastic from all streams, and its relative abundance ranged from 1.2-14.6% (Figure 17). Unclassified Betaproteobacteria was the most prevalent group in Springbrook Creek (10.8%), and *Aquabacterium* was the most common genus in Higgen's Creek (18.3%) (Figure 17). The dominant genera on plastic from the North Shore Channel, Salt Creek, and the West Branch of the DuPage River were *Zymophilus* (19.1%), *Rheinheimera* (10.9%), and *Thiobacillus* (11.0%), respectively (Figure 17). Across streams, unclassified Pseudomonadaceae, *Acinetobacter*, *Arcobacter*, and *Azospira* had relative abundances on microplastic samples ranging from 0.7-8.6%, 0.3-4.3%, 0.2-3.5%, and 0.1-4.9%, respectively.

Table 13. Water column physiochemical characteristics and nutrient concentrations upstream and downstream of the wastewater treatment plant (WWTP) effluent outfalls at our study streams. Conductivity and DO are single measurements. Nutrient concentrations are reported as mean (±SE), n=3 upstream and 3 downstream.

	Conduct. (µS)		DO (mg L^{-1})		SRP (mg L^{-1})		NO_3^- (mg L^{-1})		NH_4^+ (µg L^{-1})	
Stream	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Higgen's Cr	1227	1040	7.6	8.6	0.0(0.0)	0.7(0.0)	0.2(0.1)	6.2(0.8)	248(16)	303(30)
Springbrook Cr	952	1032	7.5	8.0	1.7(0.1)	1.7(0.2)	10.4(0.8)	15.4(1.0)	634(40)	206(3)
L Kickapoo Cr	907	960	10.5	10.5	0.2(0.0)	1.4(0.1)	4.2(0.9)	14.5(0.2)	84(15)	166(30)
Schererville Ditch	1391	1476	9.6	7.9	0.6(0.1)	1.2(0.1)	13.1(1.7)	22.7(0.6)	160(60)	145(8)
N Shore Ch.	303.3	660	6.5	7.2	0.0(0.0)	0.7(0.0)	0.2(0.0)	5.3(0.2)	134(2)	245(10)
Goose Cr	920	1001	10.2	8.1	0.0(0.0)	1.4(0.1)	0.3(0.1)	14.6(0.4)	102(5)	404(84)
DuPage R	1076	1171	11.6	8.9	0.2(0.0)	1.1(0.1)	2.8(0.5)	9.7(0.4)	69(2)	142(6)
W Br DuPage R	998	1150	9.7	9.4	1.6(0.2)	1.5(0.2)	10.6(1.2)	15.3(1.8)	72(4)	167(24)
Salt Cr	1168	1077	7.7	7.6	1.2(0.3)	2.2(0.0)	8.2(0.7)	17.9(3.0)	183(57)	170(13)
E Br DuPage R	1087	1086	11.2	10.6	0.9(0.1)	1.4(0.1)	8.1(0.7)	15.5(1.0)	96(11)	144(6)

Abbreviations: DO=dissolved oxygen, SRP=soluble reactive phosphorus, NO3-=nitrate, and NH4+=ammonium, Cr=creek, N=north, Ch=channel, R=river, W=west, Br=branch, E=east.

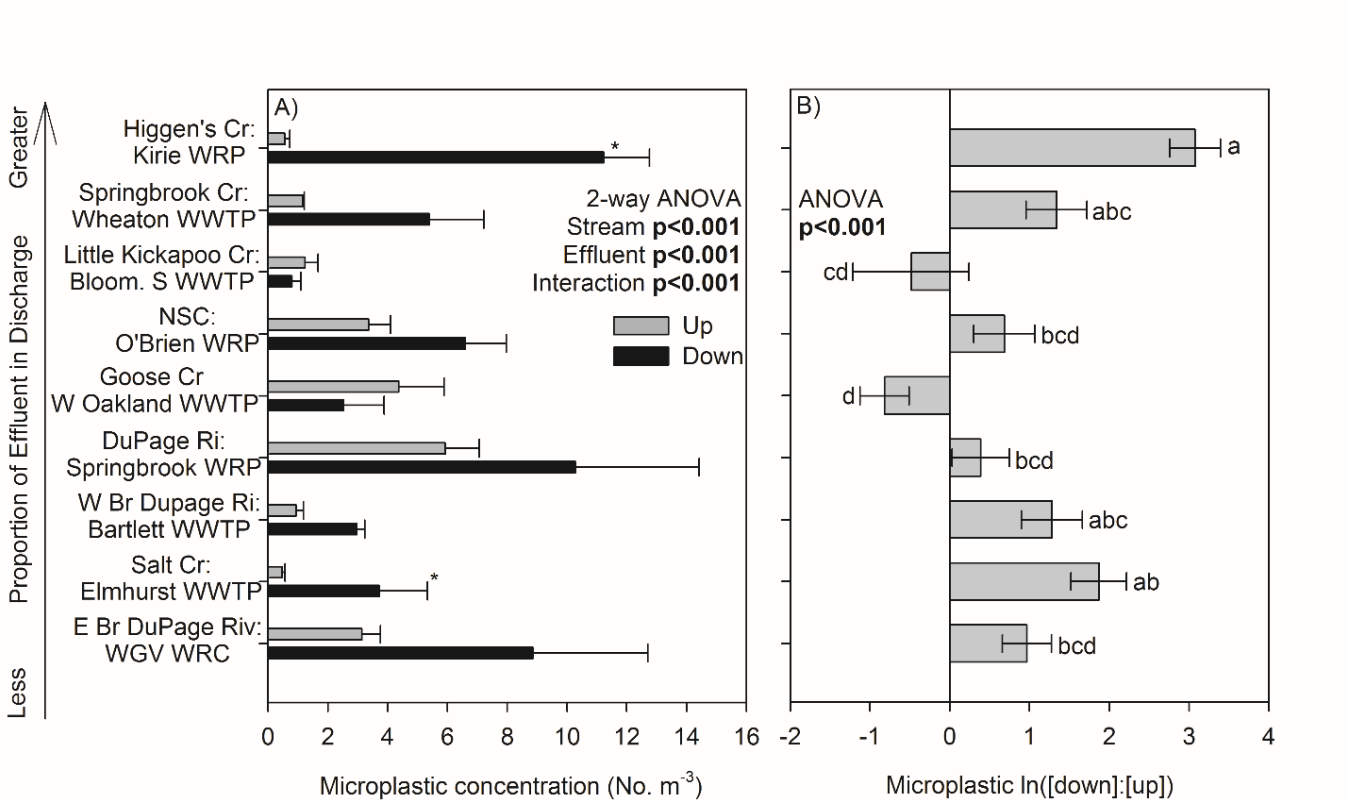

Figure 11. (A) Mean (±SE) microplastic concentration upstream and downstream of wastewater treatment plants (WWTP), water reclamation plants (WRP) or water reclamation centers (WRC) at 9 streams in Illinois (N=4 per mean). (B) Mean (±SE) ratio of microplastic concentration downstream and upstream at each site (N=4 per mean). * indicates significant difference in downstream and upstream concentrations with a Bonferroni Correction. Letter's represent Tukey's test results. Cr=creek, Bloom=Bloomington, NSC=North Shore Channel, S= south, W= west, E= east, Ri=river, Br=Branch, WGV= Woodridge Green Valley.

Table 14. Mean microplastic concentrations upstream and downstream of wastewater treatment plant (WWTP) effluent outfalls at our study streams. N=4 upstream and 4 downstream.

	Total		Fragments		Pellets		Foam		Film		Fibers	
Location	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down
Higgen's Cr	**0.57**	**11.22**	**0.17**	**2.50**	**0.13**	**6.76**	0.02	0.01	0.00	0.06	0.25	1.89
Springbrook Cr	1.17	5.39	0.43	0.91	0.45	2.41	0.00	0.03	0.00	0.02	0.29	2.02
L Kickapoo Cr	1.24	0.80	0.12	0.10	0.30	0.23	0.00	0.00	0.00	0.00	0.82	0.47
N Shore Ch.	3.36	6.60	0.94	2.19	0.45	1.57	0.04	0.03	0.02	0.07	1.92	2.75
Goose Cr	4.37	2.53	0.36	0.12	1.39	1.87	0.00	0.01	0.00	0.00	2.62	0.53
DuPage R	5.92	10.28	1.67	2.09	1.01	3.24	**0.09**	**0.01**	0.03	0.15	3.12	4.79
W Br DuPage R	0.93	2.96	0.24	0.68	**0.08**	**1.04**	0.00	0.02	0.00	0.02	0.61	1.21
Salt Cr	**0.48**	**3.73**	0.15	1.23	0.03	0.52	0.01	0.00	0.00	0.01	0.29	1.97
E Br DuPage R	3.14	8.86	0.66	0.67	0.46	2.65	0.13	0.26	0.01	0.00	1.89	5.29

Bolded values indicate a significant difference after applying a Bonferroni Correction ($\alpha=0.05/9=0.0056$). Abbreviations: Cr=creek, L=little, N=north, Ch=channel, R=river, W=west, Br=branch, E=east.

Table 15. 2-way ANOVA results for comparing microplastic concentration by study streams (site) and effluent effect (upstream and downstream sampling location).

