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Environmental Drivers of Leaf Breakdown Rate in an Urban Watershed

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ENVIRONMENTAL DRIVERS OF LEAF BREAKDOWN RATE IN AN URBAN WATERSHED

A THESIS SUBMITTED TO
THE FACULTY OF THE GRADUATE SCHOOL
IN CANDIDACY FOR THE DEGREE OF
MASTER OF SCIENCE

PROGRAM IN BIOLOGY

BY
ASHLEY RACHELLE COOK
CHICAGO, IL
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To my dad. You always have been and always will be my number one role model.
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ABSTRACT

Leaf litter breakdown is an important ecosystem process in urban streams, but conditions in urban streams may have confounding effects on breakdown rates. Reduced abundance of macroinvertebrate shredders may slow breakdown, but rates may increase if high nutrient concentrations stimulate microbial decomposers and if flooding enhances leaf fragmentation. We used the litter bag technique to measure the relative importance of multiple environmental drivers on breakdown of eastern cottonwood (*Populus deltoides*) leaves at 5 sites throughout the North Branch of the Chicago River watershed that spanned a gradient of urbanization. Although no specialized macroinvertebrate shredders were present, generalist taxa including isopods (*Asellus aquaticus*) and amphipods (*Gammarus* sp.) were abundant at all sites. Thus, we used large and small mesh bags to test macroinvertebrate effects on breakdown rate. We also measured discharge, water chemistry, organic matter standing stock, benthic macroinvertebrate community composition, and sub-watershed land-use at each site. Leaf breakdown was significantly different among sites and between bag types. Discharge and isopod abundance were positively related to leaf breakdown, while nutrient concentrations and land-use categories were unrelated to breakdown. Litterbags were ‘hot spots’ for isopods and amphipods, where their abundance was significantly higher than in benthic samples. We conducted a follow-up study in artificial streams to test the individual effects of water
velocity and isopods on leaf breakdown using conditions matching field sites. Increasing water velocity from 0.02 m/s (control) to 0.07 m/s (high velocity) increased leaf breakdown by 33%, and isopods increased leaf breakdown by 40% (density = 1,034/m²).

Measuring environmental controls on leaf breakdown is critical to advance the use of leaf breakdown as an assessment tool in urban streams. Also, advances in watershed-scale approaches for stream management require studies that examine leaf breakdown throughout watersheds. Finally, laboratory experiments are complementary tools for measuring the role of individual environmental factors on breakdown, which are inextricable using field approaches, and could help parameterize models of stream ecosystem function in urban watersheds.
CHAPTER I
INTRODUCTION

Urban Ecosystems

In the United States, 82% of the total population lives in urban areas with an estimated 1.2% increasing annual rate of change (Central Intelligence Agency, 2010). Urbanization causes many changes to surrounding terrestrial and aquatic environments such as alteration of local climate, pollution in air, water, and soil, introduced species, and reduced species richness (McDonnell and Pickett, 1990). Urban habitats are connected to ecosystems elsewhere via downstream water flow and dispersal of plants and animals (i.e., macroinvertebrates, birds, and small mammals). Thus, pollutants that accumulate in urban habitats can be transported to other, non-impacted ecosystems.

Conservation of undeveloped space in urban areas provides many critical ecosystem services. For example, habitats such as parks, wetlands, forests, and rivers in urban environments provide filtering of air pollution by vegetation, microclimate regulation, noise reduction, rainwater retention, drinking water, and sewage treatment (Bolund and Hunhammar, 1999). Green areas in urban ecosystems provide benefits for human residents including recreation, aesthetic value, and stress reduction (Bolund and Hunhammar, 1999). Therefore, research on how ecosystems in urban environments function is critical to preserve ecosystem services that sustain public and environmental health.
Urban Streams

Despite the need for ecosystem services provided by conserving natural habitats in urban settings, urban stream ecosystems face many environmental stressors (Gessner and Chauvet, 2002). The term “urban stream syndrome” is used to describe the composite of hydrological, chemical, and biological conditions typical of urban streams (Walsh et al. 2005). One of the primary drivers of degraded conditions in urban streams is impervious surface cover in densely populated areas. Impervious surfaces are materials that do not allow infiltration of water into the soil, such as paved surfaces, rooftops, bedrock outcrops, and compacted soil (Arnold and Gibbons, 1996). Impervious surface cover in urban areas inhibits deep infiltration of water into the ground, which increases water runoff directly into stormwater drains and urban streams (Arnold and Gibbons, 1996). Increased runoff into urban streams causes ‘flashy’ hydrology with increased frequency and magnitude of flooding (Paul and Meyer, 2001), leading to stream bank erosion, sedimentation, and simplification of stream channels (Arnold and Gibbons, 1996). In addition, storm and wastewater infrastructure has a strong effect on urban stream hydrology (Paul and Meyer 2001). In older cities with combined sewer overflows (CSOs), wastewater volume can exceed the capacity of the sewer system during heavy rainfall, and sewer contents can directly enter adjacent water bodies including lakes, coastal environments, and urban streams.

In addition to changes in hydrology, urban streams can become enriched with chemicals, including nutrients such as nitrogen and phosphorus (N and P), pharmaceutical and personal care products (PPCPs), and contaminants from industrial use such as polychlorinated biphenyls (PCBs) and heavy metals (Paul and Meyer, 2001;
Chemicals come from point sources, including urban and industrial wastewater effluent, and non-point sources including stormwater and lawn care chemicals (Carpenter et al., 1998). Eutrophication (i.e., nutrient enrichment) is a common condition in aquatic ecosystems worldwide and is a major focus of research in human-influenced ecosystems (Vitousek et al., 1997). Excess nutrients can stimulate increased biomass of primary producers and decomposition of biomass can reduce oxygen, thereby reducing macroinvertebrate and fish species diversity and population densities (Carpenter et al., 1998). A variety of pharmaceuticals and organic wastewater contaminants can be found in urban streams (Kolpin et al., 2004, Spongberg and Witter, 2008, Peng et al., 2008). Some examples are pesticides, caffeine, estrogens, and antibiotics. The ecosystem effects of PPCPs are less well-studied than for nutrients. Potential consequences of PPCP pollution include physiological and reproductive abnormalities and the selection of antibiotic-resistant bacteria (Kolpin et al., 2002). Finally, chemicals from industrial pollution such as polychlorinated biphenyls (PCBs) and heavy metals can also cause developmental abnormalities in fish, which can possibly affect the developmental and reproductive systems of fish predators, including humans (Wong, et al., 2000).

Physicochemical conditions typical of urban streams tend to select for specific types of biological communities. In general, the biological effects of urbanization are an increase in abundance of taxa categorized as ‘tolerant’ to anthropogenic influence, with decreases in overall community diversity (Paul and Meyer, 2001; Walsh et al., 2005). Tolerant species are those that are can survive increased frequency and magnitude of flooding, channel simplification, higher levels of eutrophication, and potential chemical
exposure, whereas ‘sensitive’ species cannot. For example, an urban stream typically will have reduced macroinvertebrate diversity, which can become dominated by tolerant taxa such as Chironomidae (non-biting midges) and Oligochaeta (worms) (Paul and Meyer, 2001). Invasive species are also common in urbanized streams (Walsh et al., 2005), as a direct result of human interaction (i.e., organisms released from aquaria or as bait) and because characteristics of invasive species help them thrive in urban stream environments, including omnivory, high fecundity, and tolerance of variable environmental conditions (Riley et al., 2005; Dukes and Mooney, 1999).

Given the suite of physical, chemical, and biological conditions that affect urban stream ecosystems, measurements of ecosystem processes such as rates of nutrient cycling, ecosystem metabolism, and leaf litter breakdown, are often suggested as useful tools for describing ‘health’ of urban streams. These metrics are useful because they integrate the activity of multiple organisms and trophic levels, and reveal interactions among physical and biological components of stream ecosystems (Palmer and Febria, 2012). Determining the drivers of aquatic ecosystem processes is a crucial step in assessing the functional integrity of a stream (Gessner and Chauvet, 2002). Studies that examine the composite physical, chemical, and biological factors that govern ecosystem processes in urban stream ecosystems are needed to develop effective conservation and restoration strategies (Wenger et al., 2009; Palmer and Febria, 2012).

