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Title: Anthropogenic litter and microplastic in urban streams: abundance, source, and fate

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Introduction

The abundance of anthropogenic litter (i.e., garbage; AL) in marine ecosystems has received attention from researchers, the public, and the media. AL has many detrimental ecological effects such as ingestion and entanglement by animals and assistance in invasive species dispersal (Moore 2008). Accumulation of AL in the environment also presents an economic burden from costs related to clogged sewers and AL removal, and may represent potential hazard to human health (i.e., injury). Rivers transport materials such as organic matter, nutrients, and pollution between terrestrial and marine ecosystems. While many studies suggest rivers are an important source of AL to marine habitats (Stefatos 1999, Galgani et al. 2000, Hammer et al. 2012), the abundance, movement, and ecosystem effects of AL in rivers are unknown.

In addition to accumulations of large AL items, high concentrations of microplastic (i.e. <5 mm particles) have been measured in oceans worldwide (Browne et al. 2011). Sources of microplastic include industrial manufacturing pellets and fragmentation of larger plastic pieces. In addition, some personal care products and cleaning agents contain microplastic abrasives (Fendall and Sewell 2009), and washing machine effluent contains microplastic fibers from synthetic textiles (Browne et al. 2011). The latter two sources enter the domestic wastewater streams and wastewater treatment plants (WWTP). Microplastic particles and fibers are often unfiltered by WWTPs due to their small size (Fendall and Sewell 2009; Browne et al. 2011). Our preliminary studies were the first to show that showed that the North Shore Channel in Chicago, IL had surface water microplastic concentrations similar to oceanic gyres (McCormick et al. 2014) and that WWTP effluent was a point source. However, more work is needed to document this pattern across a larger geographic area.

In marine ecosystems, microplastic selects for unique microbial assemblages (Zettler et al. 2013), and ingestion by consumers can transport adsorbed contaminants, block digestion, and transfer from prey to predators (Cole et al. 2011). However, the ecological effects of riverine microplastic are unknown. Microplastic is buoyant and resistant to decomposition, so it presents a novel habitat which may select and disperse bacterial assemblages with unique metabolic capabilities in river networks. Our previous research showed bacterial communities on microplastic in an urban river were distinct from those in the water column and seston (McCormick et al. 2014). This was the first assessment of microplastic-attached microbial communities in rivers. Thus, more measurements on the interactions between microplastic and microbes are required to understand its potential ecological impacts.

Project Summary

This research represents a significant contribution to the study of AL in rivers. The project had 2 parts: 1) dynamics of AL in rivers, and 2) abundance, source, and microbial communities on riverine microplastic.

Research Objectives The proposed project addresses the following questions.

1a. What is the abundance, composition, and sources of AL?
  b. How does AL move through rivers?

2a. What is the concentration of microplastic in urban rivers?
  b. Does WWTP effluent serve as a point source for their entry into rivers and streams?
  c. What types of microbes colonize microplastic in rivers and do they differ from those colonizing organic substrates?

Part 1. AL abundance, composition, and flux

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 and adjacent riparian zones 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 beginning the research.

Collection and categorization of benthic and riparian AL. We collected AL in June-October, 2014 (summer-autumn), except for 3 reaches sampled in 2013 (Table 1). Reach lengths were 50-100 m. AL was collected from the entire benthos of the reach, and from the riparian zone on one bank of the reach. We defined the riparian zone for this study as the area within 10 m of 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, which was inaccessible). To collect items, we slowly moved along the reach in teams of 2-3, picking up all AL. 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 benthos. 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' (Appendix Table 1). We used a code to classify the item's function (e.g., cutlery, clothing, and cups; Appendix Table 1). Finally, we characterized each item according to 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 instance, it is possible that an item classified as consumable, such as a beverage container, 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).

We assessed anthropogenic activity in 4 ways: the presence and distance of a walking trail from the reach, the intensity of human activity, the number of parking spots, 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 collection for Bunker Hill (Sep 16, 2014), Miami Woods (Sep 16, 2014), 26th Street Woods (Aug 4, 2014), and Pilcher Park (Sep 26, 2014). Trails were classified as near (<50 m from the stream), far (>50 m from the stream), or none (not present). We classified the intensity of human activity by the number of people observed at the reach or on a nearby reach 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 benthos and beaches. We compared our results to AL density, mass, and composition from published studies conducted in rivers and oceans. 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 AL abundance is reported by material (e.g., glass, plastic, and metal) (Rech et al. 2014, Abu-Hilal 2009) or function (i.e., food-related, dumping activities, medical/personal) (Hoellein et al. 2015). To compare AL from this study to published values, we included studies which reported the number of items or mass per unit area and used similar material classifications. Studies included marine benthic habitats in European 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), islands (Eriksson et al. 2013), and lakes (Hoellein et al. 2014).

AL flux: study sites. We examined movement of riparian zone AL at two spatial scales, seasonal (i.e., 3 times over 1 year), and biweekly (i.e., every 2 weeks during summer) in 2 riparian reaches of the North Branch of the Chicago River. The former was conducted at Bunker Hill Forest Preserve (Niles, IL) and the latter at Miami Woods (Morton Grove, IL). These 2 reaches were among the 15 sites used above, and are in the Cook County Forest Preserve network (Table 1).

AL flux: seasonal measurement. Our seasonal study measured the net accumulation of AL and export of marked AL items from the riparian zone over 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 edge.

This set a ‘blank slate’ so that any AL collected on subsequent dates represented net 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 AL. The accumulated AL was taken o 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). 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 period, we carefully searched the quadrat for the marked AL. In addition, we searched ~100 m downstream and 30 m inland from the quadrat. We recorded if the item remained in its starting location, moved within the quadrat, or was outside 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 1 year. We removed all marked AL items in the quadrat after the final date (Dec 18, 2014). Finally, we note that all marked AL 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 in units of No. d^{-1} and No. m^{-2} d^{-1}. We calculated AL export as the proportion of items 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, and calculated net flux of AL at our study site over the course of the year [Eq 1].

$$\text{Eq. 1} \qquad Flux = Net\ accumulation - Export\ rate \times Initial\ AL\ standing\ stock$$

In this equation, we multiplied the mean annual 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 output 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 zone over shorter time intervals. This study was carried out over 18 weeks during summer 2014, starting on June 2. We visited the site every ~2 weeks (mean ± SE = 15.1 ± 1.3 d). We used the same quadrat dimensions, types of AL, and methods described above. The only difference in methods for this study was that we characterized 2 types of export. We noted if the item was out of the quadrat, but in the adjacent area (export: adjacent), or was not found (export: lost). To examine patterns between AL movement and stream hydrology, we obtained discharge data from the USGS for the North Branch of the Chicago River from Jun 2-Oct 2, 2014 (http://waterdata.usgs.gov/nwis/uv?site_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 periods of 8-149 d. We combined our data with results from Smith and Markic (2013; *Figure 2* in that study).

Data Analysis. We used 2-way analysis of variance (ANOVA) to compare differences in AL density and mass among streams and between habitats (riparian and benthos). 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 percent composition data for abundance and mass. The resulting distance matrix was visualized with nonmetric multidimensional scaling (nMDS) ordinations. We determined whether there were significant differences in AL composition among sites and between habitats using analysis of similarities (ANOSIM) analyses. We calculated all 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 the density of each AL material type. We performed 2 PCA analyses for density of all 11 AL categories at our 2 habitats types (i.e., benthic and riparian). PCA was performed in PC-ORD V.6 (McCune and Mefford 2011) using a correlation matrix as our data included both environmental and AL density variables with varying units of measurement (Clarke and Warwick 2001).

Results & Discussion

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 AL in the two less urbanized watersheds was lower. There no difference in AL density between riparian and benthic zones (2-way ANOVA, $p=0.120$; Figure 1; Table 3), but there was a pattern of more AL in the riparian zone compared to the benthic zone (except Plum Creek; Figure 1A). Total AL mass (g m^{-2}) was highest at Turkey Creek and similar at other 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).

Density of AL by material type 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 variable by site (2-way ANOVA $p=0.002$; Table 3). Styrofoam and paper were more abundant in the riparian zone, but there were no differences among sites (Table 3). Ceramic density was higher in the stream benthos, 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 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 were highest in the benthos, including rubber (Kruskal Wallis p=0.039), metal (ANOVA p=0.003), and ceramic (ANOVA p=0.005; Table 3). In contrast, paper mass 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 masses of plastic, Styrofoam, glass, wood, cigarettes, and 'other' did not differ between habitats or among sites (Table 3).

AL composition among streams and between habitats. While riparian zones typically had high density of AL (Figure 1A), 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 was higher in the riparian zone (48-65%) than in the river benthos (21-46%; Table 4). Benthic habitats had a lower AL density (Figure 1B), but heavier items such as metal, wood, and ceramic had greater relative abundance than 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 no significant dissimilarity in AL composition 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 of Plum Creek strongly influenced the comparison (coordinates 2.03, -1.81; Figure 2A). This site had a very low AL density and half of the items were manufactured wood, a generally uncommon material in riparian sites elsewhere (Table 4). This 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 dissimilarity between habitats (ANOSIM R=0.267, p=0.027), although there were no significant 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 over 96% of the site's mass. As a result, the relative contribution of rubber to the overall mass at this site was much higher than at other riparian sites (Table 4).