Microplastic Type	Factor	F Stat.	p-value
Total	Site	9.67	**<0.001**
	Effluent	36.97	**<0.001**
	Interaction	6.97	**<0.001**
Fragments	Site	11.56	**<0.001**
	Effluent	22.33	**<0.001**
	Interaction	4.79	**<0.001**
Pellets	Site	5.36	**<0.001**
	Effluent	43.06	**<0.001**
	Interaction	4.15	**0.001**
Foam	Site	27.57	**<0.001**
	Effluent	1.04	0.313
	Interaction	5.41	**<0.001**
Film	Site	6.74	**<0.001**
	Effluent	15.53	**<0.001**
	Interaction	3.15	**0.005**
Fibers	Site	6.79	**<0.001**
	Effluent	11.20	**0.002**
	Interaction	3.68	**0.002**

Total concentration data were ln transformed. Concentration data for all microplastic categories were ln(x+0.5) transformed.

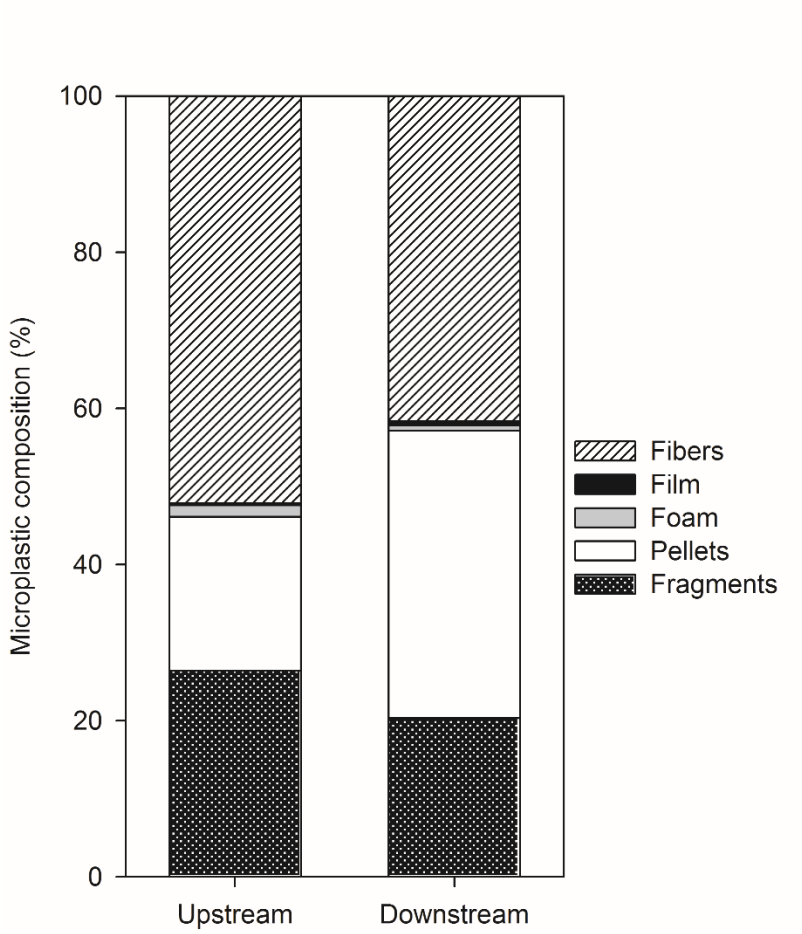

Figure 12. Mean relative abundance of each microplastic category upstream and downstream of wastewater treatment plant effluent at 9 study sites.

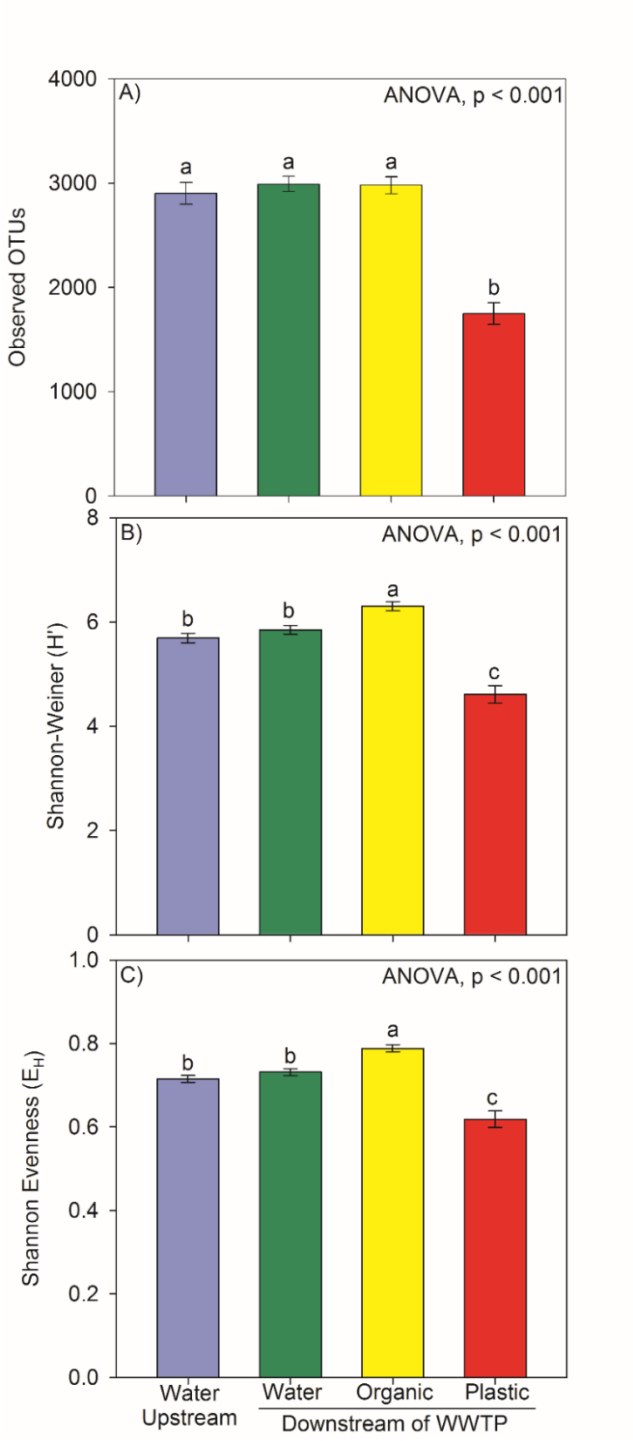

Figure 13. Mean (±SE) (A) number of observed bacterial operational taxonomic units (OTUs), (B) Shannon-Weiner diversity index (H'), and (C) Shannon evenness index (E_H) for bacterial assemblages from all study sites. P-values are from 1-way ANOVA comparing measurements among the 4 sample types. Letters show Tukey's test results. WWTP = wastewater treatment plant.

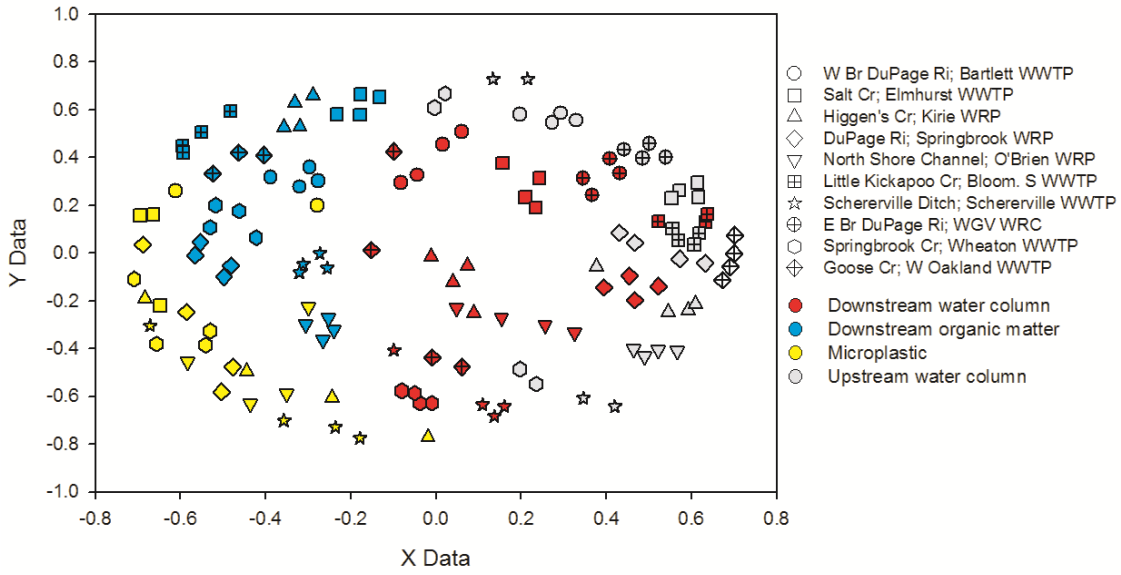

Figure 14. Non-metric multi-dimensional scaling (nMDS) ordination of 16S sequencing data (Bray-Curtis similarity index) comparing bacterial assemblages collected in 10 study streams. Note: microplastic was not visible at 3 sites (Little Kickapoo Cr, Goose Cr, and E Br DuPage Ri), thus there were no microplastic sample types from these sites for bacterial analysis. Br = Branch, Ri = river, Cr = creek, WWTP = wastewater treatment plant, WRP = water reclamation plant, WRC = water reclamation center, Bloom = Bloomington, S = south, W = west, E = east, Ri = river, Br = Branch, WGV = Woodridge Green Valley.

Table 16. Results of AMOVA analysis describing differences in bacterial community composition based on a comparison of the Bray-Curtis dissimilarity index for 4 sample types. (U=upstream water column; D=downstream water column; O=organic material; P=microplastic).