**Leaf Breakdown is an Important Ecosystem Process in Streams**

Food webs in headwater streams can be fueled by autochthonous carbon (e.g., stream algae) or allochthonous carbon (e.g., leaf litter from terrestrial plants; Doi, 2009; Rueda-Delgado et al., 2006). Both sources contribute to food webs in most streams, but
in forested headwater streams, leaf litter is the primary carbon source for the food web (Paul et al. 2006). Therefore, factors that control breakdown of leaf litter affect retention of carbon in stream biota (Benfield, 1996; Baldy et al., 1995; Imberger et al., 2013). In forested streams, leaf breakdown rate is a critical indicator of stream ecosystem function. Leaf breakdown has been suggested as a metric for categorizing overall stream ecosystem health because it combines the activity of multiple trophic levels and physicochemical conditions, and techniques for its measurement are well-established and standardized (Bärlocher, 2005; Gessner and Chauvet 2002).

Leaf litter breakdown in streams is affected by physical, chemical, and biological factors. For example, enhanced flooding from land-use development (i.e., impervious surfaces) can increase the speed of leaf breakdown via fragmentation (Paul et al. 2006). Flashy hydrology also may increase fragmentation from high velocity and rapid changes in discharge (Rueda-Delgado et al. 2006). Temperature can speed up leaf breakdown rate at several spatial scales (Royer and Minshall, 2003) by increasing microbial activity (Webster and Benfield, 1986).

Water chemistry can affect leaf litter breakdown through its influence on microbial decomposers, including bacteria and fungi. For example, leaf litter breakdown is faster in streams where microbial growth is enhanced by nutrient concentrations across streams (Pascoal et al., 2005; Young et al., 2008; Royer and Minshall, 2003) and when nutrients are experimentally added to streams (Greenwood et al. 2007). However, in streams affected by mine drainage, breakdown rates may be slower due to disruption of microbial decomposers from other changes in water chemistry such as low pH and heavy metals, which inhibit microbial growth (Hogsden and Harding, 2013).
A major focus of research on leaf litter decomposition in streams is the role of macroinvertebrate shredders. Shredders consume coarse particulate organic matter (CPOM) and produce fine particulate organic matter (FPOM) via feeding and excretion, which can be consumed by other stream organisms (Cummins et al., 1989). Many shredders have mouthparts, digestive capacity, and life history characteristics allowing them to feed exclusively on leaf litter, while others may switch among food types (i.e., fine particles, algae; Wallace and Webster, 1996; Cummins et al., 1989) and consume leaf litter when it is available in autumn and winter. Shredders obtain a portion of their nutrition from leaf litter alone, but much of the nutritive value in leaf litter is found in the microbial biofilms (Franken et al., 2005). Overall, leaf litter breakdown rates in streams are elevated with increased abundance and diversity of shredding macroinvertebrates (Cummins et al. 1989; Tank et al., 2010).

**Leaf Breakdown in Urban Streams**

Much research on leaf litter breakdown has taken place in forested headwater streams, but the influence of urban stream conditions on leaf breakdown rates is less frequently studied. The symptoms of the urban stream syndrome affect all of the hydrological, chemical, and biological factors, which, in turn, affect leaf litter decomposition. In fact, some characteristics of urban streams could increase leaf litter breakdown, and some could decrease leaf breakdown, which makes predicting the effects of urbanization on breakdown challenging. For example, reduced diversity and abundance of shredding macroinvertebrates typical of urban streams would reduce leaf breakdown rates via shredding. However, generalist macroinvertebrates (e.g., amphipods), which are abundant in urban streams, may consume leaf litter to fill that
niche. If so, leaf litter carbon could be retained within urban stream food webs, and less material would be transported downstream. However, flashy hydrology typical of urban streams could increase leaf litter breakdown and export leaf litter carbon to downstream environments. High nutrient concentrations could enhance microbial decomposers and increase leaf breakdown as well. The relative importance of mechanisms controlling leaf breakdown in urban streams are unknown.

Experimental Design: Determining the Drivers of Leaf Breakdown in an Urban Watershed

The overarching goal of my study was to measure leaf litter breakdown in an urban watershed using paired field and laboratory experiments to determine environmental controls on leaf breakdown. In the field study (chapter 2), I examined leaf litter breakdown using a watershed approach, in which I measured leaf breakdown rate and environmental drivers at multiple sites throughout an urban watershed. In the laboratory study (chapter 3), I used experimental manipulations of macroinvertebrates and water velocity to test the relationships and conclusions generated from the field study.

The field study was conducted in the North Branch of the Chicago River, an urbanized watershed north of Chicago, Illinois. Five streams of different sizes within the watershed were selected to represent a gradient of urbanization. All sites were high in nutrients and exhibited flashy hydrology. I used the standard ‘litter bag’ technique with two different mesh sizes to quantify the effect of macroinvertebrates on leaf breakdown (Bärlocher, 2005; Bo et al., 2014; Cheever and Webster, 2014). Including 5 sites in the field study allowed me to examine how physicochemical conditions, which varied among
sites, affected leaf breakdown rates. The results of the field study allowed me to further explore our findings in a laboratory setting.

The laboratory study was conducted in the artificial stream facility at Loyola University Chicago. Twelve artificial streams were used to measure leaf breakdown rate in a controlled laboratory setting. In the laboratory, I isolated our treatments (water velocity and isopod abundance) to quantify their effects on breakdown while all other environmental factors remained constant. The combined laboratory and field projects advanced the understanding of controls on leaf breakdown in urban streams.

A conceptual diagram for the drivers of leaf breakdown rate in forested streams based on the literature and our predictions for drivers of leaf breakdown rate in an urban watershed is shown in Figure 1. Macroinvertebrate shredders play a major role in leaf breakdown in forested streams that have little human impact in the watershed (Wallace et al. 1982, Flores et al., 2013). Forested streams are typically low in nutrients and do not experience flooding events as frequently as urban streams. In urban streams, I predicted that macroinvertebrate shredders would not be one of the main drivers of leaf breakdown rate, because obligate shredders are often missing. Therefore, I predicted that flooding (i.e., fragmentation) and nutrient availability (which stimulates microbial decomposition) would be the main drivers of leaf breakdown at the 5 study sites in an urban watershed.
Figure 1. Conceptual diagram of (A) drivers of leaf breakdown rate in a forested stream according to the literature and (B) my predictions for drivers of leaf breakdown rate in an urban watershed. Relative widths of arrows denote strength of interaction.
CHAPTER II
ENVIROMENTAL DRIVERS OF LEAF BREAKDOWN RATE IN AN URBAN WATERSHED: A FIELD STUDY

Introduction

Urban development has major impacts on stream biodiversity and ecosystem processes (Walsh et al., 2005). A fundamental environmental stressor in urban streams is watershed impervious surfaces (i.e., paved surfaces, rooftops, bedrock outcrops, and compacted soil) that inhibit soil infiltration and transpiration, thereby exacerbating flooding (Arnold and Gibbons, 1996). Urban stream water also has higher concentrations of solutes derived from road and lawn runoff, wastewater effluent, and combined sewer overflows (Carpenter et al., 1998). The collection of physical, chemical, and biological characteristics typical of urban streams are referred to as the “urban stream syndrome”, and includes flashy hydrology, high concentrations of nutrients and other solutes, and reduced biodiversity of biofilm taxa (i.e., algae, bacteria, and fungi), macroinvertebrates, and fish (Walsh et al., 2005; Paul and Meyer, 2001; Wenger et al., 2009).

Food webs in headwater streams can be fueled by autochthonous carbon (e.g., stream algae) or allochthonous carbon (e.g., leaf litter from terrestrial plants; Vannote et al. 1980, Tank et al., 2010). Both sources contribute to food webs in most streams, but in forested headwater streams, leaf litter is the primary carbon source for the food web (Paul et al. 2006, Hall and Meyer 1998). Leaf breakdown has been suggested as a metric for
categorizing overall stream ecosystem health because it integrates the activity of multiple trophic levels and physicochemical conditions, and techniques for its measurement are well-established and standardized (Bärlocher, 2005; Gessner and Chauvet 2002). Measuring factors that control leaf breakdown rate in streams reveals key pathways of carbon movement and retention in stream food webs (Young et al., 2008).

Leaf breakdown rate can be influenced by physical and chemical factors that differ in urban streams relative to those not impacted by human development (Paul et al., 2006; Greenwood et al., 2007; Cummins et al., 1989; Tank et al., 2010). For example, increased magnitude and frequency of flooding in urban watersheds can speed up fragmentation of leaves and overall leaf breakdown rate (Paul et al., 2006). Also, higher nutrient concentrations promote microbial growth, which increases leaf litter breakdown rate (Greenwood et al., 2007).