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

Anthropogenic factors influencing AL density. We examined relationships between 4 variables related to anthropogenic activity and the density of our 11 AL categories using principle component analysis (PCA). We performed a separate PCA for benthic and riparian AL densities. The first 3 components of each PCA explained 60.5 and 63.4% of the data variation in the benthic and riparian zones, respectively (Table 5).

The first component of the PCA (PC1) explained 27.2 and 34.6% of the variation in the benthic and riparian habitats, respectively (Table 5). The second component (PC2) explained an additional 19.5 and 16.7% of the variation in the benthic and riparian habitats, respectively (Table 5). In benthic habitats, PC1 had a significant positive relationship with 3 measures of anthropogenic activity (number of parking spots, intensity of activity, and proximity of a trail) as well as all types of AL except ceramic, cigarettes, and 'other' (Table 6; Figure 4A). PC2 had a significant negative relationship with 3 measures of anthropogenic activity (the number of parking spots, distance to a road, and proximity of a trail). PC2 was negatively related to Styrofoam density, and positively related to densities of ceramic, glass, metal, rubber, and wood (Table 6; Figure 4A). PC3 showed no significant relationship with any human activity characteristics (Table 6). The heavy items, metal, rubber, and wood were clustered on the PCA diagram, and ceramic density was uncorrelated with any anthropogenic activities (Figure 4A).

In riparian habitats there was a significant negative relationship between PC1 and 2 measures of human activity (number of parking spots and intensity of activity) as well as the densities of all AL 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. 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 had a significant negative relationship with the number of parking spots and the distance to a road and a significant positive relationship with paper and wood density in the riparian zone (Table 6). AL category vectors for plastic, rubber, glass, cloth, metal, and 'other' clustered in the PCA diagram (Figure 4B). Like 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 negative relationship with all 4 site characteristic 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, benthic AL density was higher than a majority of studies conducted in marine benthic environments (Table 7). Our 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 an order of magnitude above measurements in the marine benthos (Table 7). The only exception was marine density in the Gulf of Aqaba in the 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 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, several trends emerge when comparing relative abundance of AL among studies by material type. For example, the relative abundance of metal was typically higher in benthic habitats than aquatic-terrestrial transition habitats (i.e., riparian zone and beaches). 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%) and 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%), relatively rare in rivers (range=0-15%), and most common on beaches (range=0-41%) (Figure 5). Finally, an important difference in AL composition between the marine benthos and other habitats was the prevalence of fishing items in ocean sites (Figure 5).

Seasonal flux: net accumulation and export rates

Patterns of net accumulation and export at the seasonal scale reveal that AL is highly mobile. Across the 3 seasonal intervals, mean (±SE) net accumulation of AL was 1.114 (±0.219) items d^{-1} or 0.0028 (±0.0005) items m^{-2} d^{-1}. Mean export rate for the AL types combined was 0.379 (±0.023) % 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}), mean net accumulation rate, and mean total export rate, we calculated annual AL flux from this riparian zone site with the following calculation:

$$\begin{aligned} Flux &= Net\ accumulation\ rate - Export\ rate \\ &= 0.002785\ No.m^{-2}d^{-1} - (0.9883\ No.m^{-2})(0.003793\ d^{-1}) \\ &= -0.000964\ No.m^{-2}d^{-1} \end{aligned}$$

The net flux of AL from the study quadrat over the course of the year 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 were between 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 6), and glass was typically broken bottles. There was no clear relationship between the river discharge and changes in input rates or relative AL composition (Figure 6).

Unlike net accumulation, export of marked AL items was related to AL material type, river discharge, and proximity of each item to the river 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 7). 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 7). From that time onwards, the number of items remaining in their original locations was relatively constant (Figure 7). However, we note a later decline in the proportion of stationary plastic wrappers (Figure 7C). Overall, glass and metal were more frequently exported from the quadrat than plastic wrappers and bags (Figure 7). Exported plastic wrappers and bags that did move from their original location in the study quadrat were commonly exported near the vicinity of our quadrat (i.e., export: adjacent, Figure 7C, 7D), while glass and metal were lost. Many of the plastic items accumulated in a debris dam ~20 m inland from the study quadrat. The river discharge peaked during the first third of our study, corresponding to the period of greatest AL movement (Figure 7).

Movement of AL relative to its original location also revealed the influence of flooding on AL redistribution. The third of the quadrat closest to the water's edge had the lowest proportion of AL items remaining in their original locations. By the second date, which was 36 days into the study, only 7% of items were in their original location in the section of the quadrat within 0-3.3 m of the water's edge, and by the end of the study, 0% of AL from this section remained (Figure 8A). In contrast, for the AL items in the middle (3.3-6.7 m from the water's edge) and inland (6.7-10 m from the water's edge) sections of the quadrat, 50% and 54% of items remained in their original locations during the second sampling date, respectively (Figure 8B, 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 in the vicinity of the quadrat (export: lost; Figure 8).

We used the same approach as in our seasonal study to calculate AL flux for the summer season at our biweekly study reach. Using the original standing stock density of AL in the reach (0.037 items m^{-2}), the mean net accumulation rate, and the final export rate, we calculated summer flux with the following calculation:

$$\begin{gathered} Flux = Net\ accumulation\ rate - Export\ rate \\ = 0.008588\ No.m^{-2}d^{-1} - (0.037\ No.m^{-2})(0.005328\ d^{-1}) \\ = 0.008391\ No.m^{-2}d^{-1} \end{gathered}$$

Thus, the net flux of AL from the study quadrat was 0.008391 items m^{-2} d^{-1}. Unlike the annual value, the net flux over summer was positive, suggesting net accumulation of AL during this time.

The effect of sampling interval on AL accumulation rates. Previous research suggests there is a relationship between accumulation rate (No. items m^{-2} d^{-1}) and sampling interval as a power function (Smith and Markic 2013). Net accumulation and sampling interval showed a significant relationship (R^2=0.559, p=0.005, Figure 9). In addition, we combined these data with similar measurements for a beach in Australia which showed the same relationship (Figure 2 from Smith and Markic 2013). When all data were combined, the power function maintained this significant relationship (R^2=0.872, p<0.001; Figure 9). These data, combined with the net positive flux of AL during our seasonal study, confirm that much of the AL found at a site is in motion, and may be missed altogether if sampling intervals are too far apart.

Estimation of total AL in rivers. We scaled up our density and mass measurements of AL to estimate the total abundance of AL in each river's benthos, riparian zone (10 m from the water's edge on each bank), and the two habitats combined. This estimation suggests that some of the rivers in this study may have over 700,000 AL items (Table 11) in that relatively small part of the watershed. The estimated total AL mass in the benthic + riparian zones in the study watersheds was 21-78 metric tons (Table 11). Using the annual export rate from our seasonal flux study, we calculated that up to 2,000 items d^{-1} are exported from the riparian zones at Salt Creek and N. Branch Chicago River, with less daily export at the others sites (Table 11).

Conclusion

We found that the riparian zones typically had higher density of AL than benthic habitats, but that riparian AL assemblages typically had a higher prevalence of light-weight materials. While benthic habitats typically had relatively low density to riparian habitats, AL mass at these locations was much higher than in the riparian zone. This is reflected in the higher relative abundance of heavy material types in the river benthos. It is likely that reach-specific characteristics (i.e., our 4 anthropogenic activity variables) have a greater influence on AL abundance and composition than watershed characteristics (i.e., urban land use, watershed size). Overall, we found that AL density and mass in urban rivers is in the range of data reported from other published studies, but the density of AL in river benthos was much higher than most marine benthic studies. Finally, we demonstrate that riverine AL is highly mobile, and that hydrology influences the export of AL downstream. Despite the limited spatial scale of our measurements (i.e., 10 m adjacent to the river), the abundance and mass of AL at the watershed scale is very high, and much of it is in motion. These data support anecdotal claims that rivers are source of AL to downstream habitats.

Part I: Tables and Figures

Table 1. Locations and land use characteristics for the 15 sampled stream reaches.