Comparison	P-value
D-O-P-U	<0.001*
D-O	<0.001*
D-P	<0.001*
D-U	<0.001*
O-P	<0.001*
O-U	<0.001*
P-U	<0.001*

Bonferroni adjusted error rate: 0.008.

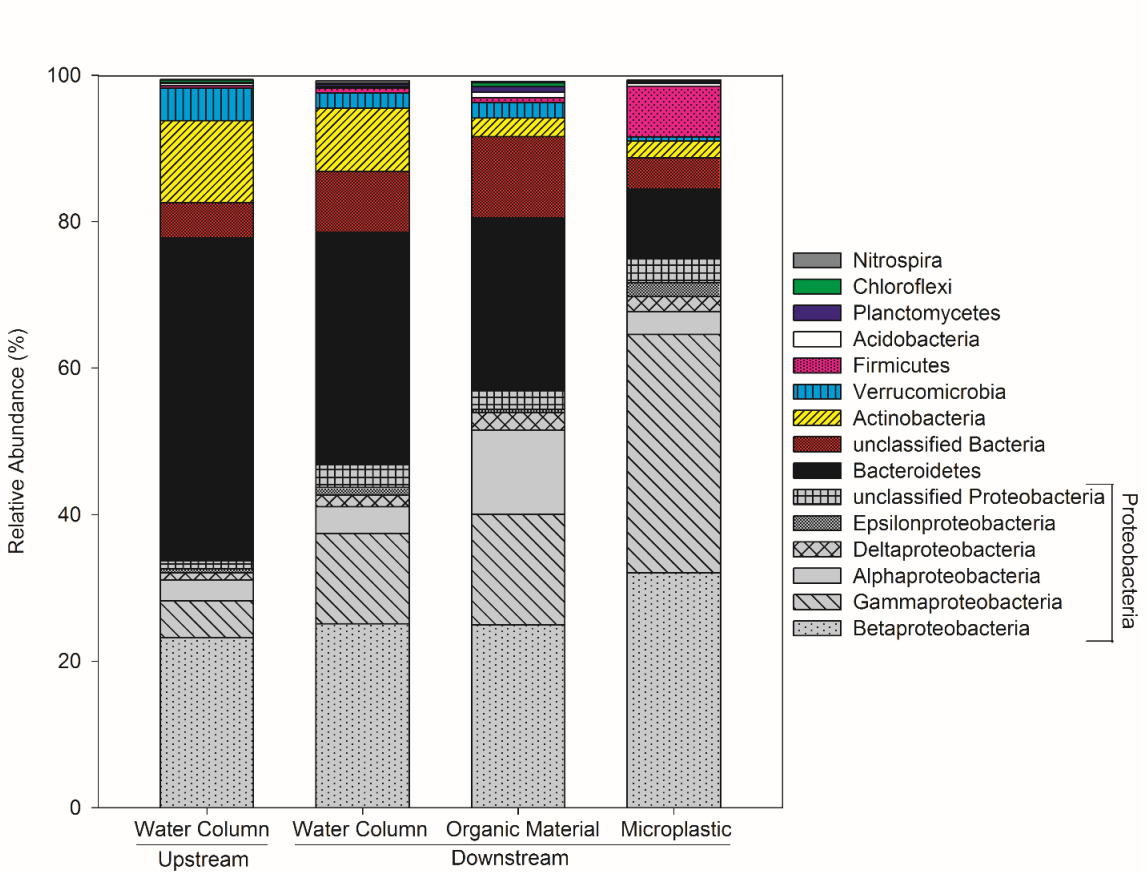

Figure 15. Relative mean abundance of the 10 most abundant phyla based on 16S sequencing data for bacterial assemblages collected in 10 study streams. Proteobacteria is represented by relative abundance of classes.

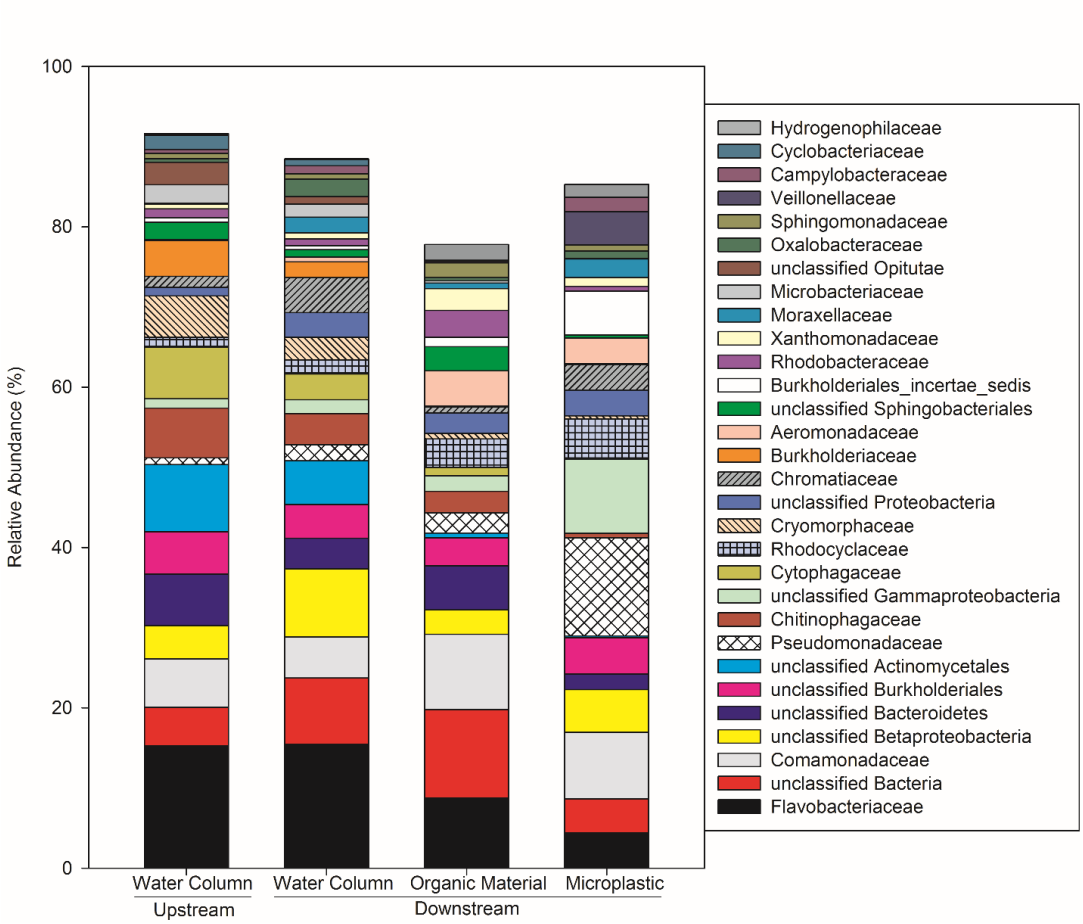

Figure 16. Relative mean abundance of the 30 most abundant families based on 16S sequencing data for bacterial assemblages collected in 10 study streams.

Table 17. 1-way ANOVA results comparing relative abundance of the 30 most common bacterial families based on 16S sequencing data among 4 sample types. Letters represent Tukey's test results.

Taxa	p-value	Water up	Water down	Organic	Plastic
Flavobacteriaceae	**0.019**	15.28^{ab}	15.42^{ab}	8.73^{bc}	4.39^{c}
unclassified Bacteria	**0.003**	4.76^{bc}	8.30^{ac}	11.07^{a}	4.26^{bc}
Comamonadaceae	0.059	6.06	5.09	9.36	8.31
unclassified Betaproteobacteria	0.076	4.17	8.52	3.04	5.29
unclassified Bacteroidetes	**<0.001**	6.42^{ac}	3.81^{bc}	5.49^{ac}	1.92^{b}
unclassified Burkholderiales	0.329	5.26	4.22	3.50	4.55
unclassified Actinomycetales	**<0.001**	8.37^{a}	5.42^{ab}	0.61^{bc}	0.24^{bc}
Pseudomonadaceae	**<0.001**	0.84^{a}	2.02^{a}	2.53^{a}	12.19^{b}
Chitinophagaceae	**0.001**	6.21^{ab}	3.92^{ab}	2.69^{bc}	0.58^{c}
unclassified Gammaproteobacteria	**0.001**	1.17^{a}	1.70^{a}	1.91^{a}	9.25^{b}
Cytophagaceae	**0.003**	6.45^{a}	3.22^{ab}	1.07^{bc}	0.16^{bc}
Rhodocyclaceae	**0.020**	1.19^{a}	1.75^{ab}	3.53^{ab}	4.84^{b}
Cryomorphaceae	**<0.001**	5.18^{a}	2.82^{b}	0.71^{c}	0.43^{c}
unclassified Proteobacteria	**0.027**	1.10	3.08	2.55	3.20
Chromatiaceae	0.073	1.34	4.35	0.75	3.14
Burkholderiaceae	**<0.001**	4.48^{a}	1.98^{b}	0.08^{b}	0.14^{b}
Aeromonadaceae	**0.009**	0.13^{a}	0.56^{a}	4.41^{b}	3.23^{ab}
unclassified Sphingobacteriales	**0.013**	2.13^{ab}	0.93^{ab}	3.02^{b}	0.36^{a}
Burkholderiales_incertae_sedis	**<0.001**	0.53^{a}	0.51^{a}	1.15^{a}	5.45^{b}
Rhodobacteraceae	**0.001**	1.14^{a}	0.84^{a}	3.40^{b}	0.62^{a}
Xanthomonadaceae	**<0.001**	0.56^{a}	0.78^{a}	2.70^{b}	1.08^{a}
Moraxellaceae	**0.023**	0.15^{a}	1.95^{ab}	0.68^{ab}	2.34^{b}
Microbacteriaceae	**0.001**	2.31^{a}	1.64^{ab}	0.26^{bc}	0.08^{c}
unclassified Opitutae	**0.038**	2.80	0.93	0.01	0.00
Oxalobacteraceae	0.220	0.47	2.17	0.45	0.86
Sphingomonadaceae	**0.007**	0.66^{a}	0.70^{a}	1.80^{b}	0.79^{ab}
Veillonellaceae	0.078	0.01	0.02	0.02	4.16
Campylobacteraceae	0.070	0.48	0.96	0.23	1.74
Cyclobacteriaceae	**0.001**	1.76^{a}	0.84^{ab}	0.08^{b}	0.03^{b}
Hydrogenophilaceae	0.526	0.20	0.05	1.95	1.65

Relative proportion data were asin(sqrt(x)) transformed.