In contrast to the effects of hydrology and nutrient availability, urban streams may have lower rates of leaf litter decomposition via reduced abundance of shredding macroinvertebrates. Shredders convert coarse particulate organic matter (CPOM, i.e., leaf litter) into fine particulate organic matter (FPOM) that can be used by other stream organisms (Cummins et al., 1989). Increased abundance and diversity of shredding macroinvertebrates enhances litter breakdown rate (Cummins et al. 1989; Tank et al., 2010). Urban streams typically have reduced macroinvertebrate diversity (Johnson et al., 2013), including a lack of specialized shredding macroinvertebrates. The diversity of macroinvertebrates is low in polluted streams (i.e., agricultural and urban streams), but the more tolerant macroinvertebrates such as isopods may be abundant and therefore affect leaf breakdown (Griffiths et al., 2012). Controls on litter breakdown are well
documented in forested streams (Flores et al., 2013; González et al., 2013; Paul et al., 2006; Suberkropp and Chauvet, 1995; Tuchman and King, 1993), and increasingly for agricultural streams (Niyogi et al., 2003; Paul et al., 2006; Tuchman and King, 1993; Goss et al., 2014; Griffiths et al., 2012; Griffiths et al., 2009). However, the mechanisms that control leaf breakdown in urban streams are less well studied.

The goal of our study was to measure environmental drivers of leaf breakdown rate in an urban watershed. To measure the effect of shredding macroinvertebrates on leaf breakdown, we used the litter bag method with two mesh sizes at each site (Bärlocher, 2005; Bo et al., 2014; Cheever and Webster, 2014). We predicted that flooding and nutrient availability would be positively related to breakdown rate, but where abundant, facultative shredding macroinvertebrates could play a secondary role in enhancing leaf breakdown.

Materials and Methods

Study sites

We addressed our research questions at 5 sites in the North Branch of the Chicago River watershed in northeastern Illinois (IL), USA (Figure 2). The watershed is 234 km², and the river flows from north to south, starting in the suburbs of Lake County, IL and joining the North Shore Channel in the City of Chicago near Foster Avenue in Cook County, IL. Previous measurements have shown that the North Branch of the Chicago River shows conditions typical of urban streams, including high nutrients and low macroinvertebrate diversity (Turek and Hoellein, in press). The riparian zone mainly consists of parkland, golf courses, and residential neighborhoods.

Our study sites are all within the North Branch watershed, and were chosen to
represent a gradient of urbanization. Three sites are separate tributaries of the river (East, Middle, and West Fork subwatersheds). The site at Harms Woods is downstream of the confluence of the East and Middle Forks, and the site at Edgebrook Woods is downstream of all confluences. We selected 5 study sites within the same watershed to minimize environmental variation that can inhibit data interpretation when sites are selected among separate watersheds. In addition, preliminary data suggested that leaf litter and potential macroinvertebrate shredders (i.e., amphipods and isopods) were present among all sites, so examining their influence on leaf litter breakdown is of potential ecological importance. However, we acknowledge that the two downstream sites do not share the same degree of equal independence as the 3 tributary sites.
Figure 2. The North Branch of the Chicago River watershed flows south from headwaters in the northern suburbs into Chicago, IL, USA. Sites: 1 Edgebrook Woods, 2 Harms Woods, 3 Middle Fork, 4 East Fork, and 5 West Fork.

**Estimating leaf litter breakdown**

We quantified breakdown rates of eastern cottonwood (*Populus deltoides*) at the 5 study sites. We selected *P. deltoides* because it is a dominant riparian tree species (Friends of the Forest Preserves & Friends of the Parks, 2002). In October 2012, naturally senesced *P. deltoides* leaves were collected from Edgebrook Woods and brought back to the laboratory, where they were spread out on a tarp to air-dry for approximately 21 d.
Small mesh (pore size= 3.3 mm) and large mesh (pore size= 6.7 mm) bags were used to measure leaf breakdown. Other studies have used much smaller mesh to exclude macroinvertebrates (Pascoal et al., 2005; Taylor and Andrushchenko, 2004; Bruder et al., 2013; Taylor and Chauvet, 2014; Cheever and Webster, 2014). However, small mesh can also restrict water flow and lead to anoxia. Our preliminary data showed the two mesh sizes each allowed for aerobic conditions in leaf packs and established a contrast in macroinvertebrate abundance rather than a complete exclusion. The small mesh bags were made of polypropylene (Cady Bag Company, Pearson, GA, USA) and the large mesh bags were constructed by hand using a sheet of black plastic aquaculture netting (Memphis Net & Twine Co., Inc., Memphis, TN, USA) held together by plastic cable ties. The small and large mesh bags had the same dimensions (30 x 15 cm).

Litterbags were filled with 8g of air-dried P. deltoides leaves in the laboratory and deployed at each of the 5 sites on October 22, 2012 (N=15 bags of each mesh type per site, 150 bags total). The bags were arranged in strings of 6, with alternating small and large mesh bags connected by plastic cable ties, where each string was secured to the streambed using rebar hammered into the substrate (Bärlocher, 2005, Entrekin et al. 2008). The strings were placed in areas with unobstructed water flow (i.e., not in pools or backwater areas).

We removed 6 bags (N=3 per mesh type) from each site on days 7, 21, 42, 77-86, and 131. After removal from the stream, the leaf bags were immediately placed in plastic zip-top bags and kept cool until back in the laboratory (Entrekin et al., 2008). In the laboratory, all bags were stored at 4 °C and processed within 3 d. For each bag, we removed all leaves and lightly rinsed each leaf with deionized water over a white
collection tray to remove aquatic organisms. All visible leaves and leaf particles were placed into brown paper bags. All macroinvertebrates were preserved in 95% ethanol. The brown paper bags containing the leaf litter were placed into a drying oven at 60°C. After 3 d, we transferred leaves from the bags into pre-ashed and weighed aluminum pans (Thermo Fisher Scientific Inc., Millville, NJ, U.S.A.) and measured leaf dry mass. The leaves were then placed in a muffle furnace (550°C) for 3 h, removed, cooled in a desiccator for 1 h, weighed, and ash-free dry mass (AFDM) was calculated.

We calculated breakdown rate (k), as exponential decay from a regression of the proportion of AFDM remaining (ln transformed) vs. time (days) (Benfield, 2006). Handling loss was calculated by bringing an additional 3 small mesh and 3 large mesh bags into the field on the deployment date (N=6), and returning them immediately to the laboratory to calculate the starting weight (Benfield, 2006). The fourth collection date was reported as 77-86 because not all bags were accessible on day 77 due to ice cover. On day 77, we collected bags at sites 2 and 3, and on day 86 we collected the bags from sites 1, 4, and 5.

Macroinvertebrates

Preserved isopods (*Asellus aquaticus*) and amphipods (*Gammarus facsiatus*) from each sample were counted, and we measured length from the base of the antennae to the tip of the tail under a dissecting microscope. We created a length-mass regression for each taxon. We selected 20 isopods and 20 amphipods that spanned the range of sizes in the study sites (isopods: 6-20 mm, amphipods: 2-14 mm), and measured dry weight and AFDM of each as described above. Length-mass regressions of AFDM (g) vs. amphipod or isopod length were constructed. The equation was \( y = 0.0007x-0.0041 \) (\( R^2 = 0.88 \)) for
isopods and $y = 0.0003x - 0.0007$ ($R^2 = 0.71$) for amphipods. We used these equations to calculate AFDM for every individual.

**Physical and chemical measurements**

We measured dissolved oxygen (DO), conductivity and discharge on the day leaf bags were deployed and each collection date. DO concentration was measured using a HQ40d portable meter (Hach Company, Loveland, CO, USA), and recorded as concentration (mg/L) and percent saturation. We measured conductivity using a YSI Model 30 conductivity probe (YSI Incorporated, Yellow Springs, OH, USA). To calculate discharge, we first suspended a meter tape across the stream at an area with no debris dams or large rocks. At 0.5 to 2 m intervals, depending on stream width, we measured depth using a top setting wading rod (Hach Company, Loveland, CO, USA) and current velocity using a Marsh-McBirney Flo-Mate 2000® Portable Velocity Flow Meter (Hach Company, Loveland, CO, USA). We calculated discharge ($Q; m^3 s^{-1}$) by multiplying width (m) by depth (m) by velocity (m s$^{-1}$) for each interval, and summing across the intervals. At sites 1 and 5, discharge measurements were obtained using nearby USGS gauging stations (Number 05536105 on the North Branch of the Chicago River and Number 05535500 on the West Fork of the North Branch, respectively).