Stream	Urban Land Use (%)	Pop. Density (No. km^{-2})	Reach	Function	Date Sampled	City	County (State)	Latitude, Longitude
Salt Cr.	73*	1236*	26th St Woods	Forest Preserve	28-Oct-13	Berwyn	Cook (IL)	41.8426265, -87.8595240
			Sleepy Hollow Park	Residential	31-Jul-14	Elmhurst	DuPage (IL)	41.8809199, -87.9584902
			Bemis Woods	Forest Preserve	4-Aug-14	W. Springs	Cook (IL)	41.8266232, -87.9106167
Turkey Cr.	53^+	333^+	Hidden Lake	County Park	6-Jun-14	Merrillville	Lake (IN)	41.5035670, -87.3277269
			Broadway St	Commercial	6-Jun-14	Merrillville	Lake (IN)	41.5031540, -87.3367570
			Hidden Lake	County Park	7-Jun-14	Merrillville	Lake (IN)	41.5041679, -87.3305381
N. Br. Chi.R.	48*	572*	Bunker Hill	Forest Preserve	23-Sep-13	Niles	Cook (IL)	42.0004406, -87.7835676
			Miami Woods	Forest Preserve	2-Jun-14	Morton Grove	Cook (IL)	42.0274486, -87.7937181
			LaBagh Woods	Forest Preserve	30-Jul-14	Chicago	Cook (IL)	41.9780194, -87.7427133
Hickory Cr.	21*	352*	Pilcher Park	Nature Cent/Park	28-Oct-13	Joliet	Will (IL)	41.5262440, -88.0070321
			Hillcrest Rd	Residential	26-Sep-14	Joliet	Will (IL)	41.5251124, -88.0409162
			Schoolhouse Rd	Intersection	26-Sep-14	Joliet	Will (IL)	41.5169885, -87.9333094
Plum Cr.	8*	88*	Plum Cr For. Pres.	Forest Preserve	14-Aug-14	Beecher	Will (IL)	41.3931726, -87.6243616
			Goodenow Nat. Pres.	Forest Preserve	14-Aug-14	Beecher	Will (IL)	41.4036569, -87.6091785
			Ridgeland Ave	Residential	28-Sep-14	Chicago Heights	Cook (IL)	41.4827058, -87.5319383

* indicates data were obtained from Fitzpatrick et al. 2005. + indicates data were obtained from Northwestern Indiana Regional Planning Commission 2012.

Table 2. Site characteristics for the 15 reaches in the 5 study streams.

Reach	Distance to trail (m)	Parking spots	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 at the reach.

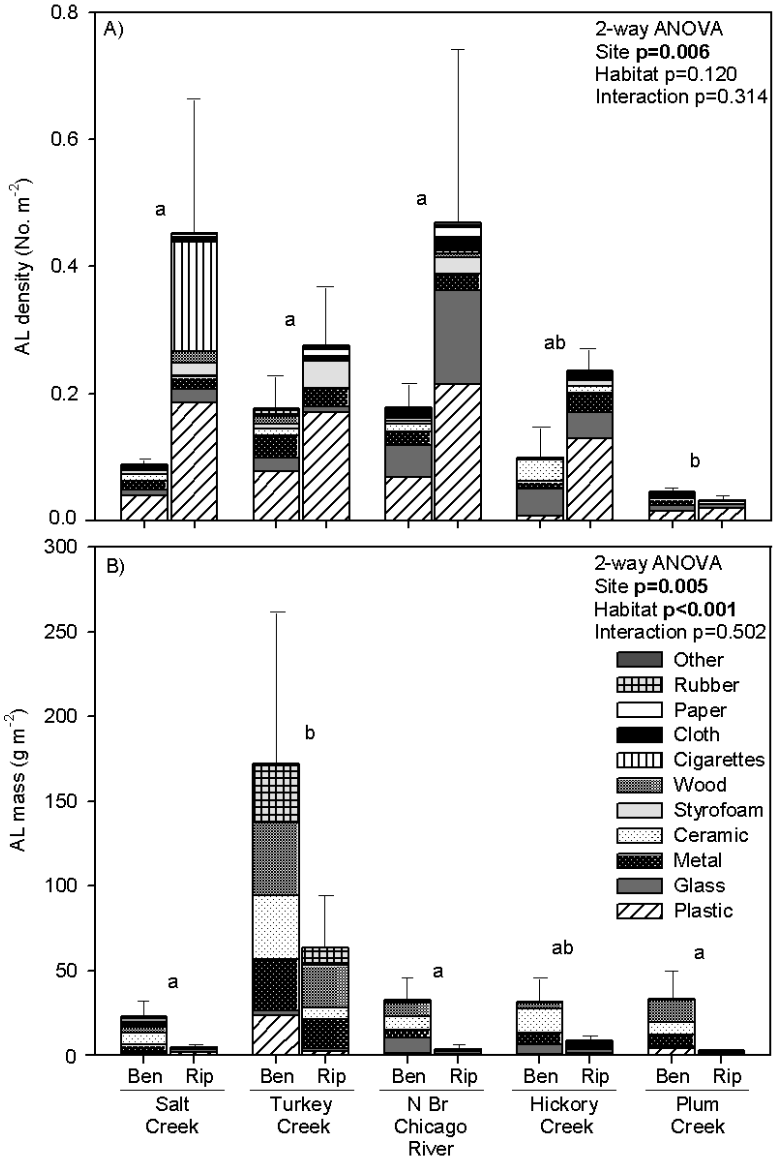

Figure 1. Density (A) and mass (B) of anthropogenic litter (AL) in 5 streams and 2 habitats (river benthos and riparian zone). Bars represent mean density and mass of combined AL categories with standard error bars. Each bar section represents the mean density or mass of that category in the reach. Letters indicate a difference between AL density or mass among sites using Tukey's test ($p \leq 0.05$).

Table 3. Summary of differences in AL density and mass between habitats and across sites using 2-way ANOVA and when necessary, the non-parametric Kruskal Wallis Test.

		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	-	-	-	-

Patterns in total AL density and mass and each category were compared between habitats (riparian and benthos) and across sites. Test statistics are ANOVA F-rations unless noted with #, which indicates Test Statistic from the non-parametric Kruskal Wallis Test. Symbol * indicates ln(x) transformation; + indicates ln(x+0.5) transformation.

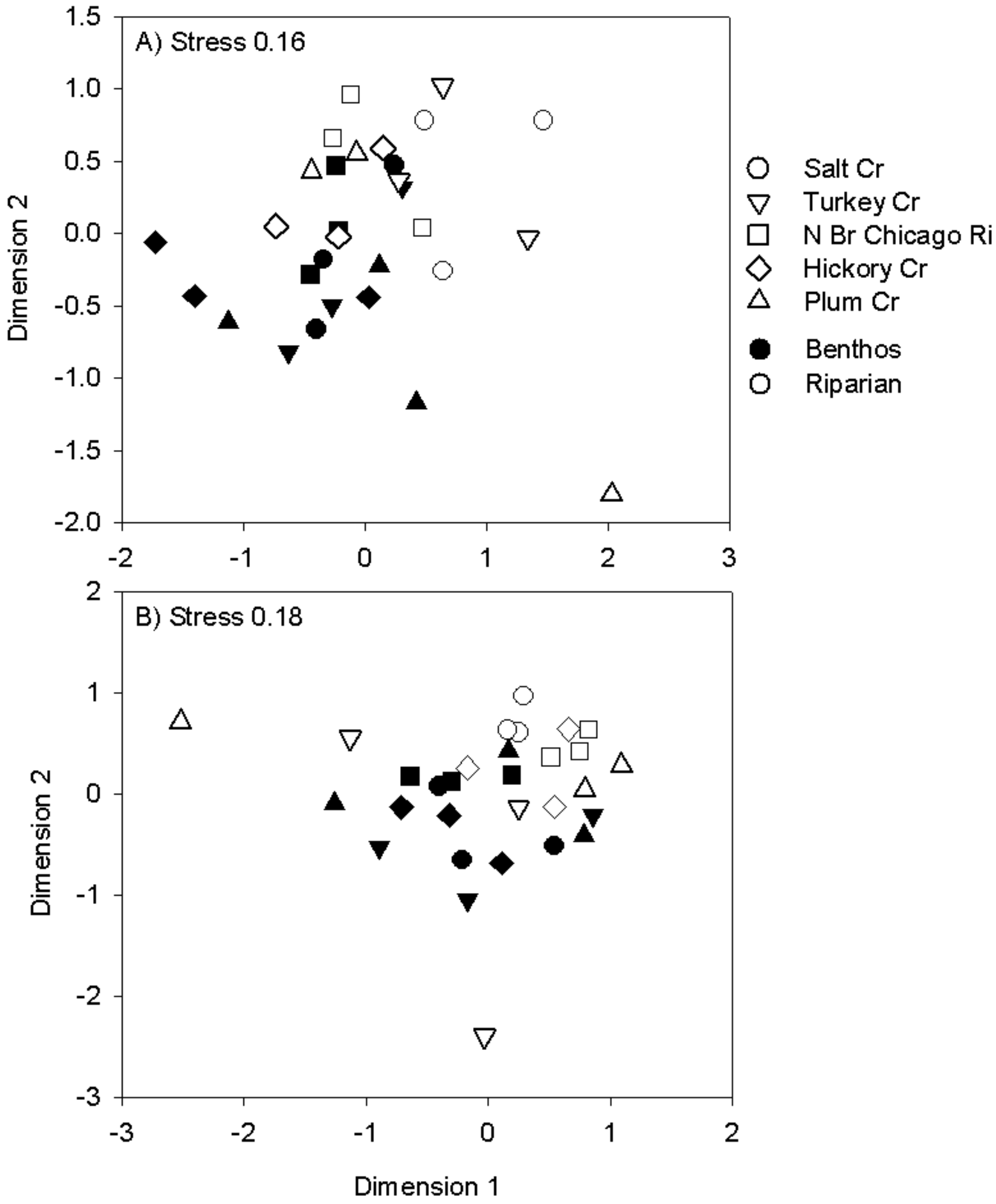

Figure 2. Non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis dissimilarity of anthropogenic litter (AL) composition in 5 streams and 2 habitats based on relative abundance (A) and relative mass (B) of the 11 AL categories. Percent composition data for AL abundance and mass was log(x+1) transformed.