Table 18. Bacterial operational taxonomic units (OTUs) making the most significant contribution to variation between communities from plastic and organic material collected downstream of WWTPs. Each data point is the mean relative abundance. P-value based on a t-test comparison of plastic and organic material samples.

Taxon	Organic material	Plastic	p-value	Contr. to var (%)	Cumul. contr. to var (%)
unclassified Gammaproteobacteria	1.90	10.12	0.007	6.92	6.92
unclassified Bacteria	11.12	3.81	<0.001	6.23	13.15
Pseudomonas	0.87	7.58	0.001	5.21	18.36
Flavobacterium	7.97	4.04	0.001	4.30	22.66
Aquabacterium	0.92	5.28	0.015	3.60	26.26
unclassified Pseudomonadaceae	0.86	4.85	0.008	3.22	29.48
unclassified Betaproteobacteria	3.02	5.47	0.029	3.01	32.49
unclassified Bacteroidetes	5.51	1.82	<0.001	2.90	35.39
unclassified Sphingobacteriales	3.04	0.31	<0.001	2.09	37.48
Rheinheimera	0.75	2.56	0.038	1.92	39.40
unclassified Rhodobacteraceae	2.40	0.40	<0.001	1.55	40.95
Acinetobacter	0.31	2.05	0.007	1.47	42.42
unclassified Rhizobiales	1.86	0.35	<0.001	1.19	43.61
unclassified Chitinophagaceae	1.88	0.38	<0.001	1.18	44.79
unclassified Alphaproteobacteria	1.67	0.39	<0.001	1.01	45.80
Arcobacter	0.22	1.42	0.002	0.98	46.78
Azospira	0.07	1.30	0.010	0.97	47.75
unclassified Xanthomonadaceae	1.42	0.64	<0.001	0.84	48.59
unclassified Sphingomonadaceae	1.17	0.41	0.002	0.82	49.41
Cellvibrio	0.82	0.39	0.030	0.63	50.04
Arenimonas	0.80	0.11	0.004	0.56	50.60
unclassified Cytophagaceae	0.72	0.07	<0.001	0.52	51.12
Prosthecobacter	0.71	0.08	<0.001	0.52	51.64
Rhodobacter	0.71	0.08	<0.001	0.50	52.14
Methylophilus	0.70	0.14	<0.001	0.49	52.63
unclassified Flavobacteriaceae	0.67	0.06	0.032	0.47	53.10
Deefgea	0.65	0.18	0.006	0.47	53.57
Thiothrix	0.52	0.09	0.017	0.41	53.98
unclassified Actinomycetales	0.62	0.22	0.014	0.40	54.38
unclassified Saprospiraceae	0.45	0.03	<0.001	0.32	54.70
unclassified Planctomycetaceae	0.46	0.09	<0.001	0.32	55.02
Sulfurospirillum	0.02	0.41	0.002	0.31	55.33
Haliea	0.38	0.06	<0.001	0.28	55.61

Haliscomenobacter	0.39	0.05	<0.001	0.27	55.88
3_genus_incertae_sedis	0.41	0.09	<0.001	0.26	56.14
unclassified Sphingomonadales	0.33	0.10	0.021	0.26	56.40
unclassified Hyphomicrobiaceae	0.33	0.04	<0.001	0.24	56.64
unclassified Deltaproteobacteria	0.35	0.12	<0.001	0.23	56.87
Ohtaekwangia	0.32	0.03	<0.001	0.23	57.10
unclassified Burkholderiales_incertae_sedis	0.05	0.32	0.002	0.22	57.32
Ferruginibacter	0.30	0.04	<0.001	0.21	57.53
unclassified Actinobacteria	0.29	0.04	<0.001	0.21	57.74
Bacteroides	0.05	0.24	0.024	0.20	57.94
Sediminibacterium	0.29	0.08	<0.001	0.19	58.13
unclassified Verrucomicrobiaceae	0.27	0.03	<0.001	0.19	58.32
Porphyrobacter	0.25	0.05	0.004	0.18	58.50
Catellibacterium	0.24	0.07	0.018	0.18	58.68
unclassified Methylococcaceae	0.22	0.06	0.025	0.17	58.85
unclassified Acidimicrobiales	0.25	0.03	<0.001	0.17	59.02
Nitrospira	0.22	0.06	<0.001	0.17	59.19
unclassified Verrucomicrobia	0.25	0.03	<0.001	0.17	59.36
Caldilinea	0.23	0.02	<0.001	0.16	59.52
unclassified Microbacteriaceae	0.24	0.06	0.001	0.16	59.68
Bosea	0.23	0.04	0.002	0.16	59.84
Sphingomonas	0.21	0.06	0.002	0.15	59.99
Gp4	0.21	0.04	0.001	0.15	60.14
Novosphingobium	0.23	0.06	<0.001	0.15	60.29
Cloacibacterium	0.04	0.20	0.014	0.14	60.43
Byssovorax	0.17	0.04	0.010	0.14	60.57
Silanimonas	0.18	0.03	0.008	0.13	60.70

Abbreviations: contr=contribution, var=variation, cumul=cumulative

Table 19. Bacterial operational taxonomic units (OTUs) making the most significant contribution to variation between communities from upstream and downstream water column samples. Each data point is the mean relative abundance. P-value based on a t-test comparison of upstream and downstream water column samples.

Taxon	Down water column	Up water column	p-value	Contrib. to var. (%)	Cumul. contrib. to var. (%)
unclassified Actinomycetales	5.30	8.37	0.015	6.55	6.55
unclassified Betaproteobacteria	8.60	4.17	0.003	5.85	12.40
unclassified Cytophagaceae	2.89	5.07	0.029	4.94	17.34
unclassified Bacteria	8.36	4.76	<0.001	4.30	21.64
Rheinheimera	4.43	1.28	0.001	4.13	25.77
Sediminibacterium	2.16	4.34	0.004	3.93	29.70
unclassified Bacteroidetes	3.69	6.42	<0.001	3.85	33.55
Polynucleobacter	1.83	4.39	<0.001	3.35	36.90
unclassified Opitutae	0.77	2.80	0.002	2.98	39.88
unclassified Cryomorphaceae	2.07	3.68	0.001	2.79	42.67
unclassified Proteobacteria	3.14	1.10	<0.001	2.50	45.17
unclassified Microbacteriaceae	1.51	2.18	0.050	1.82	46.99
unclassified Sphingobacteriales	0.91	2.13	0.009	1.81	48.80
Algoriphagus	0.78	1.75	0.001	1.55	50.35
Undibacterium	1.44	0.04	0.009	1.45	51.80
Fluviicola	0.64	1.48	<0.001	1.08	52.88
Acinetobacter	1.07	0.06	<0.001	1.05	53.93
unclassified Gammaproteobacteria	1.72	1.17	0.001	0.92	54.85
Zoogloea	0.87	0.07	0.001	0.83	55.68
unclassified Moraxellaceae	0.66	0.06	<0.001	0.62	56.30
unclassified Pseudomonadaceae	0.70	0.18	0.008	0.61	56.91
Mycobacterium	0.63	0.09	<0.001	0.57	57.48
Pseudomonas	0.48	0.16	0.001	0.47	57.95
Aeromonas	0.53	0.10	0.002	0.46	58.41
Porphyrobacter	0.43	0.02	0.037	0.43	58.84
unclassified Alphaproteobacteria	0.62	0.28	<0.001	0.39	59.23
Gordonia	0.36	0.00	0.037	0.37	59.60
Nitrospira	0.39	0.04	<0.001	0.36	59.96
unclassified Oxalobacteraceae	0.47	0.31	0.049	0.35	60.31
Shewanella	0.29	0.02	0.006	0.29	60.60
unclassfied Myxococcales	0.34	0.07	<0.001	0.28	60.88
Armatimonas_Armatimonadetes_gp1	0.05	0.29	<0.001	0.28	61.16

unclassified Xanthomonadaceae	0.41	0.26	0.003	0.28	61.44
Aquabacterium	0.39	0.19	<0.001	0.28	61.72
unclassified Methylococcaceae	0.06	0.29	0.004	0.27	61.99
3_genus_incertae_sedis	0.18	0.33	0.019	0.25	62.24
Bdellovibrio	0.36	0.13	<0.001	0.25	62.49
Luteolibacter	0.08	0.25	0.009	0.23	62.72
unclassified Anaerolineaceae	0.06	0.25	0.043	0.23	62.95
Albidiferax	0.27	0.15	0.007	0.22	63.17
Alkanindiges	0.23	0.02	<0.001	0.22	63.39