We also collected triplicate water samples for measuring inorganic nutrient concentrations on each leaf bag collection date and on day 0 ($N=30$ per site). Water from each site was filtered in the field (glass microfiber filter; GF/F; Sigma-Aldrich Co., St. Louis, MO, USA) into a 20 mL, acid-washed Wheaton plastic scintillation vial (Thermo Fisher Scientific Inc., Millville, NJ, USA), and frozen until analyzed. All water samples were analyzed for soluble reactive phosphorus (SRP), ammonium ($NH_4^+$), and nitrate
We measured SRP using the antimonyl tartrate technique (Murphy and Riley, 1962), NH$_4^+$ with the phenol hypochlorite technique (Solorzano, 1969), and NO$_3^-$ with the cadmium reduction technique (APHA, 1988). NO$_2^-$ was measured using the same technique as NO$_3^-$, but with the cadmium column deactivated.

Coarse benthic organic matter and benthic macroinvertebrate community

Coarse benthic organic matter (CBOM) and benthic macroinvertebrate community measurements were conducted at each of the 5 sites on December 3, 2012 (day 42). First, we marked 100 m of stream reach either upstream or downstream of the leaf bag sites. We used a random number generator to determine the meter mark of the 3 replicate CBOM sampling sites. In addition, each of the 3 replicates was randomly assigned to the left, right or center of the channel. We used the same randomly assigned sampling locations at all 5 sites. We used a plastic corer (511 area cm$^2$) to collect CBOM. To obtain a sample, the corer was pushed into the substrate approximately 10 cm, the substrate was vigorously stirred, and collected using a 1-mm sieve (Benfield, 2006). Organic matter obtained using the sieve was collected in Uline 6 Mil Poly Bags (Uline, Pleasant Prairie, WI, USA). In the laboratory, the samples were preserved in 95% ethanol and sealed until analyzed. When analyzed, the samples were sorted into 3 categories: wood, coarse benthic organic matter (CBOM), and shells. The sorted samples were then measured for AFDM as described above.

We used a Hess Sampler (Wildlife Supply Company, Saginaw, MI, USA) to collect benthic macroinvertebrates. We collected 3 random samples from each of the 5 streams as described for CBOM. For each collection, we pushed the Hess Sampler
approximately 10 cm into the substrate and vigorously stirred the sediment.

Macroinvertebrates were suspended in the water and moved into the collecting net by the stream current. We emptied each sample into Uline 6 Mil Poly Bags, brought the samples to the lab, and we preserved them with 95% ethanol as described above. We picked, counted, and identified all macroinvertebrates from each sample. The isopods and amphipods were viewed under a dissecting microscope and measured to the nearest millimeter and biomass was calculated using length-mass regressions. We did not measure biomass of other macroinvertebrates, which included Chironomidae, Annelida, Corbicula fluminea, Gastropoda, Zygoptera, Tipulidae, Ephemeroptera, Epiprocta, and Coleoptera.

*Temperature*

Temperature was measured every hour for the duration of the experiment using HOBO® data loggers (Onset Computer Corporation, Pocasset, MA, U.S.A.). One logger was left on a string of bags at each site. The mean temperature for each site was calculated by averaging all hourly temperature measurements. The temperature logger was lost at site 1, so we used temperature data from the nearby USGS gauging station (05536105), and found that site 2 had the most similar temperature pattern. Therefore, for each collection date we compared the USGS temperature data for site 1 with our HOBO data from site 2 to find the mean difference between sites. We then extrapolated the data from site 2 using this value to estimate site 1 temperatures.

*Land-use*

We used ArcGIS 10.1 (Environmental Systems Research Institute, Redlands, California, USA) to analyze land-use data for the watersheds surrounding each
subwatershed drained by the 5 study sites. We imported the most recent data (2005) from the Chicago Metropolitan Agency for Planning (CMAP) into ArcGIS. For each subwatershed, we obtained data for total area and area covered by land-use types including impervious surface cover, agriculture, forest/grassland, water, wetland, commercial, industrial, institutional, residential, transportation, and utilities. We calculated the ‘urban’ category by combining commercial, industrial, residential, transportation, and utilities land-use. ArcGIS 10.1 was also used to create the site map (Figure 2).

Data Analysis

We used a nested analysis of covariance (ANCOVA) to determine if the breakdown rates were statistically different among small and large mesh bags and among sites where day was the covariate (sensu Griffiths et al., 2009). A 2-way ANOVA was used to determine if there was a difference in the number of isopods + amphipods per bag in the small mesh versus the large mesh. We used an additional 2-way ANOVA to determine if there was a difference in isopods + amphipods (No. m$^{-2}$) between small mesh, large mesh, and the benthic samples. A one-way ANOVA was used for nutrient concentrations (SRP, NO$_3^-$, and NH$_4^+$), DO, conductivity, discharge, temperature, CBOM, and wood to determine if there was a difference across the 5 sites. We used a correlation to measure the relationship between land-use and nutrient concentrations.

To determine which factors were having the greatest effect on breakdown rate we used a forward stepwise multiple regression analysis. The dependent factor was the breakdown rate, and 23 measurements were entered as the independent factors. The independent factors were physicochemical measurements (NO$_3^-$, NO$_2^-$, NO$_3^- +$ NO$_2^-$, ...
SRP, NH₄⁺, dissolved inorganic nitrogen (DIN), DO, conductivity, Q, and temperature),
macroinvertebrate shredder measurements (density and biomass of isopods, amphipods,
and isopods + amphipods), and land-use metrics (percent cover of agriculture,
forest/grassland, water, wetland, urban, impervious surface cover, and watershed area).
We did not include CBOM and wood as independent variables in the regression. Those
measurements were completed only once during the 131 day decomposition period, while
all other metrics were measured on each collection date (e.g., water column nutrients) or
did not change during the decomposition measurement (i.e., land-use). We used
SYSTAT 13 for all statistical analyses (Systat Software, Cranes Software International
Ltd., Chicago, IL). A p-value of 0.05 was the threshold for statistical significance.

Results

Leaf litter breakdown

Mean leaf litter turn over time among sites and bag types ranged from 130 to 238
days (slope = 0.0042- 0.0077 d⁻¹) (Figure 3). Litter breakdown rates were significantly
different by site and mesh size (Table 2; Figure 4). Overall, small mesh bags had a
significantly faster leaf breakdown rate than large mesh bags (ANCOVA p=0.002; Table
2; Figure 4). Leaf breakdown rates were faster at the two most downstream sites and
slower in the tributaries (ANCOVA p<0.00; Table 2; Figure 4).

Shredding macroinvertebrates

Density and biomass of potential macroinvertebrate shredders in leaf bags (i.e.,
isopods and amphipods) were significantly different among sites and between leaf bag
types. For example, across all 5 sites, there were significantly more isopods + amphipods
per bag in the small mesh relative to the large mesh bags (2-way ANOVA; p=0.023;
When considering isopods and amphipods separately, however, there was no significant difference in the number of isopods by bag type (2-way ANOVA; p = 0.063) or number of amphipods by bag type (2-way ANOVA; p = 0.077), although isopod and amphipod density were each significantly different among sites (2-Way ANOVA, p=0.006 and p=0.004, respectively; data not shown). We also compared the density of isopods + amphipods in the leaf bags to the benthic density (i.e., via Hess sampler; Figure 6). Isopod + amphipod density was significantly higher the small mesh bags relative to the benthic samples (2-way ANOVA p<0.001), and there was no difference among sites (2-way ANOVA; p = 0.121; Figure 6). Considered individually, isopod density was significantly different among the leaf packs and benthos (2-way ANOVA p = 0.017) and among the 5 sites (2-way ANOVA p = 0.012). Similarly, amphipod density was significantly different among the 3 habitat types (2-way ANOVA, p = 0.007) and among the 5 sites (2-way ANOVA, p = 0.007; data not shown).