Table 4. AL composition by abundance and mass for benthic and riparian habitats for the 5 streams (n=3 reaches per stream). Data values represent the mean percent contribution of each AL category for each site.

	Benthos					Riparian				
Density	Salt Cr	Turkey Cr	N Br Chi R	Hickory Cr	Plum Cr	Salt Cr	Turkey Cr	N Br Chi R	Hickory Cr	Plum Cr
Ceramic	12.5	8.3	5.1	28.3	7.5	0.1	0.4	0.3	5.2	0.7
Cigarette	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

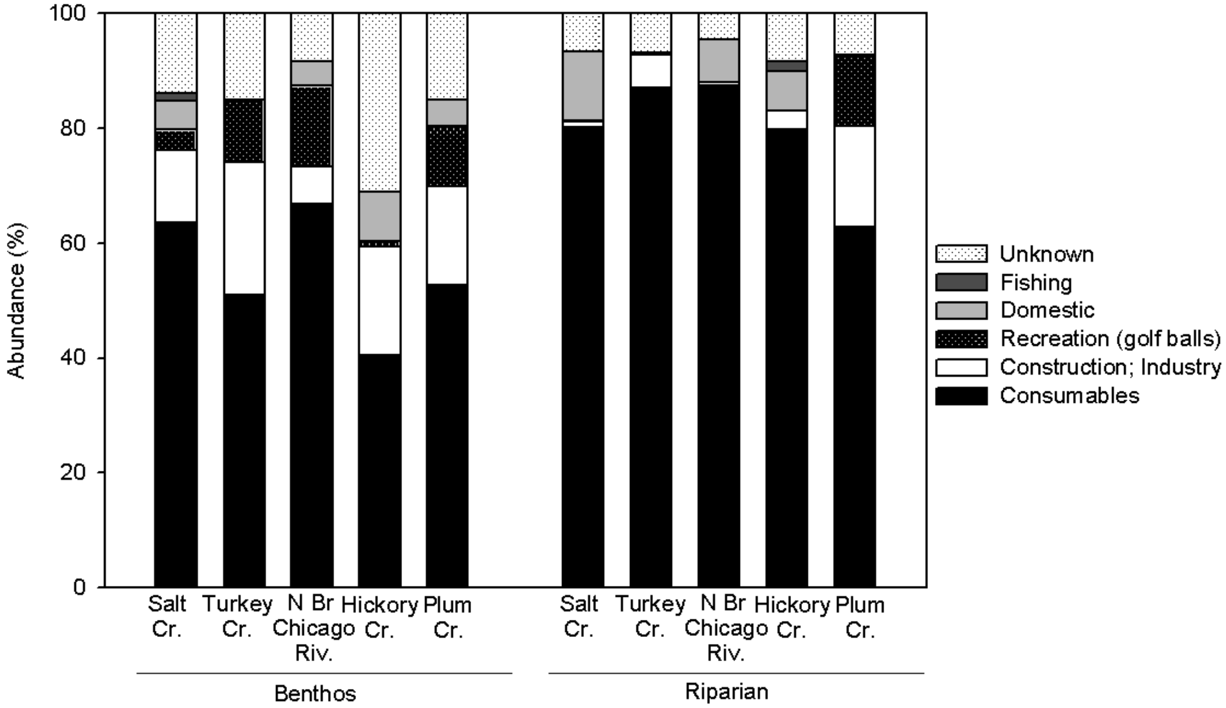

Figure 3. Mean relative proportion of sources contributing to anthropogenic litter (AL) collected from 5 streams.

Table 5. Contribution of first 3 PCA components in explaining variation in AL density and mass in stream habitats.

Density, benthos			*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

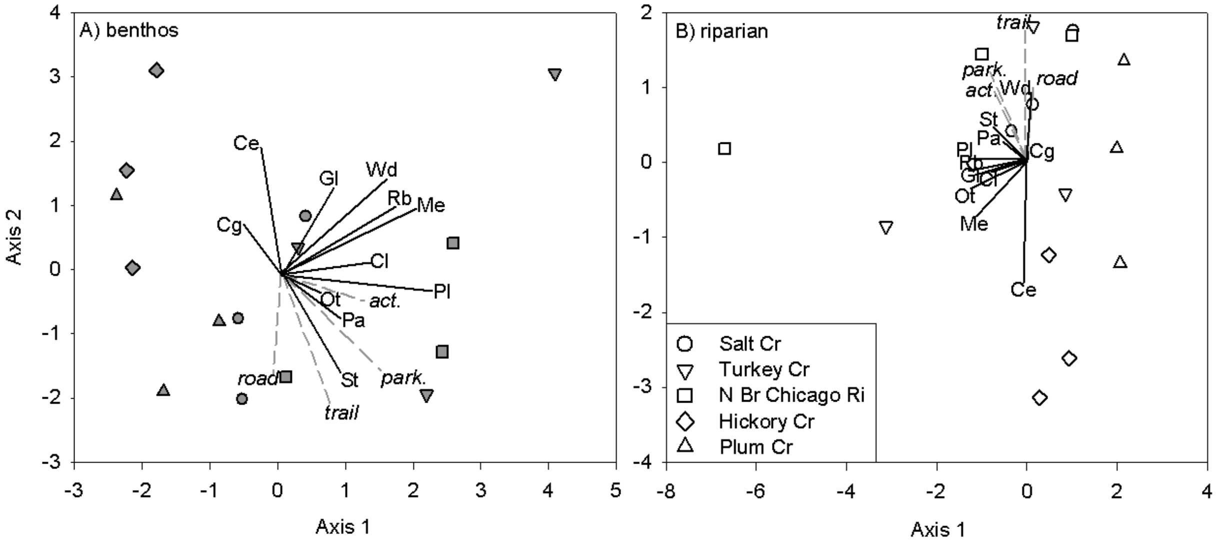

Figure 4. Principle component analysis (PCA) of site characteristics (presence and distance of a trail, number of parking spots, distance to a road, and level of human activity) (gray, dashed lines) and AL abundance at the 15 sampling sites. Abbreviations: park.=parking, act.=activity, Ce=ceramic, Cg=cigarettes,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

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

	Benthos			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**

Table 7. Published AL densities for worldwide benthic and aquatic-land transitional habitats.

Location	Ecosystem	Habitat	N	Measurement	AL Density (No. m^{-2})	Source
Benthic habitats						
N. Illinois/Indiana, USA	River	Benthos	15	Mean (±SE)	0.117 (0.021)	*This study*
N. Br. Chicago R., USA	River	Benthos	3	Mean (±SE)	0.076 (0.018)	Hoellein et al. 2014
Gulf of Aqaba, Red Sea	Marine	Benthos	6	Mean (Range)	2.8 (0.9-5.9)	Abu-Hilal and Al-Najjar 2009
Mediterranean Sea	Marine	Benthos	2	Mean	0.000165	Stefatos et al. 1999
Caribbean Islands	Marine	Benthos	24	Mean (Max)	0.0027 (0.0046)	Debrot et al. 2014
Condor Seamount, PT	Marine	Benthos	NR	Mean	0.00098	Pham et al. 2013
NW Hawaiian Islands	Marine	Benthos	2	Mean (Range)	0.000033	Donohue et al. 2001
European Seas	Marine	Benthos	18	Range	0-0.101	Galgani et al. 2000
Atlantic Ocean	Marine	Benthos	21	Range	0.0003-0.0032	Pham et al. 2014
Mediterranean Sea	Marine	Benthos	10	Range	0.0004-0.0032	
Arctic Ocean	Marine	Benthos	1	Mean	0.00136	
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., US	River	Riparian	3	Mean(±SE)	0.095 (0.017)	Hoellein et al. 2014
Lake Michigan, US	Lake	Beach	3	Mean(±SE)	0.007(0.002)	
Lake Michigan, US	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	
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 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	Benthos	15	Mean(±SE)	58.40 (16.74)	*This study*
N. Br. Chicago R., USA	River	Benthos	3	Mean(±SE)	13.43 (0.65)	Hoellein et al. 2014
Gulf of Aqaba, Red Sea	Marine	Benthos	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)	
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)	