Abbreviations: contr=contribution, var=variation, cumul=cumulative

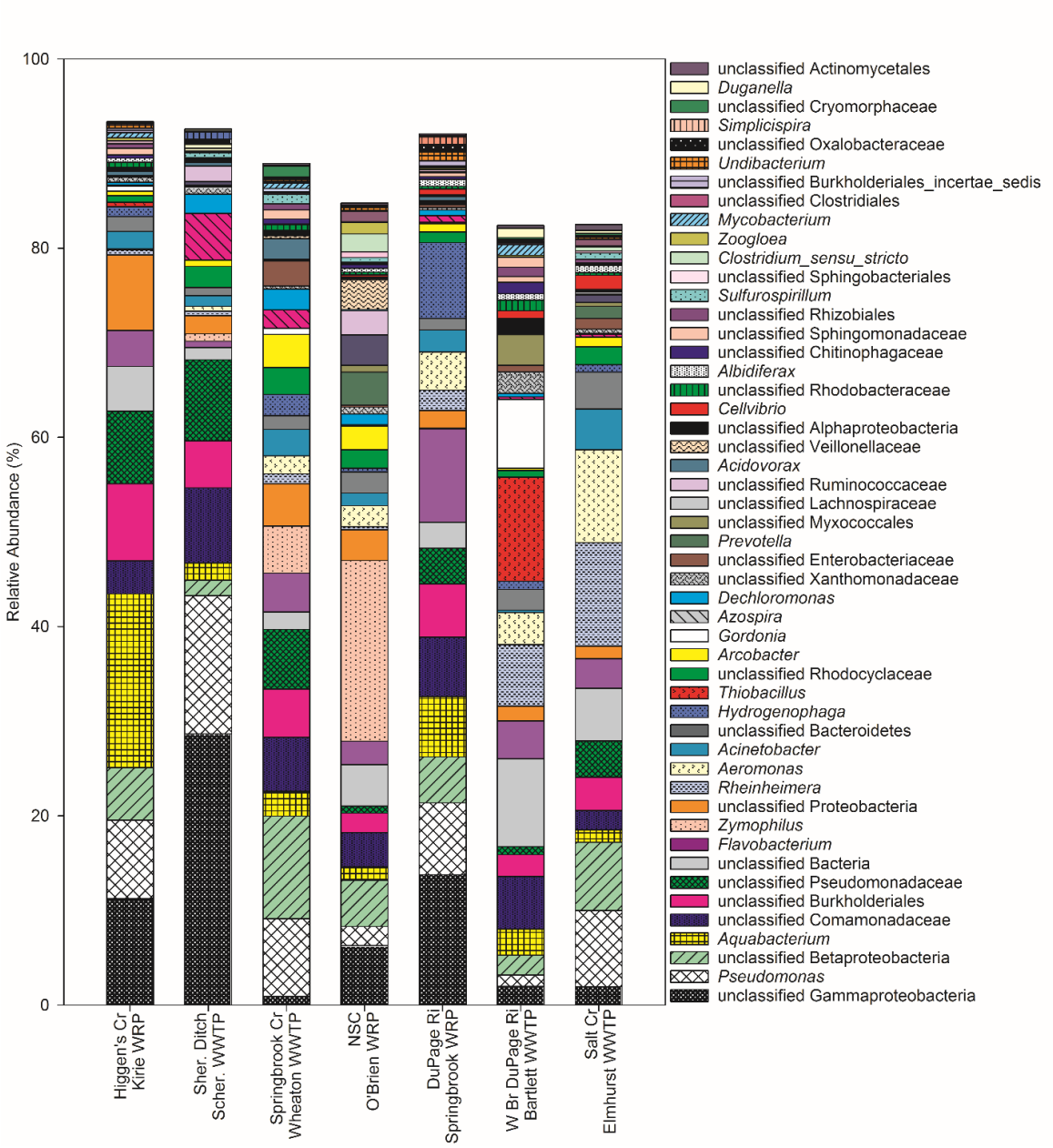

Figure 17. Relative mean abundance of 50 most abundant taxa based on 16S sequencing data for microplastic bacterial assemblages from our study sites. Note: microplastic was not visible at 3 sites (Little Kickapoo Cr, Goose Cr, and E Br DuPage Ri), thus there were no microplastic sample types from these sites for bacterial analysis. Cr = creek, Br = Branch, Ri = river, WWTP = wastewater treatment plant, WRP = water reclamation plant, W = west, NSC = North Shore Channel, Scher = Schererville.

Discussion

Microplastic concentrations in urban rivers

Our results for microplastic concentration and the composition of microplastic types suggest that WWTP effluent is an important source of microplastic to urban rivers. Microplastic concentrations were higher downstream of WWTPs than upstream at all but two sites. Pellets, which are associated with personal care products that enter WWTPs (Fendall and Sewell 2009), had a higher relative abundance downstream, and their concentration was higher downstream at all but one site. While the relative abundance of fibers was higher upstream than downstream, fibers made up a large proportion of microplastic from both upstream and downstream locations, and the concentration of fibers was higher downstream at all but two sites. Previous research showed microplastic fibers are deposited in coastal sediment via treated wastewater (Browne et al. 2011). Some fibers are retained in WWTP sludge products, which are applied as fertilizer (Habib et al. 1998; Zubris and Richards 2005). Therefore, we suspect fibers also enter aquatic systems as runoff.

Microplastic concentrations showed high variation among streams, which is consistent with previous research showing microplastic concentrations are spatially and temporally heterogeneous (Yonkos et al. 2014; Dubaish and Liebezeit 2013; Gilfillan et al. 2009; Goldstein et al. 2013). Differences in microplastic concentrations among streams could be explained by variation in landscape features such as the number of WWTPs, combined sewer overflows (CSO), impervious surface cover, dams, and stream geomorphology. These features could enhance microplastic delivery or deposition. For instance, the DuPage River in Naperville, IL and the North Shore Channel in Chicago, IL

had relatively high microplastic concentrations at downstream and upstream sampling sites. The East and West Branches of the DuPage River contain several WWTPs, and they join to form the DuPage River ~730 m upstream of the Springbrook Water Reclamation Plant (WRP). Additionally, water from Lake Michigan, which contains treated effluent from various municipalities including Milwaukee, WI, flows into the North Shore Channel. Eriksen et al. (2013a) measured microplastic concentrations in 3 of the Great Lakes and found high concentrations near urban centers, so it is likely that the nearshore waters of Lake Michigan are a source of microplastic to the North Shore Channel. During heavy rainfall, the North Shore Channel also receives untreated wastewater via CSOs that can contribute to microplastic accumulation.

Microplastic concentrations were also variable among replicate net samples within each sampling site (i.e., net samples were collected simultaneously or in sequence), suggesting microplastic distribution within a stream is spatially and temporally heterogeneous. Microplastic is a composite of different types of polymers, at different stages of biofilm colonization, and of different sizes. Microplastic pieces collected in surface water may be recently suspended from sediment, in the processes of deposition, or permanently buoyant (i.e., polystyrene). To our knowledge, no previous work has measured distribution of microplastic at multiple sites through the water column simultaneously to determine the extent to which a net collecting surface water accurately represents the instantaneous microplastic flux in the river water column. These assessments represent an important line of questioning for future research.

Although WWTP effluent influenced microplastic concentrations at almost all of our sites, it had no effect at 2 streams in Bloomington, IL (Goose and Little Kickapoo

Creeks; Figure 11). We propose two possible explanations for this pattern: sand filtration and upstream hydrology. Both plants utilize sand filtration as a tertiary treatment method which may effectively retain microplastic particles. Locations with sand filters (n=5) and without sand filters (n=4) had mean downstream to upstream microplastic concentration ratios of 0.43 and 1.71, respectively. While the ratio was lower at sites using sand filtration, there was no significant difference, because the ratios were highly variable among the 5 sites with sand filters. Furthermore, our study was not explicitly designed to test the effect of tertiary treatment methods, such as sand filtration, on microplastic concentrations in WWTP effluent. Studies comparing microplastic concentration in sewage influent, WWTP effluent, and various steps in the wastewater treatment process are warranted and would illustrate effective methods for microplastic retention. On the other hand, the upstream sampling location at Goose Creek had very low discharge, and the downstream discharge was 31 times higher than upstream (the largest difference among sites). In addition, the water upstream at Goose Creek was very shallow, so that only ~1/3 of the net was submerged. This resulted in a low volume of water collection, and thus the low number of microplastic generated very high concentration. By absolute number, we collected ~6.5 times more microplastic particles downstream in Goose Creek than upstream.

Microplastic concentration in urban rivers is higher than other ecosystems

We compared our data to global microplastic concentrations from a variety of ecosystems which used the same size range for microplastic collection. Similar to our previous research (McCormick et al. 2014), we found that riverine microplastic concentrations are among the highest in the literature. Mean upstream and downstream

microplastic concentrations from this study were higher than mean concentrations from studies in the open ocean, and our maximum concentration was higher than almost all measurements from the open ocean (Table 20). Coastal regions are considered areas of high microplastic concentration, and mean riverine microplastic concentrations from this study were comparable to mean coastal measurements. Our riverine measurements were also higher than estuarine studies, and equal to or higher than concentrations from lakes (Table 20). The mean downstream microplastic concentration was similar to maximum concentrations reported in the Great Lakes (Eriksen et al. 2013a), and riverine concentrations were 15-40 times higher than the maximum concentration from a remote lake in Mongolia (Free et al. 2014). Finally, our results were in the range of other riverine microplastic data. However, during the wet season, Moore et al. (2011) documented higher microplastic concentrations in the San Gabriel River, CA, USA. Additionally, the maximum concentration from the Danube River (Lechner et al. 2014) was 6 times higher than our maximum measurement. If we consider studies that used a larger size range than our equipment (0.08-0.33 mm plastic particles), the maximum measurements from the Seine River (Dris et al. 2015b) were higher than our values.