Water chemistry and sub-watershed land use

All 5 study sites exhibited characteristics typical of urban streams, but there were significant differences in several physicochemical measurements among sites. As expected, discharge was significantly higher at the downstream sites (Table 1). However, differences in temperature and water chemistry did not show a clear longitudinal trend. For example, temperature was significantly higher at sites 2 and 3 compared to sites 1, 4, and 5 (ANOVA p<0.001; Table 1). Nitrate concentrations were significantly higher at sites 1, 2, 3, and 5 compared to site 4 (ANOVA p<0.001; Table 1). In contrast, SRP concentrations were significantly higher at site 4 compared to sites 1, 2, 3, and 5.
ANOVA p<0.001; Table 1). Finally, NH₄⁺ concentrations were not significantly different among sites (ANOVA p = 0.488; Table 1).

As with physicochemical measurements, land-use data indicated a high degree of urbanization across all 5 study sites (56%-78% urban land use), but several characteristics were different among locations (Table 1). For example, site 4 had higher proportion of land-use as agriculture, grassland, and wetland than sites 1, 2, 3 and 5 (Table 1). Impervious surface cover, which is often used as an indicator of watershed urbanization, ranged from 21% at site 4 to 37% at site 5 (Table 1). We used correlations to determine if nutrient concentrations were related to land-use. The land-use categories that were positively correlated with SRP concentrations were agriculture (p=0.013), forest/grassland (p=0.011), and wetland (p=0.009). Unexpectedly, there were no significant correlations between NO₃⁻ and NH₄⁺ with any land-use categories.

Controls on breakdown rate: multiple linear regression

We ran a stepwise multiple linear regression to determine what factors had the greatest effect on leaf breakdown rate (Table 3). Despite the large number of independent variables entered in the multiple regression, only two factors were significant in the final equation. Discharge had the greatest effect on leaf breakdown rate (F-ratio = 63.155, p<0.001), followed by isopod density (No. m⁻²) (F-ratio = 10.906, p = 0.016; Table 2). Together, the two factors explained 91.6% of the variation (p=0.001). We further explored the results of the multiple linear regression by creating a simple linear regression between discharge (Q) and breakdown rate (Figure 7A), which showed that discharge explained 71.3% of variation in the breakdown rate. We then graphed the
residuals from that regression with isopod density, which showed that isopods account for 10.8% of the remaining variation in leaf breakdown rate (Figure 7B).
Table 1. Site characteristics for the 5 sites along the North Branch of the Chicago River. Critical p-values are listed for the measurements in which a 1-way ANOVA was performed, significant values are in bold (p<0.05). Standard error values are in parenthesis. Impervious = impervious surface cover, Q = discharge, Temp = temperature, Cond = conductivity, DO = dissolved oxygen, SRP = soluble reactive phosphorus, CBOM = coarse benthic organic matter.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
<th>F-ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Latitude/Longitude</td>
<td>41°59'23.5&quot;N</td>
<td>42°03'30.6&quot;N</td>
<td>42°05'15.8&quot;N</td>
<td>42°05'16.3&quot;N</td>
<td>42°03'49.0&quot;N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area (km²)</td>
<td>233.94</td>
<td>106.34</td>
<td>66.49</td>
<td>39.85</td>
<td>62.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Land-use (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impervious</td>
<td>33.93</td>
<td>25.45</td>
<td>27.64</td>
<td>21.80</td>
<td>36.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>71.52</td>
<td>60.55</td>
<td>63.13</td>
<td>56.24</td>
<td>78.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agriculture</td>
<td>1.68</td>
<td>2.94</td>
<td>1.17</td>
<td>5.88</td>
<td>1.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest/grassland</td>
<td>4.52</td>
<td>7.33</td>
<td>4.07</td>
<td>12.77</td>
<td>4.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>1.41</td>
<td>2.32</td>
<td>2.36</td>
<td>2.23</td>
<td>1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wetland</td>
<td>0.95</td>
<td>1.84</td>
<td>1.20</td>
<td>2.91</td>
<td>0.44</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physicochemistry and organic matter standing stock</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q (m³ s⁻¹)</td>
<td>1.230 (0.440)</td>
<td>0.717 (0.216)</td>
<td>0.574 (0.126)</td>
<td>0.141 (0.071)</td>
<td>0.214 (0.051)</td>
<td>3.51</td>
<td>0.026</td>
</tr>
<tr>
<td>Temp (°C)</td>
<td>4.1 (0.4)</td>
<td>5.6 (0.3)</td>
<td>6.8 (0.3)</td>
<td>3.2 (0.3)</td>
<td>4.2 (0.3)</td>
<td>19.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cond (µS cm⁻¹)</td>
<td>1709 (788)</td>
<td>1316 (357)</td>
<td>1182 (261)</td>
<td>1841 (505)</td>
<td>2078 (817)</td>
<td>0.43</td>
<td>0.785</td>
</tr>
<tr>
<td>DO (mg L⁻¹)</td>
<td>9.9 (1.0)</td>
<td>9.0 (0.8)</td>
<td>9.2 (1.0)</td>
<td>10.1 (0.9)</td>
<td>9.6 (1.1)</td>
<td>0.23</td>
<td>0.917</td>
</tr>
<tr>
<td>NO₃⁻ (µg N L⁻¹)</td>
<td>4232 (1057)</td>
<td>5297 (719)</td>
<td>7639 (772)</td>
<td>77 (741)</td>
<td>2584 (741)</td>
<td>16.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SRP (µg P L⁻¹)</td>
<td>303 (49)</td>
<td>523 (107)</td>
<td>476 (70)</td>
<td>1300 (255)</td>
<td>183 (39)</td>
<td>10.69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NH₄⁺ (µg N L⁻¹)</td>
<td>132 (54)</td>
<td>146 (49)</td>
<td>72 (9)</td>
<td>124 (40)</td>
<td>176 (44)</td>
<td>0.88</td>
<td>0.488</td>
</tr>
<tr>
<td>CBOM (g m⁻²)</td>
<td>307 (126)</td>
<td>406 (294)</td>
<td>184 (108)</td>
<td>10 (8)</td>
<td>92 (45)</td>
<td>1.10</td>
<td>0.408</td>
</tr>
<tr>
<td>Wood (g m⁻²)</td>
<td>6 (1)</td>
<td>770 (722)</td>
<td>30 (22)</td>
<td>23 (23)</td>
<td>12 (7)</td>
<td>1.08</td>
<td>0.415</td>
</tr>
</tbody>
</table>
Figure 3. Breakdown of *P. deltoides* leaves in small and large mesh bags over a period of 131 days. The slope of each line is the breakdown rate (k·d$^{-1}$). Data points are means ± SE from replicate bags (n = 3 bags per data point). The dashed line represents the small mesh bags and the solid line represents the large mesh bags.
Figure 4. Breakdown rate of small and large mesh bags across 5 sites. The letters indicate a difference between breakdown rates among sites using Tukey’s test ($p \leq 0.05$).

Table 2. We analyzed leaf litter breakdown using an analysis of covariance (ANCOVA), with bag types nested within site and day as the covariate. Significant values are in bold ($p < 0.05$). We found significantly different breakdown rates for both site ($p < 0.001$) and bag type ($p = 0.002$).

<table>
<thead>
<tr>
<th>ANCOVA</th>
<th>d.f.</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>1</td>
<td>413.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Site</td>
<td>4</td>
<td>17.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bag type</td>
<td>1</td>
<td>9.77</td>
<td>0.002</td>
</tr>
<tr>
<td>Site x bag interaction</td>
<td>4</td>
<td>0.26</td>
<td>0.901</td>
</tr>
</tbody>
</table>
Figure 5. Mean (±SE) number of isopods + amphipods per bag across 5 sites. Data are averaged over time (n = 4 dates for site 1, n = 5 dates for all other sites).
Figure 6. Means (±SE) density of isopods + amphipods in small mesh bags, large mesh bags, and benthic density (via Hess sampler) across 5 sites. The letters in the legend indicate a difference between habitat types using Tukey’s test ($p \leq 0.05$).

Table 3. We analyzed the controls on leaf litter decomposition among sites using a forward stepwise multiple linear regression ($R^2 = 0.916$, $p = 0.001$).