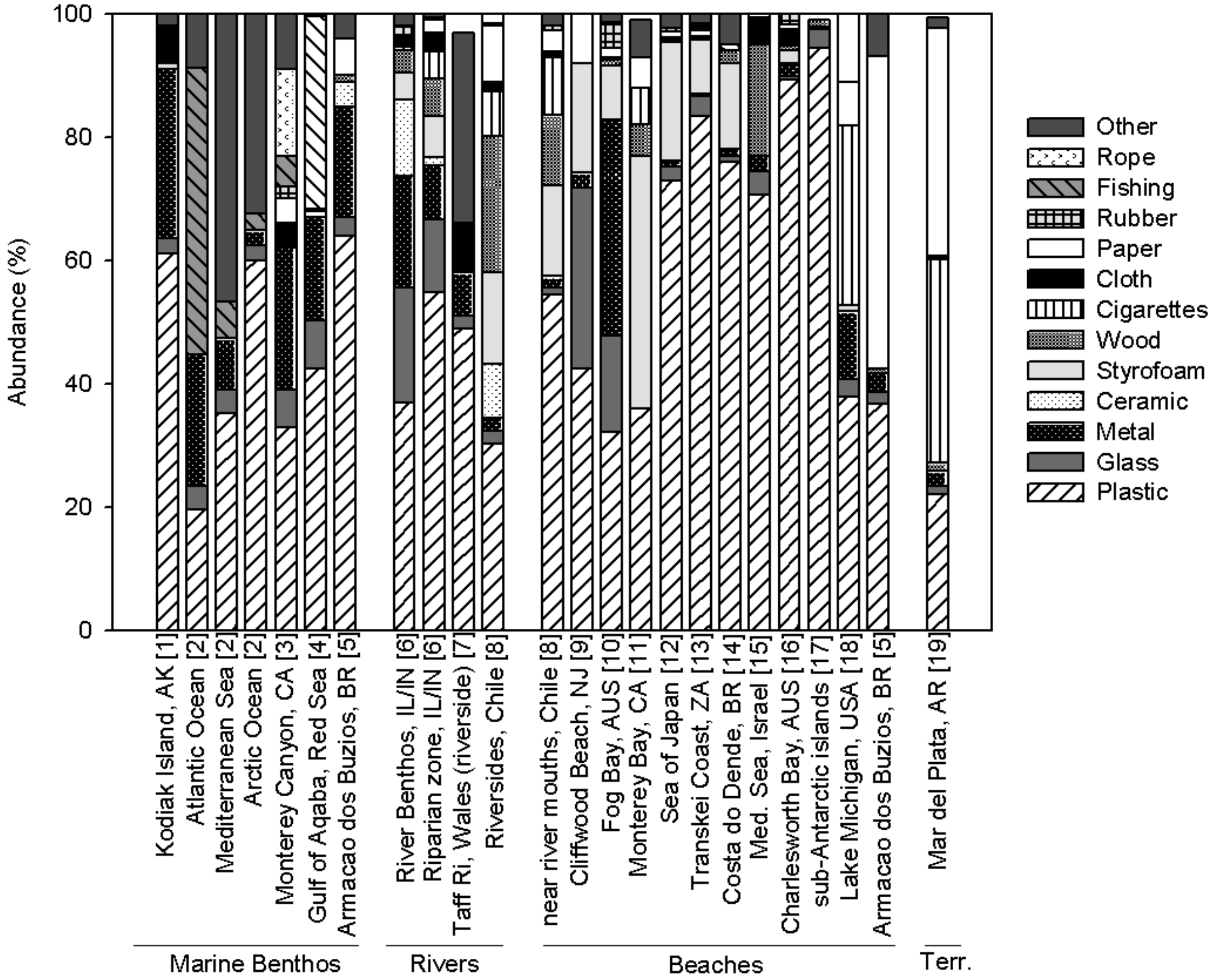

Figure 5. Relative abundance of anthropogenic litter (AL) categories in marine benthos, river, beach, and terrestrial habitats. Bars from this study represent the overall mean relative abundances for all riparian data combined and all benthic data combined**.** Numbers in brackets refer to the following sources: [1] Hess et al. 1999; [2] Pham et al. 2014; [3] Schlining et al. 2013; [4] Abu-Hilal and Al-Najjar 2009; [5] Oigman-Pszczol and Creed 2007; [6] this study; [7] Williams and Simmons 1999; [8] Rech et al. 2014; [9] Thornton and Jackson 1998; [10] Whiting 1998; [11] Rosevelt et al. 2013; [12] Kusui and Noda 2003; [13] Madzena and Lasiak 1997; [14] Santos et al. 2009; [15] Bowman et al. 1998; [16] Smith and Markic 2013; [17] Eriksson et al. 2013; [18] Hoellein et al. 2014; [19] Seco Pon and Becherucci 2012.

Table 9. Summary of net accumulation and export rates for the seasonal flux study.

Sampling Interval			Net Accumulation		Export rate (% d^{-1})					Export
Start	End	Days	No. d^{-1}	No. m^{-2} d^{-1}	Glass	Metal	Wrapper	Bag	Total	No. 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	0.0039797
28-May-14	16-Sep-14	111	1.5405	0.0039	0.2815	0.4505	0.4204	0.4851	0.4022	0.0039748
16-Sep-14	18-Dec-14	93	0.9892	0.0025	0.3584	0.5376	0.0597	0.4032	0.3332	0.0032928

Table 10. Estimated turnover time for each type of item based on results from the seasonal flux study.

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

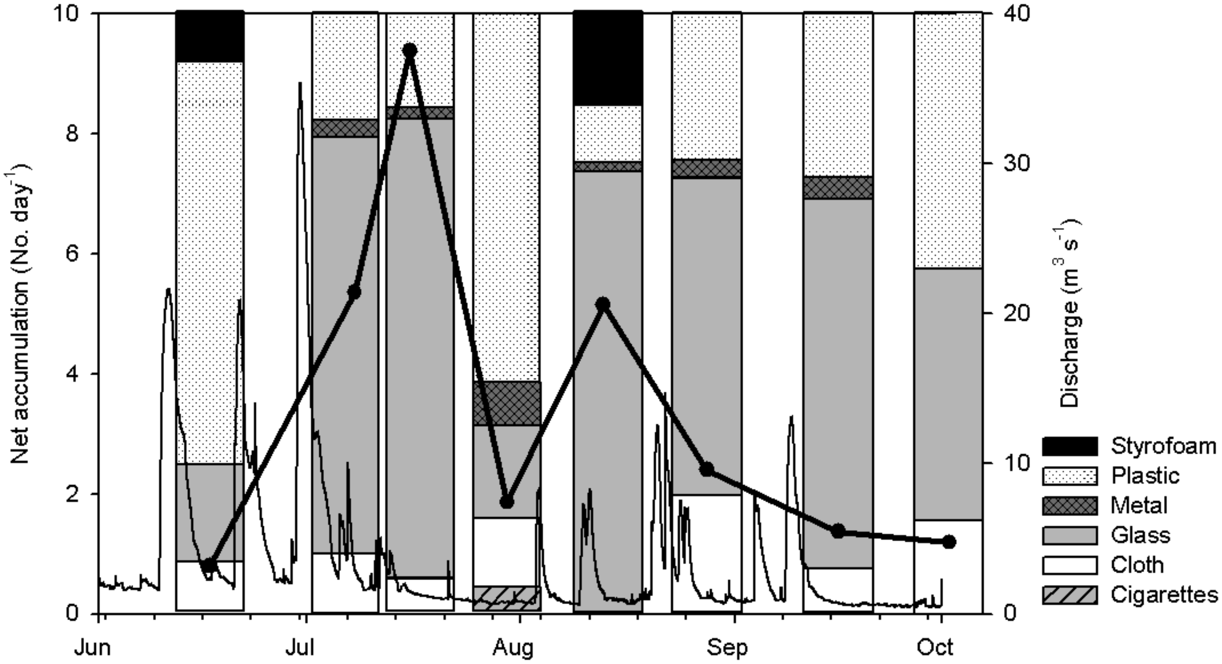

Figure 6. Composition of anthropogenic litter (AL) net accumulation and net accumulation rates for the biweekly flux study conducted in the riparian zone of the North Branch of the Chicago River in Miami Woods.