Several methodological variations limited the number of studies to which we could compare our data. For instance, sampling methods impact the size range of collected microplastic. Lozano and Mouat (2009) reported that microplastic concentrations were up to 100,000 times higher when a net with 80 µm mesh was used compared to 450 µm mesh, and Song et al. (2014) collected much higher microplastic concentrations with sampling techniques that isolated 1 µm particles in comparison to a neuston net with 330 µm mesh (Table 20). Therefore, we only directly compared our data

to studies that used a size threshold of 330-335 µm when sampling. Comparing results across published studies is also complicated by differences in concentration units (Hidalgo-Ruz et al. 2012). Some studies report concentrations in terms of surface area (i.e., No. km^{-2}) (Free et al. 2014; Law et al. 2010; Eriksen et al. 2013b; Yamashita and Tanimura 2007), while others use volume (i.e., No. m^{-3}) (Lechner et al. 2014; Gilfillan et al. 2009; Moore et al. 2002; Lozano and Mouat 2009; Lattin et al. 2004). Some studies provide a depth of net submergence (Yonkers et al. 2014; Goldstein et al. 2013), which allows the conversion of concentration data between areal and volumetric units. We recommend that future studies report mean concentrations in terms of area and volume to facilitate cross-ecosystem comparisons.

Bacterial assemblages colonizing microplastic are unique from natural habitats

Habitat and WWTP effluent were major drivers of bacterial assemblages, which were significantly different on microplastic, organic material, and the upstream and downstream water column. Few studies have examined microplastic's effects on microbial communities, but our results showing microplastic selects for a unique community of bacteria is consistent with results from other studies (Zettler et al. 2013; McCormick et al. 2014). In particular, community richness and diversity on microplastic was low compared to natural substrates, consistent with data from urban rivers (McCormick et al. 2014).

The differences between the bacterial assemblages on organic material and plastic is of particular interest as these microbial habitats exist in close proximity in rivers and were collected simultaneously in the same net. Several taxa were more abundant on plastic, but the mechanism for the selection of bacteria by microplastic is unknown.

These taxa may utilize the hard surface of microplastic as habitat, or they may have the capacity to metabolize the plastic polymers as a carbon source (McCormick et al. 2014). In the Atlantic Ocean, microbial digestion of microplastic was evidenced by pits which conform to bacterial cell shapes on microplastic surfaces (Zettler et al. 2013).

We used the identity of bacterial taxa to infer that microplastic selects for both biofilm-forming organisms and those with the capacity to break down plastic compounds. Among the most significant distinctions between plastic and other habitats was the relatively high abundance of Pseudomonadaceae and unclassified Gammaproteobacteria. Previous research has shown Gammaproteobacteria are early biofilm colonizers of non-natural substrates in marine habitats (Lee et al. 2008), and these bacteria also are prevalent in biofilms located downstream of WWTPs (Marti et al. 2013). In particular, the Gammaproteobacteria genus *Pseudomonas* had significantly higher abundance on microplastic than organic material. *Pseudomonas* was also prevalent on microplastic-associated bacterial assemblages from our previous work in the North Shore Channel (McCormick et al. 2014), and it is common genus in other urban waterways (Ibekwe et al. 2013). *Pseudomonas* has been associated with degradation of plastic polymers such as high-density polyethylene (HDPE) (Balasubramanian et al. 2010), low-density polyethylene (LDPE) (Tribedi et al. 2015), polythene (Kathiresan 2003), polypropylene (Cacciari et al. 1993; Arkatkar et al. 2010), and polyvinyl alcohol (PVA) (Shimao 2001). Strains of *Pseudomonas* produce enzymes such as serine hydrolases, esterases, and lipases which assist in plastic biodegradation (Bhardwaj et al. 2013). Furthermore, previous studies have shown plastic degradation by *Pseudomonas* is rapid. Strains collected from plastic waste disposal sites contributed to a 15% weight loss of HDPE

after a 30 day incubation experiment (Balasubramanian et al. 2010), and *Pseudomonas* spp. degraded over 20% of polythene in 30 days (Kathiresan 2003). While our data does not identify specific strains or species of *Pseudomonas*, its noticeable presence on microplastic substrates in this study and previous research (McCormick et al. 2014) suggests that these taxa may be selected by microplastic for their ability to digest plastic compounds.

Aquabacterium (family Comamonadaceae) also had a high relative abundance on microplastic, and previous research identified this taxa as a dominant member of biofilms that formed on plastic substrates in drinking water facilities (Kalmbach et al. 2000). Drinking water is oligotrophic and dark in comparison to WWTP effluent and the water column of urban rivers, so their abundance may be related to the presence of plastic polymers. Some members of the *Aquabacterium* genus metabolize plasticizers used in soft-PVC (Kalmbach et al. 1999), so it is possible that these taxa have plastic-degrading capabilities.

In addition to biofilm-forming and plastic-degrading bacteria, some of the taxa common to microplastic assemblages are associated with pathogenic bacteria. For instance, Campylobacteraceae had higher relative abundance on microplastic than all other habitats in this study and in our previous research (McCormick et al. 2014). This family is known to include several pathogens (On et al. 2001; Lu and Lu 2014). In particular, *Arcobacter*, which is a member of Campylobacteraceae, was significantly higher on microplastic than organic material, and it is a genus containing pathogenic species (Lu and Lu 2014; Engberg et al. 2000) that is abundant in sewage influent (Newton et al. 2013). The ability of microplastic to transport pathogenic bacteria from

WWTPs to rivers poses a potential threat to human and ecosystem health. It is possible that pathogenic bacteria are most abundant on microplastic recently emerging from the WWTP, as the environmental conditions in rivers are not typically suitable for their survival. However, more research on the capacity of microplastic to transport pathogenic bacteria longer distances downstream than natural surfaces is needed.

Like concentration of microplastic among study streams, bacterial assemblages on microplastic were variable among sites. The chemical properties of the various microplastic polymers and the environmental differences among streams likely facilitated this pattern. Previous studies on microplastic-associated bacterial assemblages also show variation in community composition. For instance, Zettler et al. (2013) described a diverse 'plastisphere' assemblage on microplastic in the marine pelagic environment, where *Vibrio* was a dominant member of bacterial assemblages. With an incubation experiment using marine sediment, Harrison et al. (2014) found that after 14 d bacterial communities on LDPE were almost exclusively dominated by two genera: *Arcobacter* and *Colwellia*. We found no *Vibrio* or *Colwellia* in our samples, but *Arcobacter* was significantly more abundant on microplastic than suspended organic matter. Studies on the interactions between microbes and microplastic are lacking (Harrison et al. 2011), and further research is necessary to understand microplastic's ecological impacts. In particular, more research that further identifies strains that metabolize microplastic and the potential for live pathogens to persist on microplastic is needed.

WWTP effluent influences water column bacteria assemblages

We found that WWTP effluent influenced bacterial communities in the upstream and downstream water column habitats. At the phylum level, the most noticeable

difference was that Bacteroidetes abundance was higher upstream, and Proteobacteria abundance was higher downstream. Several genera of interest such as *Mycobacterium*, *Acinetobacter*, and *Aeromonas* were significantly more abundant in the downstream than upstream water column. *Mycobacterium* was also found in high abundances in effluent samples from a WWTP in Hong Kong (Ye and Zhang 2013), and this genus is known to contain a variety of pathogenic bacteria (Bibby et al. 2010; Ye and Zhang 2011; Ibekwe et al. 2013). Previous research has shown that *Acinetobacter* and *Aeromonas* are prevalent taxa in biofilms near WWTP effluent outfalls (Marti et al. 2013) and that *Acinetobacter* is abundant in sewage influent (Newton et al. 2013). Additionally, *Aeromonas* is known to contain pathogenic taxa (Ye and Zhang 2011; Bibby et al. 2010), and taxa from this genus in urban waterways can have resistance to some antibacterial agents (Cattoir et al. 2012).

The fate of riverine microplastic

Urban rivers contain high microplastic concentrations in surface waters compared to other habitats, and converting concentrations to flux measurements showed rivers in our study can transport over 4.5 million microplastic pieces d^{-1} (Table 21). However, we know little about the downstream movement and deposition of microplastic in rivers. It is unclear what portion of riverine microplastic travels downstream and what portion is deposited to the benthic zone. Some microplastic is likely transported long distances, as several recent studies report high concentrations of microplastic in estuaries and other coastal habitats and implicated rivers as major microplastic sources to the ocean (Yonkers et al. 2014; Moore et al. 2002; Dubaish and Liebezeit 2013; Lima et al. 2014; Sadri and Thompson 2014). However, some microplastic is deposited into sediments, as

microplastic concentrations in St. Lawrence River sediments were ~137,590 No. m^{-3} (Castañeda et al. 2014) and microplastic concentrations in sediment were up to 15,000 times higher than surface water samples in the North Shore Channel (T. Hoellein, unpublished data). Biofilm formation may decrease the buoyancy of microplastic and thus contribute to its accumulation in sediments (Castañeda et al. 2014), but we suspect that microplastic depositional patterns are also driven by hydrology (i.e., storms), geomorphology (i.e., depositional zones and dams), and location with a river network (i.e., headwater streams to large rivers).