<table>
<thead>
<tr>
<th>Effect</th>
<th>F-ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q ($m^3 \text{ s}^{-1}$)</td>
<td>63.155</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Isopods (No. m$^{-2}$)</td>
<td>10.906</td>
<td>0.016</td>
</tr>
</tbody>
</table>
Figure 7. Graphical illustrations of variation in results from the multiple linear regression show (A) discharge (Q) explains 71.3% of the breakdown rate and (B) isopod density (No. m$^{-2}$) account for 10.8% of the remaining variation after accounting for the influence of discharge. The points are displayed as site number (S) and mesh type of litter bags (L= large, S= small).
Discussion

Leaf breakdown: physicochemical drivers

Overall, leaf breakdown was significantly faster at downstream sites and slower in tributaries, which can be attributed to biological properties (i.e., macroinvertebrates; see below) and physicochemical characteristics of the study sites, especially discharge. Discharge can drive faster leaf breakdown through enhanced leaf fragmentation (Gessner et al., 1999), which appears to be a critical factor for leaf breakdown when comparing among the 5 sites examined in this study. Previous studies have also suggested that higher water velocity (i.e., m s\(^{-1}\)) and/or stream discharge (i.e., m\(^3\) s\(^{-1}\)) increase fragmentation of leaf litter, especially when considered among streams of different sizes. For example, breakdown of red maple leaves (*Acer rubrum*) was positively correlated with discharge and current velocity in a study of 18 streams with varying catchment size, discharge, and degree of urbanization along the St. Johns River, Florida (Chadwick et al., 2006). Water velocity was also positively related to leaf fragmentation of a mixture of leaf species in a laboratory experiment using artificial channels to simulate upstream and downstream tributaries (dos Santos Fonseca et al., 2013). In addition to fragmentation, dos Santos Fonseca et al (2013) suggested higher water velocity stresses leaf fibers, thereby increasing leaching of solutes from leaf tissue. In contrast, Tiegs et al. (2009) found that current velocity was not strongly correlated with *Populus nigra* leaf decomposition, but high variability of velocity within streams was suggested as a reason for the absence of a strong correlation.

In addition to average discharge, variability of discharge (i.e., rapid changes from low to high discharge) during leaf breakdown can affect leaf fragmentation. Fluctuation
in discharge was highly correlated with leaf decomposition of 3 leaf species in a tropical stream, suggesting variable hydrology during leaf decomposition can drive breakdown rate (Rueda-Delgado et al., 2006). We did not have continuous discharge measurements at our study sites, so we were unable to calculate variation in discharge and may have missed the scouring effects of periodic flood events on leaf breakdown. However, among the 5 dates where we measured discharge, the mean and variability of discharge was highest at site 1, which also had the highest breakdown rates.

Other physicochemical factors including temperature, SRP, and NO$_3^-$ concentrations were significantly different among the 5 study sites, but were unrelated to leaf breakdown rates. Increased temperature is generally thought to increase leaf breakdown by stimulating microbial decomposition (Webster and Benfield, 1986). However, previous research on temperature and leaf litter decomposition in streams has shown equivocal results, even when comparing across sites which span a larger gradient of temperature variation than our study. For example, leaf breakdown was strongly affected by temperature when comparing across sites in Canada and Norway (Taylor and Andrushchenko, 2014). However, Bruder et al. (2013) found temperature did not affect leaf breakdown, despite comparisons across temperate and tropical streams. Although temperature was significantly different among our study sites (p<0.001), it is possible that the differences among our study streams were not great enough for temperature to significantly affect decomposition rate.

We predicted that high nutrient concentrations, which are typical of urban streams, would be positively related to leaf breakdown due to increased microbial activity (Pascoal et al., 2005; Niyogi et al., 2003; Pascoal et al., 2003; Paul and Meyer, 2001). In
low-nutrient streams, small increases in nutrient concentrations can affect ecosystem processes, including leaf breakdown, by stimulating activity of microbial decomposers (Rosemond et al., 2002). Breakdown of yellow poplar (*Liriodendron tulipifera*) leaf litter was positively related to nutrient availability across a gradient of enrichment in streams in Alabama, where nutrients enhanced growth of leaf-decomposing fungi (Suberkropp and Chauvet, 1995). Despite patterns elsewhere, nutrient concentrations and leaf breakdown were unrelated in our study. This result may be attributed to the magnitude of nutrient concentrations in the North Branch of the Chicago River watershed, which may have exceeded a threshold above which nutrients no longer affect leaf breakdown. Pérez et al. (2013) also found that higher nutrient concentrations were unrelated to leaf breakdown in an urban stream. Determining the nutrient ‘threshold’ that drives leaf breakdown would require comparing these sites to low nutrient sites elsewhere, or performing laboratory analyses of breakdown across a nutrient enrichment gradient. Overall, the effect of nutrient thresholds on leaf litter breakdown in urban streams has not been well established and merits further research attention.

We expected urban land-use would be positively related to leaf breakdown rate, but found no relationships between any land-use categories and leaf breakdown. Other studies have also found leaf breakdown rates were unrelated to watershed land-use. For example, red maple (*Acer rubrum*) leaf breakdown rates were not significantly affected by agricultural land-use in the southern Appalachian region even though agriculture increased nutrient levels (Hagen et al., 2006). In addition, increasing impervious surface area at study sites in Australia did not affect leaf abrasion, suggesting the flashy hydrologic pattern did not influence breakdown rate (Imberger et al., 2008). Our 5 study
sites had relatively high coverage of impervious surface and urban land-use (i.e., \( \geq 56\% \)), which was possibly above a threshold of urbanization that could influence leaf breakdown. We may have documented an effect if we had compared non-urban streams to urban streams. Since all of our study sites were urban, factors that were variable among sites and leaf bag types (i.e, discharge and macroinvertebrates) were more important drivers of leaf breakdown rates.

\textit{Leaf breakdown: influence of macroinvertebrates}

Small mesh bags showed faster breakdown rate than large mesh bags (\( p = 0.002 \)), and the data suggested that the difference between bag types was due to the abundance of isopods and amphipods. Isopods and amphipods are often categorized as shredders (Cummins \textit{et al.}, 1989), however, their functional feeding group classification is not always clear. MacNeil \textit{et al.} (1997) stated that the term “shredders” is too specific since amphipods have been shown to consume animal matter, diatoms, filamentous algae, particles from bryophytes, and fine and coarse detritus including leaf litter (Felten \textit{et al.}, 2008). Some species of freshwater amphipods prefer animal prey and tissue to other food sources (Bacela-Spychalska and Van Der Velde, 2012), and one species is cannibalistic, even when other food sources were available (Jormalainen and Shuster, 1997). Aquatic isopods also consume a wide variety of food including detritus, dead or injured animals, and live or decaying plants (Voshell, 2002). Aquatic isopods have been reported to belong to many functional feeding groups such as collector-gatherers, shredder-detritivores, shredder-herbivores, and engulfer-predators (Voshell, 2002). Regardless of their assignment to generalist or shredder feeding groups, the role of isopods and amphipods in leaf breakdown in urban streams is not well documented.
In forested streams, macroinvertebrate shredders are critical for leaf litter processing and breakdown (Cummins et al., 1989; Herbst, 1980; Flores et al., 2013) and our results suggest a role for isopods, and potentially amphipods, in leaf litter decomposition in urban streams. Urban stream ecosystems typically have reduced diversity of macroinvertebrates, including specialist shredders (Paul and Meyer, 2001), even though leaf litter can be locally abundant in urban streams (Pascoal et al., 2005). Our study sites were low in macroinvertebrate diversity. Aside from chironomids, isopods and amphipods were the only taxa present likely to consume leaf litter (Turek and Hoellein, in press). Although isopods and amphipods may be generalist feeders, consumption of leaf litter may be a critical component of leaf litter processing in urban streams. To our knowledge, this is the first time this relationship has been demonstrated. Because both taxa are known to be consumed by fish (Covich et al., 1999), their role as facultative shredders may thereby serve as a previously overlooked mechanism for retaining riparian carbon within urban stream food webs.

Leaf litterbags represented ‘hot spots’ of isopod and amphipod activity in this study, as their abundance in small mesh bags was significantly higher than the benthic samples. During the time period covered by breakdown measurements (autumn through spring), both taxa were more ‘attracted’ to the food resource and habitat offered by decomposing leaves in the bags than to other benthic surfaces. The leaf bags may represent an ideal environment for these facultative shredders because leaf litter was abundant and the bags were relatively stable. In contrast, benthic samples were representative of the stream surface with periphyton, leaves, and fine benthic sediment as feeding options. Benthic samples are often used to assess overall macroinvertebrate
abundance and diversity in streams (Felten et al., 2008; Hogsden and Harding, 2013; Johnson et al., 2013). The spatial distribution of isopod and amphipod abundance in small mesh litter bags relative to benthic samples further supports their role in leaf litter breakdown in this urban watershed.