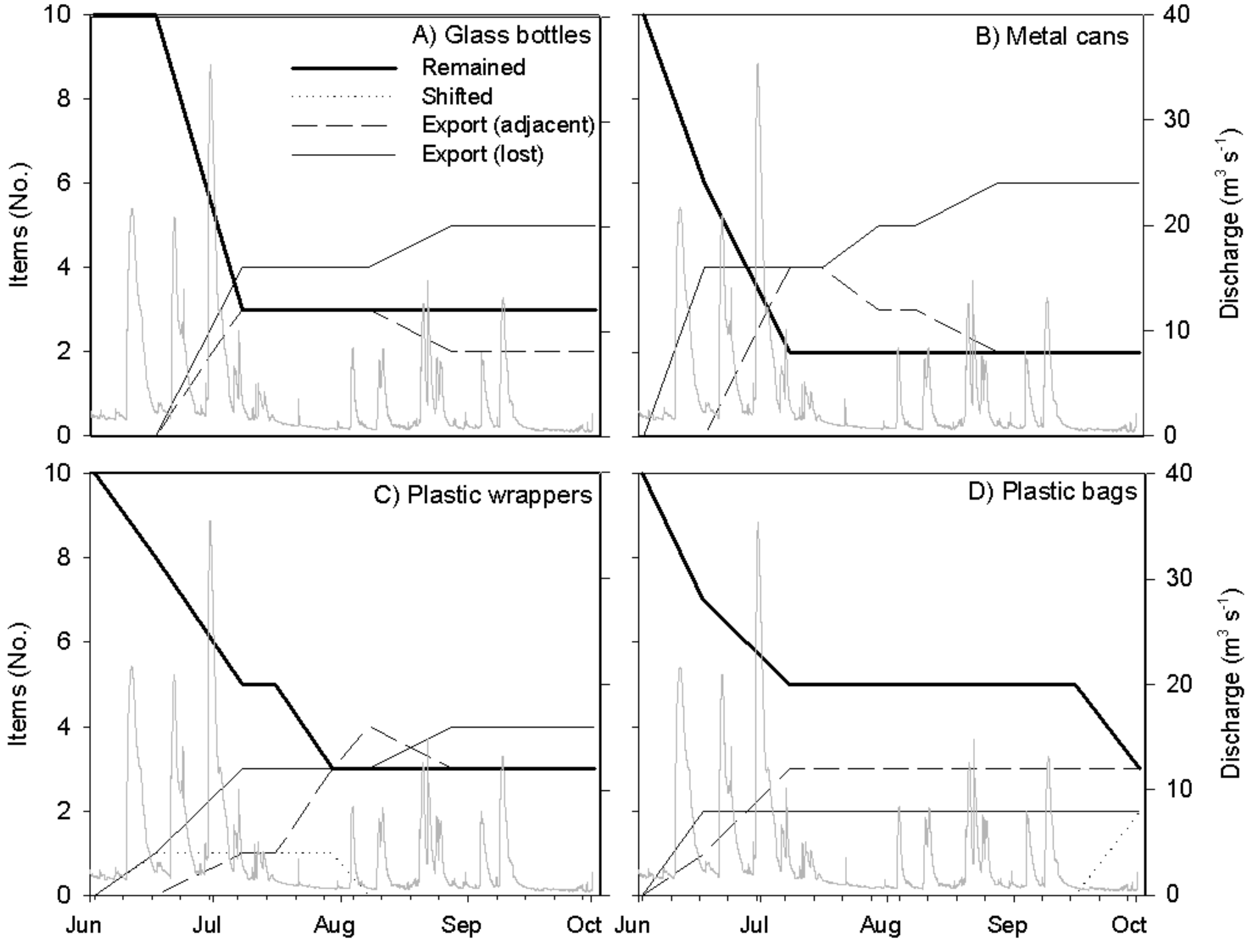

Figure 7. Movement patterns of marked glass bottles (A), metal cans (B), plastic wrappers (C), and plastic bags (D) during the biweekly flux study conducted in the riparian zone of the North Branch of the Chicago River in Miami Woods. ‘Remained’ indicates that the item remained in its original location between sampling periods. ‘Shifted’ indicates that the item moved between sampling periods but remained within the study quadrat. ‘Exported (near)’ indicates that the item was exported out of the quadrat but remained in the vicinity (within 100 m downstream and 40 m inland) of the study area. ‘Exported (lost)’ indicates the item was exported from the quadrat and was not in the vicinity of the study area.

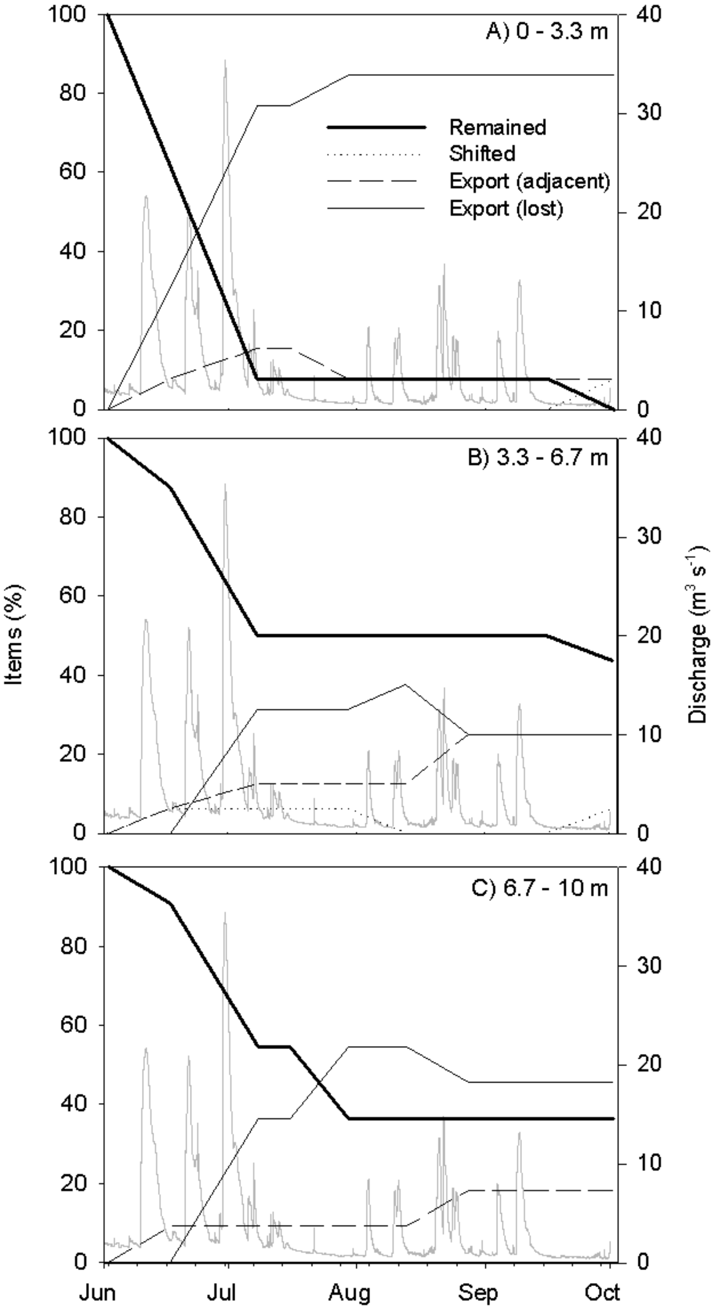

Figure 8. Movement patterns of marked anthropogenic litter (AL) items near the water's edge (0-3.3 m inland) (A), the middle (3.3-6.7 m inland) of the quadrat (B), and the most inland portion of the quadrat (6.7-10 m inland) (C) during the biweekly flux study conducted in the riparian zone of the North Branch of the Chicago River in Miami Woods. 'Remained' indicates that the item remained in its original location between sampling periods. 'Shifted' indicates that the item moved between sampling periods but remained within the study quadrat. 'Export (near)' indicates that the item was exported out of the quadrat but remained in the vicinity (within 100 m downstream and 40 m inland) of the study area. 'Export (lost)' indicates the item was exported from the quadrat and was not in the vicinity of the study area.

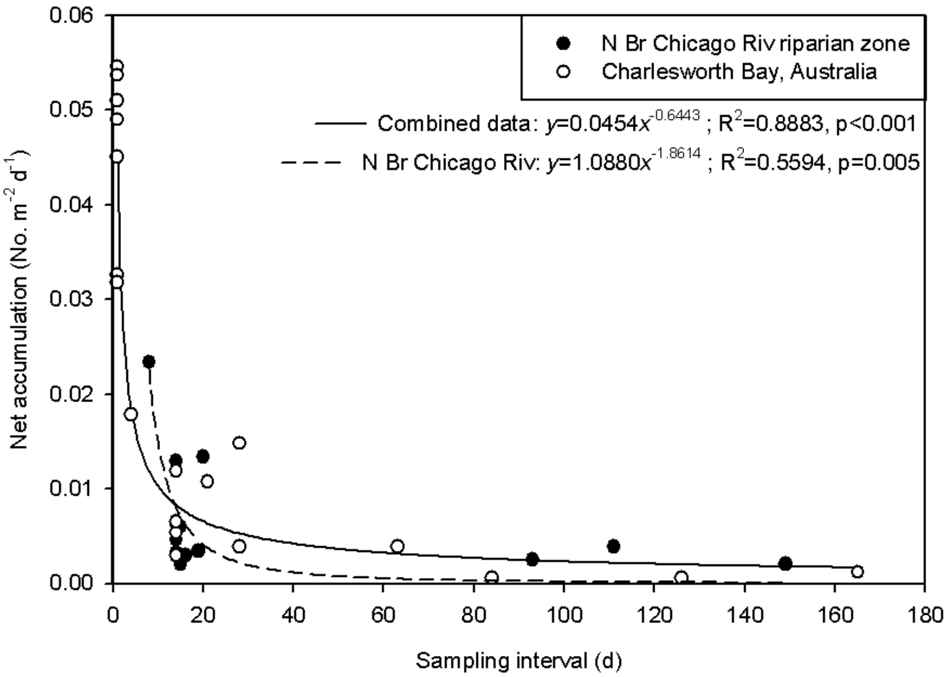

Figure 9. Plot of estimated daily accumulation rate of anthropogenic litter compared to time between sampling periods. The graph displays data from this study as well as data from Figure 2 of Smith and Markic 2013.