Future research and management implications

Results from this study provide an experimental framework and intellectual justification for continuing research on microplastic-associated biofilms in freshwaters and exploring their impact on higher trophic levels. For example, while marine organisms (i.e., filter-feeders, grazers, and predators) ingest microplastic (Wright et al. 2013), consumer ingestion of microplastic in freshwater ecosystems is largely unknown (Wagner et al. 2014; Imhof et al. 2013). Microplastic ingestion by freshwater organisms has been recorded for wild gudgeons (*Gobio gobio*) in French Rivers (Sanchez et al. 2014), and in a controlled experiment, de Sá et al. (2015) demonstrated that gobies (*Pomatoschistus microps*) collected from estuaries ingested microplastic which reduced their predatory performance. In a laboratory experiment, Imhof et al. (2013) showed that a variety of freshwater invertebrates from different trophic levels ingest artificially ground fluorescent microplastic. Invertebrates and fish play key components in aquatic food webs, and future studies on micoplastic's effects on organism fitness, secondary production, and life history are warranted.

Microplastic accumulation in the environment is an emerging topic of concern to the scientific community and the general public. Recent legislation from some European countries and several states in the USA proposed bans on the sale of personal care products containing microplastic (www.beatthemicrobead.org). While legislation may reduce pellets and microbeads in the domestic wastewater stream, these laws will not affect fiber or fragment components of microplastic assemblages. In addition, attempts to diminish microplastic inputs at the source may reduce the amount of new microplastic entering aquatic environments. However, microplastic input is ongoing and plastic polymers are recalcitrant, so analyses regarding the ecological fate of microplastic accumulations in river ecosystems are needed which span long-time scales to craft dynamic management protocols.

Table 20. Worldwide surface water and water column microplastic concentrations.

Location	Eco-system	Size range (mm)	Measurement	Reported concentration	Other standard units	Citation
Freshwater						
Urban streams, IL	River	0.333-4.75	Up Mean (±SE)	2.36 (0.37) m^{-3}	673,583 km^{-2}	This study
			Down Mean (±SE)	5.73 (0.85) m^{-3}	1,758,340 km^{-2}	This study
			Max	22.41 m^{-3}	7,116,587 km^{-2}	This study
Seine R, FRA[+]	River	>0.080	Range	3 - 106 m^{-3}	N/A	Dris et al. 2015b
		>0.330	Range	0.28 - 0.45 m^{-3}	N/A	Dris et al. 2015b
Three Gorges Res.	River	>0.112	Range	3,407,700 - 13,617,500 km^{-2}	N/A	Zhang et al. 2015
N Shore Ch, IL	River	0.333-2	Up Mean (±SE)	2.06 (±0.82) m^{-3}	775,214 km^{-2}	McCormick et al. 2014
			Down Mean (±SE)	18.00 (±11.07) m^{-3}	6,725,888 km^{-2}	McCormick et al. 2014
Danube R	River	0.5-20	Mean(±SD)	0.317(4.665) m^{-3}	N/A	Lechner et al. 2014
			Range	0-141.6 m^{-3}	N/A	Lechner et al. 2014
San Gabriel R, CA	River	1-4.75[†]	Mean wet, dry	153, <1 m^{-3}	N/A	Moore et al. 2011
Coyote Cr, CA	River		Mean wet, dry	<1, 5 m^{-3}	N/A	Moore et al. 2011
Los Angeles R, CA	River		Mean wet, dry	9, 0 m^{-3}	N/A	Moore et al. 2011
Great Lakes, USA	Lake	0.333 - 4.75	Mean	43,157 km^{-2}	0.54 m^{-3}	Eriksen et al. 2013
			Max	466,000 km^{-2}	5.83 m^{-3}	Eriksen et al. 2013
L. Hovsgol, MNG	Lake	0.333 - 4.75	Mean	20,264 km^{-2}	N/A	Free et al. 2014
			Range	99 - 44,435 km^{-2}	N/A	Free et al. 2014
L. Geneva, CHE	Lake	unknown[#]	Mean	51,556 km^{-2}	N/A	Faure et al. 2012
			Max	82,713 km^{-2}	N/A	Faure et al. 2012
Estuarine						
Yangtze R. Est.	Est.	>0.032	Mean	4,137 (2,462) m^{-3}	N/A	Zhao et al. 2014

			Range	500 - 10,200 m^{-3}	N/A	Zhao et al. 2014
North Sea, DEU	Bay	> 0.04	Granules Mean (±SD)	64 (194) L^{-1}	64,000 m^{-3}	Dubaish & Liebezeit 2013
			Granules Range	0 - 1,770 L^{-1}	0 - 1,770,000 m^{-3}	Dubaish & Liebezeit 2013
			Fibers Mean (±SD)	88 (82) L^{-1}	88,000 m^{-3}	Dubaish & Liebezeit 2013
			Fibers Range	0 - 650 L^{-1}	0 - 650,000 m^{-3}	Dubaish & Liebezeit 2013
Yangtze R. Est.	Est.	>0.112	Range	192,500 - 11,889,700 km^{-2}	N/A	Zhang et al. 2015
Goiana Est, BRA	Est.	0.300 - 5	Mean	0.260 m^{-3}	N/A	Lima et al. 2014
			Mean (w/o paint)	0.185 m^{-3}	N/A	Lima et al. 2014
Tamar Est, ENG	Est.	0.300 - >5	Mean	0.028 m^{-3}	N/A	Sadri & Thompson 2014
Chesapeake Bay *Coastal*	Est.	>0.330	Mean	94,701 km^{-2}	0.627 m^{-3}	Yonkers et al. 2014
S. Coast Korea	Coast	0.001 - 1	Mean	16,272 m^{-3}	13 m^{-2}	Song et al. 2014
		0.05 - >2	Mean	1,143 (3,353) m^{-3}	N/A	Song et al. 2014
		0.330 - >2	Mean	47 (192) m^{-3}	N/A	Song et al. 2014
BC, CAN	Coast	0.062 - 5	Min Mean (±SD)	1,710 (1,110) m^{-3}	N/A	Desforges et al. 2014
			Max Mean (±SD)	7,630 (1,410) m^{-3}	N/A	Desforges et al. 2014
			Max	9,180 m^{-3}	N/A	Desforges et al. 2014
West SWE	Coast	>0.08	Range	150 - 2,400 m^{-3}	N/A	Lozano & Mouat 2009
Coast Portugal	Coast	>0.180	Min Mean (±SD)	0.002 (0.001) m^{-3}	N/A	Frias et al. 2014
			Mean (±SD)	0.036 (0.027) m^{-3}	N/A	Frias et al. 2014

Bay of Calvi, FRA	Coast	0.2 - 10	Mean	0.062 m^{-2}	0.31 m^{-3}	Collignon et al. 2014
			Max	0.688 m^{-2}	3.44 m^{-3}	Collignon et al. 2014
S. California Coast	Coast	0.333 - 4.75	Before storm	3 m^{-3}	N/A	Moore et al. 2002
			After storm	12 m^{-3}	N/A	Moore et al. 2002
			Mean	7.25 m^{-3}	N/A	Moore et al. 2002
S. California Coast	Coast	0.333 - 4.75	Before storm	<1 m^{-3}	N/A	Lattin et al. 2004
			After storm	18 m^{-3}	N/A	Lattin et al. 2004
			Mean	3.92 m^{-3}	N/A	Lattin et al. 2004
Coastal AUS	Coast	>0.333	Mean	4256.4 km^{-2}	N/A	Reisser et al. 2013
Gulf of Maine	Coast	> 0.335	Mean	1,534 (200) km^{-2}	N/A	Law et al. 2010
Caribbean Sea	Coast	> 0.335	Mean	1,414 (112) km^{-2}	N/A	Law et al. 2010
East China Sea	Coast	>0.5	Mean	0.167 (0.138) m^{-3}	N/A	Zhao et al. 2014
			Range	0.030 - 0.455 m^{-3}	N/A	Zhao et al. 2014
Sardinian Sea	Coast	>0.500	Mean	0.15 m^{-3}	N/A	de Lucia 2014
S. California	Coast	> 0.505	Medians	0.011 - 0.033 m^{-3}	N/A	Gilfillan et al. 2009
			Max	3.141 m^{-3}	N/A	Gilfillan et al. 2009
Pelagic						
NE Pac. Ocean	Ocean	0.062 - 5	Mean (±SD)	279 (178) m^{-3}	N/A	Desforges et al. 2014
NE Atl. Ocean	Ocean	0.25 - >10	Mean	2.46 m^{-3}	N/A	Lusher et al. 2014
Med. Sea	Sea	>0.200	Mean	243,853 km^{-2}	0.975 m^{-3}	Cózar et al. 2015
Sargasso Sea	Ocean	>0.330	Mean	3,537 km^{-2}	N/A	Carpenter & Smith 1972
			Range	47 - 12,080 km^{-2}	N/A	Carpenter & Smith 1972