The use of leaf litter bags with different mesh sizes is a common technique for studying leaf breakdown rates (Bärlocher et al., 2005; Taylor and Andrushchenko, 2014; Bruder et al., 2013; Taylor and Chauvet, 2014; Cheever and Webster, 2014), where small mesh bags exclude macroinvertebrates to test their influence on leaf breakdown. However, the small mesh size used in our study (3.3 mm) was similar to the large mesh size used in previous research. For example, Taylor and Andrushchenko (2014), excluded macroinvertebrates using small mesh bags with mesh size 0.25 mm, while their large mesh bag was 2.5 mm. Other studies have also used small mesh sizes including 0.28 mm (Bruder et al., 2013), 0.5 mm (Taylor and Chauvet, 2014), and 1 mm (Cheever and Webster, 2014). While successful at excluding macroinvertebrates and often leading to reduced breakdown rates in small mesh bags, a major concern with such small pore sizes is that lack of water flow can lead to anoxia (Battle and Golladay, 2001).

In contrast to previous research, our study was designed to establish a difference in macroinvertebrate abundance between bag types, not an exclusion. Thus, leaf litter breakdown rate and isopod + amphipod abundance were higher in the small mesh bags relative to the large mesh bags. We observed very large isopods and amphipods inside the small mesh bags, and it is possible that the macroinvertebrates entered the small mesh bags then grew to a size where they could not escape. In contrast, the large mesh bags had larger openings and appeared to allow macroinvertebrates to move freely in and out
of the bags. These results were consistent with our pilot studies, which showed that small mesh selected for higher abundance of isopods and amphipods. We acknowledge that leaf litter breakdown in large mesh bags in our study (mesh size = 6.7 mm) would be more strongly impacted by loss of leaf fragments than the small mesh bags because particles could more easily escape. However, breakdown in large mesh bags was slower, suggesting the difference in breakdown rates between mesh types was not due to fragmentation, but attributable to differences in macroinvertebrates. In addition, our approach minimized the potential for anoxia affecting breakdown rate in the small mesh litter bags.

Magnitude of leaf breakdown rates compared to literature values

Our leaf breakdown rate values were similar to literature values. Leaf breakdown rates among our study sites ranged from 0.0042-0.0077 d\(^{-1}\), which are categorized as slow (k < 0.005 d\(^{-1}\)) to medium breakdown (k = 0.005-0.001 d\(^{-1}\)) (Peterson and Cummins 1974). We found relatively few studies that have used P. deltoides in leaf breakdown studies in streams, but Herbst (1980) conducted a study in a forested, low discharge stream with P. deltoides, and found slightly lower breakdown rates of 0.0020-0.0060 d\(^{-1}\). Bruder et al. (2013) measured breakdown of various leaf species and found a wide range of breakdown rates from 0.0038-0.0506 d\(^{-1}\) in temperate streams, and 0.0047-0.0210 d\(^{-1}\) in tropical streams.

Watershed approach for measuring leaf breakdown in urban streams

Our results suggested that the two most important drivers of leaf breakdown at the 5 study sites were discharge and macroinvertebrates, rather than factors that drive breakdown in other studies including nutrient concentrations, temperature, and land-use
patterns. Unlike most previous studies, which typically compare leaf litter breakdown among streams of the same size (González et al., 2013; Greenwood et al., 2007; Griffiths et al., 2009; Taylor and Andrushchenko, 2014) and use leaf bags that exclude macroinvertebrates, we used a watershed approach with nested subwatersheds as study sites and mesh sizes that established differences in macroinvertebrate abundance. While not conventional, our experimental design narrowed the set of driving factors for leaf litter breakdown by reducing among watershed variation and avoiding anoxic conditions, thereby explaining a relatively high proportion of breakdown among locations (83% of variation in small mesh bags).

Our study design also positions our results well to inform urban watershed management, which requires simultaneous measurements of stream ecosystem structure and function that occur throughout a watershed, including upstream and downstream locations (Hoellein et al. 2011). Leaf litter breakdown is an important ecosystem process, and measuring the rate of breakdown at urban sites is integral for understanding anthropogenic impacts on ecosystem function in urban streams (Webster and Benfield, 1986). Our results contribute to the literature describing the relative importance of physical and biological processes, which break down and retain leaf litter C within an urban watershed. These data will inform conservation or restoration strategies, which can enhance beneficial processes such as C retention, including tributary and mainstem sites throughout an urban watershed (Wenger et al., 2009).
CHAPTER III
ENVIRONMENTAL DRIVERS OF LEAF BREAKDOWN RATE IN AN URBAN WATERSHED: A LABORATORY STUDY

Introduction

Stream biodiversity and ecosystem processes are negatively affected by urban development (Walsh et al., 2005). A primary environmental stressor in urban streams is storm water runoff from watershed impervious surfaces (i.e. roads, rooftops, bedrock outcrops, and compacted soil), wastewater effluent, and combined sewer overflows (Arnold and Gibbons, 1996; Carpenter et al., 1998). Because of these changes to water movement off the landscape, urban streams often display flashy hydrology, high concentrations of nutrients and other solutes, increased erosion of streambed, and reduced biodiversity of macroinvertebrates and fish (Walsh et al., 2005; Paul and Meyer, 2001; Wenger et al., 2009). This collection of characteristics is referred to as the “urban stream syndrome”.

Autochthonous carbon (e.g., stream algae) and allochthonous carbon (e.g., leaf litter) fuel food webs in headwater streams (Tank et al., 2010). Leaf breakdown rate has been suggested as a metric for categorizing overall stream ecosystem health, including for urban streams, because it integrates the activity of multiple trophic levels and physicochemical conditions, and techniques for its measurement are well-established and standardized (Bärlocher, 2005; Gessner and Chauvet 2002). Measuring leaf litter breakdown rate in a stream is crucial to understanding the way in which energy moves
through the food web (Young et al., 2008), however, the relative importance of environmental drivers of leaf breakdown in urban streams is unknown.

Leaf litter breakdown in streams is affected by physical, chemical, and biological factors. Impervious surfaces and storm water infrastructure inhibit soil infiltration and transpiration by vegetation (Arnold and Gibbons, 1996). In urban streams, enhanced flooding from developed landscapes can speed the leaf breakdown process via fragmentation (Paul et al. 2006). Also, higher nutrient concentrations promote microbial growth, which also increases leaf litter breakdown rate (Greenwood et al., 2007).

A major focus of research on leaf litter decomposition in forested streams is the role of shredding macroinvertebrate. In urban streams, reduced diversity of shredding macroinvertebrates could reduce leaf breakdown rates from shredding. However, our field study (Chapter 2) showed that isopods (Asellus aquaticus) were one of the main drivers of leaf breakdown rate in the North Branch of the Chicago River, an urbanized watershed. These results were unique because no specialized shredders were present in the watershed, and it appears omnivorous amphipods filled the role of shredding macroinvertebrates to some extent. Results suggest that the relative importance of generalist macroinvertebrates to leaf litter processing in urban streams may have been overlooked and merit further study.

Leaf breakdown rate is a useful index because it integrates the action of physical, chemical, and biological factors. However, determining the relative importance of each driver individually requires careful experimental manipulation. Experiments designed to isolate drivers of leaf breakdown in the field include addition of nutrients (Greenwood et
al., 2007; Gulis and Suberkropp, 2003), macroinvertebrate exclusion (Pascoal et al.,
2005; Taylor and Chauvet, 2014; Cheever and Webster, 2014), and changes in leaf
species diversity (Battle and Golladay, 2001; Bruder et al., 2013). Researchers have also
used a variety of laboratory environments such as microcosms, glass flumes, and plastic
chambers to conduct leaf breakdown studies (Duarte et al., 2006; Dos Santos Fonseca et
al., 2013; Swan and Palmer, 2006). However, few studies of leaf decomposition include
paired field and laboratory analyses, which are valuable to examine ecological processes
under controlled settings.

We conducted two studies in artificial streams to test the role of individual drivers
on leaf breakdown rate that were significantly correlated to leaf breakdown from field
measurements throughout an urban watershed (see Chapter 2). The goals were to
measure 1) the effect of isopod abundance on leaf breakdown rate and 2) the effect of
water velocity on leaf breakdown rate. For the isopod experiment, we determined our
target isopod densities in the artificial streams from field measurements in the North
Branch of the Chicago River. For the velocity experiment, we chose current velocities
that would represent low and high velocities in the field.