Table 11. Estimated total abundance of AL in the study streams. Riparian export rates are based on the mean export rate from our seasonal flux study.

	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
Benthos 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})	22.694	172.166	32.453	31.637	33.066
Benthos Mass (g m^{-2})	4.923	63.673	3.379	8.830	2.892
Riparian River (kg)	27,848	67,327	38,213	25,270	20,621
Benthos River (kg)	5,630	11,043	3,008	5,240	500
Total River (kg)	33,478	78,371	41,221	30,510	21,121
Total River (metric ton)	33	78	41	31	21
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

*Lengths were obtained from U.S. Geological Survey. National Hydrography Dataset high-resolution flowline data. The National Map, accessed March 10, 2015.

Part II. Microplastic concentration and bacterial colonization

Methods *Study sites.* During Jul-Oct 2014, we sampled 8 streams in Chicago metropolitan area and 2 in central Illinois (IL) (N=10 total) that receive treated WWTP effluent. Streams varied in their discharge and relative influence of effluent. The WWTPs were variable in the size of municipalities which they service, volume of treated effluent released, and treatment methods used for filtration and disinfection.

Microplastic collection and quantification. Microplastic was collected from streams with neuston nets (0.52 × 0.36 m) of 333 µm mesh. In the North Shore Channel, we deployed nets behind a stationary boat. All of the other streams were wadeable. At these streams, we stood behind the nets, took care not to disturb the net tail, and held the nets at the water's surface. Deployment time (typically 15-20 minutes) and depth of net submergence were recorded. Water velocity was measured at the center of each net during each deployment (Marsh-McBirney Flo-Mate Model 2000 Portable Flowmeter, Loveland, CO). After 15-20 min, all collected material was rinsed from the net into 1 L plastic containers (N=4 downstream and 4 upstream) with unfiltered site water, and then placed into a cooler on ice for transport to the laboratory where they were stored at 4ºC until measurement of microplastic concentrations.

To collect samples for bacterial measurements, additional net samples were collected downstream. Material from the nets was rinsed onto a sterile white tray. Individual microplastic particles were picked using sterilized forceps and placed in a 160 mL sterile specimen container with ~20 mL of site water. Organic material from the sample was removed in the same fashion. To measure water column bacteria, 2 L of unfiltered site water from the water column at the upstream and downstream sites were collected. The specimen containers and 2 L water column samples were transported on ice to the laboratory where they were stored at 4ºC until processing. Samples for DNA extraction were processed within 72 h, and samples for microplastic counts were processed over 6-8 weeks. Also collected were 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.

A protocol designed for the quantification of marine samples to measure microplastic concentrations was adapted for this study (Baker et al. 2011, Eriksen et al. 2013). Samples from the net collections were first run through 4.75 mm and 330 µm stacked sieves. The remaining 0.330-4.75 mm fractions were stored in glass beakers in a drying oven at 75 ºC. Organic material was degraded through a wet peroxide oxidation (0.05 M Fe(II) and 30% hydrogen peroxide) at approximately 75ºC. Plastic is resistant to wet peroxide oxidation (Baker et al. 2011, Eriksen et al. 2013). Samples then went through a salinity-based density separation using sodium chloride, where microplastic floated and heavier inorganic material was drained from the sample (Baker et al. 2011). Microplastic was filtered and counted under a dissecting microscope. The microplastic type (i.e., fragment, pellet, foam, film, or fiber) was recorded for each particle in each field of view. All particles of fragments, pellets, foam, and film were counted individually. Due to the abundance of fibers and their tendency to stick to the filter, microplastic fiber particles were counted using a sub-sample approach. For each sample, 3 random subsamples of each quadrat of the filter were counted. 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. Concentration was calculated by dividing the number of particles by water volume (No. items m^{-3}), or 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) No microplastic contamination of fragments, pellets, or foam was found. Mean procedural contamination by microplastic fibers was 4.67 per sample, which was 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 organic matter samples, material collected manually from the net samples was placed into 2 mL microcentrifuge tubes for DNA extraction. For the water column samples, 500 mL of 2 L water samples was filtered using 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).

Bacterial assemblages were profiled via next-generation amplicon sequencing of 16S rRNA genes. PCR amplification was performed using primers CS1_515F and CS2_806R, which amplify the V4 hypervariable region of bacterial and archaeal 16S rRNA genes (Caporaso et al. 2011). For all samples, successful DNA amplification was confirmed by agarose gel electrophoresis. Amplicons were sequenced in a 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 (Schloss et al. 2011). 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.

Data analysis. We used 2-way analysis of variance (ANOVA) to compare differences in total microplastic concentration among streams and between sampling locations (upstream and downstream, or whether WWTP effluent was present). We applied a natural log transformation to ensure concentration data met the assumptions of ANOVA. After applying a Bonferroni correction ($\alpha=0.05/9=0.006$), we then performed multiple comparison of microplastic concentration at each stream, since there was a significant interaction between stream and sampling location in our 2-way ANOVA. Since the proportion of effluent entering our study streams was variable, we also calculated the ratio of microplastic downstream to upstream. Upstream and downstream samples were independent of one another, so one replicate each from downstream and upstream were randomly paired to calculate the ratios. We performed a 1-way ANOVA on the natural log of this ratio to detect differences among streams. We followed this 1-way ANOVA with Tukey's multiple comparison test. All ANOVAs and Tukey's tests were completed in SYSTAT 13.0 (Systat, Inc. Chicago, IL).

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 observed numbers of OTUs, Chao1 richness, and the inverse Simpson and Shannon-Weiner (H') indices were calculated for each sample using MOTHUR.

Principle findings/results. *Microplastic concentration.* There was a significant interaction between stream and presence of effluent (i.e., upstream versus downstream) (2-way ANOVA, p<0.001; Figure 10A). Microplastic concentration was significantly different among streams (2-way ANOVA p<0.001; Figure 10A). At all sites microplastic concentration downstream of the WWTP effluent was higher than upstream (except Little Kickapoo and Goose Creeks; Figure 10A). The only stream that had significant differences in downstream and upstream microplastic concentration with a Bonferroni Correction applied was Higgen's Creek (Figure 10A). There were significant differences in the ratios of downstream to upstream microplastic concentration (Figure 10B). Sampling methodologies may explain the pattern observed at Goose Creek. The upstream sampling location in this creek had a lower discharge compared than all other upstream sites, except Little Kickapoo and Springbrook Creeks. The water upstream at Goose Creek was also very shallow, so that only one-third of the net was submerged. This resulted in a low volume of water being collected, and thus a low number of microplastic particles generated a potentially artificially-high concentration.

Bacterial communities on microplastic. There were distinct differences in the bacterial assemblages among sample types (Figure 11). Bray-Curtix index scores were significantly differen when comparing all sample types (AMOVA, p value <0.001) and when comparing any one category to another (Table 12). To complete this part of our analysis, we will use one-way ANOVA to assess the effects of sample type on microbial diversity, which we will follow Tukey's multiple comparison test. During March 15-31, 2015, we will be identifying bacterial taxa genera which make the largest contributions to the dissimilarities between sample types (based on the Bray-Curtis index) with a SIMPER analysis run in Primer 6 (Primer-E Ltd., Plymouth, United Kingdom). Multiple analyses are ongoing, and take long processing time due to the high number of microbial sequences in each sample. We will compare microplastic to organic material, microplastic to non-plastic downstream substrates, and microplastic to the upstream water column. We will compare our results to previous work which identified *Pseudomonas* as a common bacteria genus on plastic (McCormick et al. 2014). Initial results suggest that variation in community composition within microplastic samples may be greater than the within variation of other substrates. We will also analyze whether the dominant bacteria genera on microplastic differ among sites.

Conclusion. Overall, our results suggest that WWTP effluent is a point source of microplastic to rivers. Furthermore, this research and previous studies (McCormick et al. 2014, Zettler et al. 2013, Harrison et al. 2014) demonstrate that microplastic supports unique bacterial communities in comparison to natural substrates. The plastic may provide a novel habitat for bacteria, or if taxa colonizing microplastic have plastic-degrading metabolic capabilities, microplastic may provide a novel carbon source (McCormick et al. 2014). Research on the ecological impacts of microplastic in freshwater environments is lacking in comparison to marine ecosystems. This research provides a foundation for future studies analyzing biofilm activity on microplastic and its effect on higher trophic levels.