NW Med. Sea	Sea	0.333-5	Mean	0.116 m^{-2}	1.16 m^{-3}	Collignon et al. 2012
			Range	0 - 0.892 m^{-2}	0 - 8.92 m^{-3}	Collignon et al. 2012
Atl. Ocean	Ocean	0.3 - >5	Mean	0.01 m^{-3}	N/A	Ivar do Sul et al. 2013
N Pac. Ocean	Ocean	0.333 - 4.75	Mean 2000	0.43 m^{-3}	N/A	Moore et al. 2005
			Mean 2002	1.52 m^{-3}	N/A	Moore et al. 2005
NE Pac. Ocean	Ocean	> 0.333	Median 2009	0.448 m^{-2}	2.24 m^{-3}	Goldstein et al. 2013
			Max 2009	6.553 m^{-2}	32.765 m^{-3}	Goldstein et al. 2013
			Median 2010	0.021 m^{-2}	0.105 m^{-3}	Goldstein et al. 2013
			Max 2009	0.910 m^{-2}	4.55 m^{-3}	Goldstein et al. 2013
N Pac. Ocean	Ocean	0.330 - >11	Mean	174,000 km^{-2}	N/A	Yamashita & Tanimura 2007
N Atl. Subtr. Gyre	Ocean	> 0.335	Mean	20,328 km^{-2}	N/A	Law et al. 2010
			Max	580,000 km^{-2}	N/A	Law et al. 2010
S Pac. Subtr. Gyre	Ocean	0.333 - 4.75	Mean	26,898 km^{-2}	N/A	Eriksen et al. 2013 b
			Max	396,342 km^{-2}	N/A	Eriksen et al. 2013 b
N Pac. Cent. Gyre	Ocean	0.333 - 4.75	Mean	334,271 km^{-2}	2.23 m^{-3}	Moore et al. 2001
			Range	31,982 - 969,777 km^{-2}	6.65 m^{-3}	Moore et al. 2001
N Pac. Ocean	Ocean	0.505 - 10	Range	0.004 - 0.19 m^{-3}	N/A	Doyle et al. 2011

Up and down for this study and McCormick et al. 2014 refer to sampling locations upstream and downstream of wastewater treatment plants (WWTPs). *Individual site data provided in Tables 14 and 21; + data obtained from Dris et al. 2015a; † data from manta nets in Moore et al. 2011; # data obtained from Free et al. 2014. Abbreviations: IL=Illinois, R=river, FRA=France, Res.=reservoir, N=north, Ch=channel, CA=California, L.=lake, Est.=estuary, S.=south, DEU=Germany, ENG=England, BC= British Columbia, CAN=Canada, SWE=Sweden, AUS=Australia, Pac.=Pacific, Atl.=Atlantic, Med.=Mediterranean, Subtr.=subtropical, Cent.=central

Table 21. Estimated daily flux of microplastic at each study stream.

River	Mean downstream concentration No. m^{-3}	Flux No. d^{-1}
Higgen's Cr	11.22	857,758
Springbrook Cr	5.39	185,317
L Kickapoo Cr	0.80	15,520
N Shore Ch.	6.60	4,721,709
Goose Cr	2.53	214,449
DuPage R	10.28	3,520,277
W Br DuPage R	2.96	217,570
Salt Cr	3.73	364,692
E Br DuPage R	8.86	1,951,522

Cr = creek, R = river, E = east, Br = branch

APPENDIX A

SUPPLEMENTAL ANTHROPOGENIC LITTER AND MICROPLASTIC TABLES

Table A1. Classification of AL by material and item type. Classification categories were adapted from Cheshire et al. 2009.

Material	Item Code	Litter form (examples)
Ceramic	CE01	Construction material (brick, cement, pipes)
Ceramic	CE02	Bottles & Jars
Ceramic	CE03	Ceramic fragments
Ceramic	CE04	Other (specify)
Cigarettes	CG01	Cigarettes, butts & filters
Cloth	CL01	Clothing, shoes, hats & towels
Cloth	CL02	Backpacks & bags
Cloth	CL03	Canvas, sailcloth & sacking
Cloth	CL04	Rope & string
Cloth	CL05	Carpet & furnishing
Cloth	CL06	Other cloth (including rags)
Glass	GL01	Bottles & jars
Glass	GL02	Tableware (plates & cups)
Glass	GL03	Light bulbs
Glass	GL04	Fluorescent light tubes
Glass	GL05	Glass buoys
Glass	GL06	Glass fragments
Glass	GL07	Other
Metal	ME01	Tableware (plates, cups & cutlery)
Metal	ME02	Bottle caps, lids & pull tabs
Metal	ME03	Aluminium drink cans
Metal	ME04	Other cans (< 4 L)
Metal	ME05	Gas bottles, drums & buckets (> 4 L)
Metal	ME06	Foil wrappers
Metal	ME07	Fishing related (sinkers, lures, hooks, traps & pots)
Metal	ME08	Fragments
Metal	ME09	Wire, wire mesh & barbed wire
Metal	ME10	Other, including appliances
Paper & Cardboard	PC01	Paper (including newspapers & magazines)
Paper & Cardboard	PC02	Cardboard boxes & fragments
Paper & Cardboard	PC03	Cups, food trays, food wrappers, cigarette packs
Paper & Cardboard	PC04	Tubes for fireworks
Paper & Cardboard	PC05	Other
Plastic	PL01	Bottle caps & lids
Plastic	PL02	Bottles < 2 L
Plastic	PL03	Bottles, drums, jerrycans & buckets > 2 L

Plastic	PL04	Knives, forks, spoons, straws, stirrers, (cutlery)
Plastic	PL05	Drink package rings, six-pack rings, ring carriers
Plastic	PL06	Food containers and wrappers
Plastic	PL07	Plastic bags (opaque & clear)
Plastic	PL08	Toys
Plastic	PL09	Gloves
Plastic	PL10	Cigarette lighters
Plastic	PL12	Syringes
Plastic	PL13	Baskets, crates & trays
Plastic	PL14	Plastic buoys
Plastic	PL15	Mesh bags (vegetable, oyster nets, mussel bags)
Plastic	PL16	Sheeting (tarpaulin or other woven plastic bags, palette wrap)
Plastic	PL17	Fishing gear (lures)
Plastic	PL18	Monofilament line
Plastic	PL19	Rope
Plastic	PL20	Fishing net
Plastic	PL21	Strapping
Plastic	PL22	Fibreglass fragments
Plastic	PL23	Resin pellets
Plastic	PL24	Other
Rubber	RB01	Balloons, balls & toys
Rubber	RB02	Footwear (flip-flops)
Rubber	RB03	Gloves
Rubber	RB04	Tires
Rubber	RB05	Inner-tubes and rubber sheet
Rubber	RB06	Rubber bands
Rubber	RB07	Condoms
Rubber	RB08	Other
Styrofoam	FP01	Foam sponge
Styrofoam	FP02	Cups & food packs
Styrofoam	FP03	Foam buoys
Styrofoam	FP04	Insulation & packaging
Styrofoam	FP05	Other
Wood	WD01	Corks
Wood	WD02	Fishing traps and pots
Wood	WD03	Ice-cream sticks, chopsticks & toothpicks
Wood	WD04	Processed timber and pallet crates
Wood	WD05	Matches & fireworks
Wood	WD06	Other

Other	OT01	Paraffin or wax
Other	OT02	Sanitary (diapers, cotton buds, feminine hygiene)
Other	OT03	Appliances & Electronics
Other	OT04	Batteries
Other	OT05	Other

Table A2. Results of AMOVA analysis describing differences in bacterial community composition based on a comparison of the Bray-Curtis dissimilarity index for 10 sites.

Comparison	p-value
All sites	**<0.001***
Bart-Elm	**<0.001***
Bart-Kir	**<0.001***
Bart-NSC	**<0.001***
Bart-Nap	**0.001***
Bart-SBL	**<0.001***
Bart-Scher	**<0.001***
Bart-WGV	**<0.001***
Bart-Wheat	**<0.001***
Bart-Woak	**<0.001***
Elm-Kir	**0.001***
Elm-NSC	**<0.001***
Elm-Nap	0.010
Elm-SBL	0.017
Elm-Scher	**<0.001***
Elm-WGV	0.001*
Elm-Wheat	**<0.001***
Elm-Woak	0.011
Kir-NSC	**<0.001***
Kir-Nap	0.009
Kir-SBL	**<0.001***
Kir-Scher	**<0.001***
Kir-WGV	**<0.001***
Kir-Wheat	**<0.001***
Kir-Woak	0.009
NSC-Nap	0.002
NSC-SBL	**<0.001***
NSC-Scher	**<0.001***
NSC-WGV	**<0.001***
NSC-Wheat	**<0.001***
NSC-Woak	**0.001***
Nap-SBL	0.018
Nap-Scher	**<0.001***
Nap-WGV	**<0.001***
Nap-Wheat	**<0.001***
Nap-Woak	0.031
SBL-Scher	**<0.001***

SBL-WGV	0.002
SBL-Wheat	**<0.001***
SBL-Woak	0.025
Scher-WGV	**<0.001***
Scher-Wheat	**<0.001***
Scher-Woak	**<0.001***
WGV-Wheat	**<0.001***
WGV-Woak	**<0.001***
Wheat-Woak	**<0.001***

Site abbreviations: Bart = West Branch DuPage River, Bartlett WWTP; Elm = Salt Creek, Elmhurst WWTP; Kir = Higgen's Creek, Kirie WRP; NSC = North Shore Channel, O'Brien WRP; Nap = DuPage River, Springbrook WRP; SBL = Little Kickapoo Creek, Bloomington South WWTP; Scher = Schererville Ditch, Schererville WWTP; WGV = East Branch DuPage River, Woodridge Green Valley WRC; Wheat = Springbrook Creek, Wheaton WWTP; Woak = Goose Creek, West Oakland WWTP. WWTP = wastewater treatment plant, WRP = water reclamation plant, WRC = water reclamation center.

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