Part II: Tables and Figures

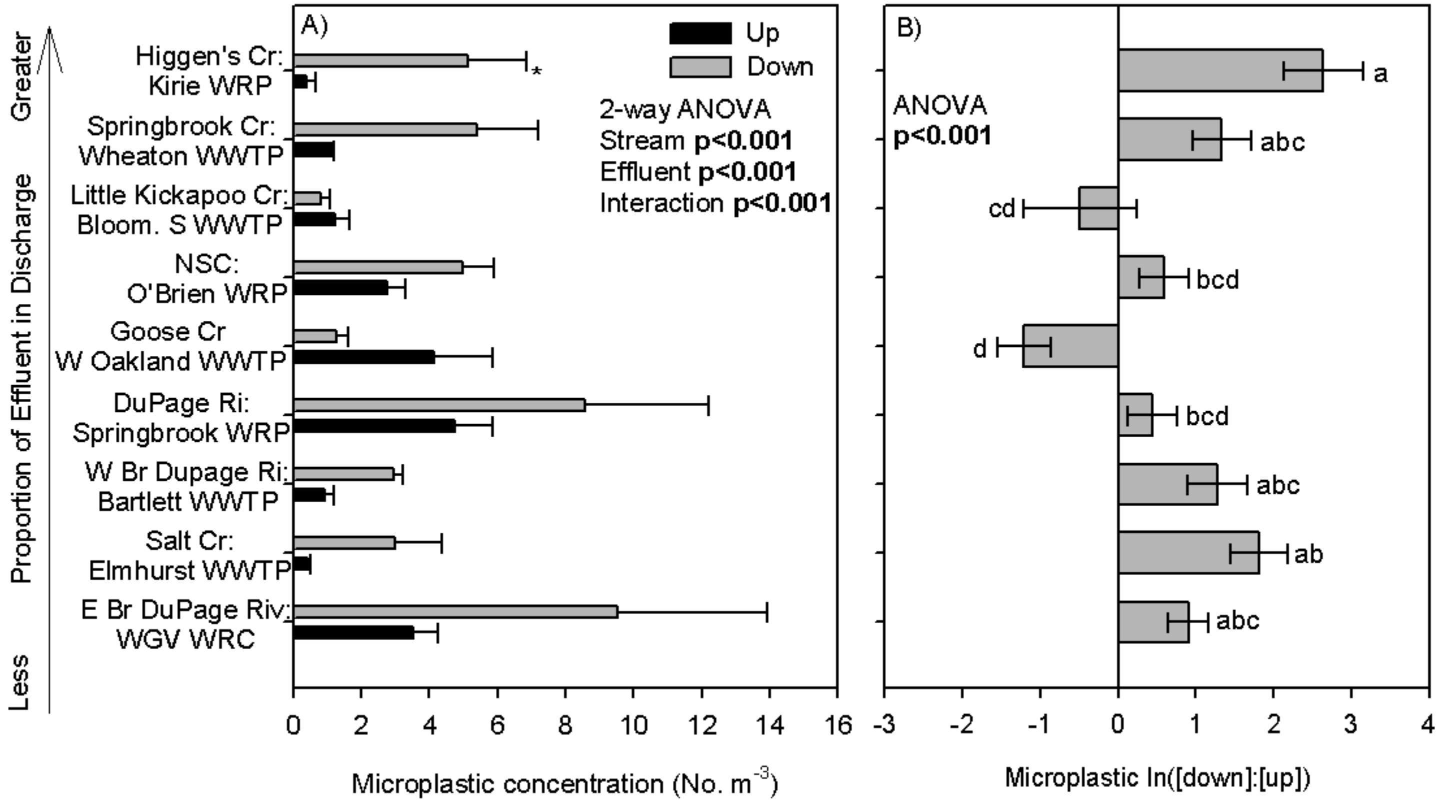

Figure 10. A) Microplastic concentration upstream and downstream B) ratio of microplastic concentrations downstream and upstream at each of our study streams. Bars represent mean (SE). * indicates significant difference in downstream and upstream concentrations with a Bonferroni Correction. Letter’s represent Tukey’s test results.

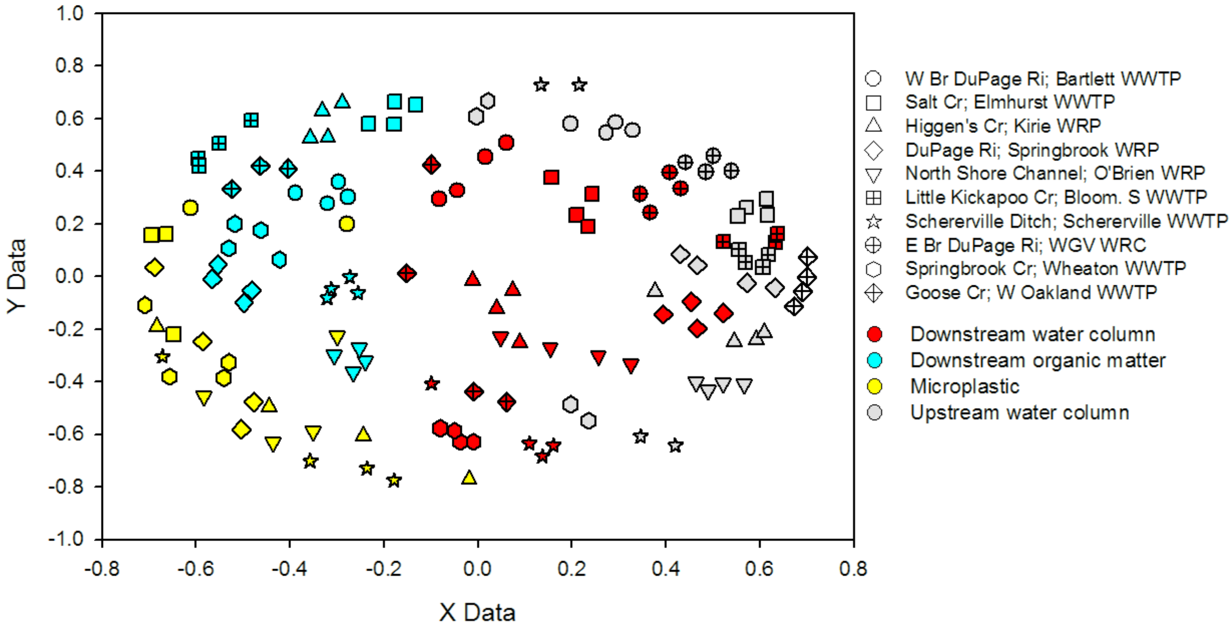

Figure 11. nMDS ordination of 16S sequencing data (Bray-Curtis dissimilarity) comparing community structures of bacteria collected in our 10 study sites.

Table 12. 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). P-values were calculated using the AMOVA run within MOTHUR

Comparison	p-value
All sample types	<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

Student information

1. Amanda McCormick, *MS student, Loyola University Chicago*
Ms. McCormick worked on this project as her MS thesis (2013-2015) at Loyola. She is currently writing together the thesis which will be published in two manuscripts. Her anticipated date of graduate with the MS is August 2015.

2. Joshua Hittie, *undergraduate student, Loyola University Chicago*
Mr. Hittie assisted in collecting AL and microplastic samples during the summer of 2014. He also assisted in categorizing AL and processing microplastic samples in the lab. He is a senior and will graduate in May 2015.

3. Melaney Dunne *undergraduate student, Loyola University Chicago*
Ms. Dunne assisted in processing microplastic samples. She is a senior and will graduate in May 2015.

Communication of results

A. Publication: We plan to publish this research in 2 peer-reviewed publications with submission in summer 2015.

1. The first paper will consist of the research described in Part 1 above (AL abundance, composition, and flux

2. The second paper will include the data in Part 2: microplastic concentration and microbial colonization research.

B. Presentations:

Completed

1. McCormick, A. and T.J. Hoellein. Oct 15, 2014. Anthropogenic litter and microplastic in urban streams: abundance, source, and fate. Illinois Water Conference. Champaign, IL

Upcoming

2. The microplastic research will also be presented in an oral presentation by Amanda McCormick at the annual Society for Freshwater Science meeting in Milwaukee, WI in May 2015.

3. The AL density and flux data will be presented in an oral presentation by Timothy Hoellein at the annual Society for Freshwater Science meeting in Milwaukee, WI in May 2015.

4. Data on microplastic abundance and microbial community composition will be presented at Loyola University Chicago, Weekend of Excellence (dedicated to undergraduate research) by Joshua Hittie.

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Appendix Table 1. Classification of AL by material and item type.

Material	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	Aluminum 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, nets, bags)
Plastic	PL16	Sheeting (tarp or 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