Materials and Methods

Artificial streams

The laboratory experiment in the artificial stream facility at Loyola University
Chicago began on July 12, 2013 and spanned 123 days. The laboratory study consisted
of 12 artificial streams organized into 2 separate experiments: (1) the effect of isopod
abundance on leaf breakdown and (2) the effect of water velocity on leaf breakdown.
Artificial streams are re-circulating channels with a paddle wheel, where channel width = 14 cm and total flowpath length = 2 m (Hoppe et al., 2012). The streams were filled to 12 cm depth with dechlorinated tap water. Water evaporated at a rate of approximately 1 L day$^{-1}$ and additional dechlorinated tap water was added daily. At the start of the experiment, we added 100 mL of sediment-water slurry collected from the North Branch of the Chicago River as a microbial inoculum.

**Experimental conditions**

The experiment investigating the effect of isopod abundance on leaf breakdown consisted of 6 streams: 3 with isopods present and 3 control streams (no isopods). Mean ($\pm$SE) water velocity was 0.050 ($\pm$0.002) m s$^{-1}$. For the 3 streams with isopods present, 145 isopods (mean size approximately 7 mm) collected from the North Branch of the Chicago River were placed into each stream at the beginning of the study. Pilot experiments suggested approximately 30% mortality per week, so we placed 50 additional isopods into each stream once per week for the first 5 weeks. During the third collection date (week 6) we observed an increase in the isopod population, so we did not add any isopods from that point forward. In addition, starting on week 7 and every week thereafter, we selected a random leaf bag in each of the 3 streams to count the number of isopods present in one bag and determine if the population was stable (Figure 7). The target number of isopods in one bag was 32, which was the highest number of isopods we found in one bag during the field study. Some reproduction occurred during the litter breakdown study, but density remained at or above 32 individuals bag$^{-1}$ throughout the study (Figure 7).
The experiment investigating the effect of velocity consisted of 6 streams: 3 streams set at a low velocity and 3 streams set at a high velocity. Mean (±SE) water velocity was 0.023 (±0.003) m s\(^{-1}\) for the low velocity streams and 0.066 (±0.005) m s\(^{-1}\) for the high velocity streams. The goal for the high velocity streams was 0.086 m s\(^{-1}\), which was based on our field site 3, a fast moving stream. However, the maximum velocity for the artificial streams was approximately 0.070 m s\(^{-1}\). At greater velocities, water in the artificial streams was continuously lost over the paddle wheel and stream edges.

*Estimating leaf litter breakdown*

On day 0, 15 small mesh leaf bags containing 4 g of senesced, air-dried Eastern Cottonwood (*Populus deltoides*) leaves were placed into each artificial stream. Three additional bags were prepared, but not placed into the artificial streams and were used to calculate handling loss (Benfield, 2006). We covered each stream with 2 layers of wire screening material to minimize exposure to light. This created shaded conditions (approximately 75%), which minimized algal growth, although some visible algal growth occurred during the experiment. Three bags were collected from each stream on day 7, 19, 41, 84, and 123. On each collection date, we measured water velocity at each leaf bag location in the artificial stream using a Marsh-McBirney Flo-Mate 2000® Portable Velocity Flow Meter (Hach Company, Loveland, CO, U.S.A.).

Leaves were taken out of each bag and gently rinsed with deionized water to remove any isopods. We also rinsed all leaves from the control streams to account for any effect of rinsing on fragmentation. We placed the leaves into brown paper bags to be
dried and then ashed using the same procedure described in Chapter 2. We calculated breakdown rate (k), as exponential decay from a regression between the proportion AFDM remaining (ln transformed) and time (days) (Benfield, 2006).

When sorting isopods from leaf bags, we classified the isopods as either small or large. An isopod was classified as adult if it was longer than 3 mm from the base of the antennae to the tip of the tail. Only adult isopods were recorded and all isopods were placed back in their respective stream. However, we preserved a subset of isopods to measure length and calculate biomass under a dissecting microscope (N= 3 individuals on dates 7 and 19, and N=15 individuals on dates 41, 84, and 123). Individuals were collected that spanned the range of size from 3.0-13.5 mm, preserved in 95% ethanol, and length was measured.

At the same time we began leaf breakdown experiments, we set up 2 additional artificial streams without isopods and with identically prepared leaf bags (N=15 bags per stream). Every time a leaf bag was removed from the streams for the isopod experiment, it was replaced with a bag from the additional streams. These replacement leaves were at the same stage of decomposition. This ensured that the only change in leaf mass during the experiment was due to decomposition, and not attributable to the periodic removal of leaf litter. This also ensured isopods would be evenly distributed among 15 leaf bags throughout the course of the experiment. For the velocity experiment, removal of the leaf bags affected stream flow, so the leaf bags removed for measuring decomposition were replaced with empty bags.
Data Analysis

We used an analysis of covariance (ANCOVA), with day as the covariate, for both the isopod experiment and the velocity experiment to determine if breakdown rates \((-k)\) were different among treatments (Griffiths et al., 2009). We used a t-test to compare the mean breakdown rate between the 3 isopods and 3 control streams, and to compare breakdown between the 3 low and 3 high velocity streams. We used SYSTAT 13 for all statistical analyses (Systat Software, Cranes Software International Ltd., Chicago, IL). A p-value of 0.05 was the threshold for statistical significance.

Results

Isopod density and water velocity in ratification streams

Measurements taken during the laboratory study indicate we successfully maintained our isopod density and water velocity target values, which were similar to conditions in the field (Figure 8). For the isopod experiment, mean (±SE) isopod density was 55 (±4) individuals bag\(^{-1}\), which was just above our target of 32 individuals bag\(^{-1}\). For the velocity experiment, our mean (±SE) water velocity in the high velocity stream was 0.066 (±0.005) m s\(^{-1}\), which was slightly below our target of 0.086 (±0.017) m s\(^{-1}\) from site 3, and our low velocity was maintained at 0.023 (±0.003) m s\(^{-1}\).

Effects of isopods and water velocity on leaf litter breakdown

In the isopod experiment, leaf litter turn over time among treatments ranged from 101 to 161 days (slope = 0.0062-0.0099 d\(^{-1}\)). Leaf breakdown was faster in all streams containing isopods than in the control streams (Figure 9A). The ANCOVA showed that isopods significantly increased breakdown rates (p<0.001; Table 4). The mean
breakdown rate for the streams with isopods was significantly higher than the mean breakdown rate for the control streams (Figure 10A; t-test; p=0.006). Finally, within the 3 isopod streams there was a difference in final isopod density. The stream with the largest final isopod community (2,399 isopods) also had the fastest breakdown rate (k = 0.0099). The stream with the second largest final isopod community (601 isopods) had the second fastest breakdown rate (k = 0.0093). The stream with the smallest final isopod community (208 isopods) had the third fastest breakdown rate (k = 0.0082).

In the water velocity experiment, leaf litter turnover time among treatments ranged from 125 to 213 days (slope = 0.0047- 0.0080 d\(^{-1}\)). Leaf breakdown was faster in all 3 replicate high velocity streams than in the low velocity streams (Figure 9B). The ANCOVA showed that higher current velocity significantly increased breakdown rates (p<0.001; Table 4). The mean breakdown rate for the high velocity streams was significantly higher than the low velocity streams (Figure 10B; t-test; p=0.023).
Figure 8. Measurements of isopod density and water velocity were taken in artificial streams during leaf breakdown measurements over a period of 123 days. (A) Mean (±SE) number of isopods bag$^{-1}$ (n = 3 bags per data point). (B) Mean (±SE) water velocity measurements taken on each bag collection date, measured at the location of the bag removed (n = 3 velocity measurements for each treatment). The solid horizontal line on each panel represents the target values based on data from the field study.
Figure 9. Breakdown of *P. deltoides* leaves in the artificial streams over a period of 123 days. Data points are means ± SE from replicate bags (n = 3 bags per data point in each stream). (A) The dashed lines represent the 3 replicate streams with isopods and the solid lines represent the 3 replicate control streams. (B) The dashed lines represent the high velocity streams and the solid lines represent the low velocity streams.
Figure 10. Mean (±SE) breakdown rate of *P. deltoides* leaves in artificial streams (n = 3 streams per treatment). (A) The breakdown rate for streams with isopods is significantly higher than the control stream (p = 0.006). (B) The breakdown rate for streams with low velocity is significantly lower than streams with high velocity (p = 0.023).
Table 4. Analysis of covariance (ANCOVA) for leaf litter breakdown rate in two experiments, isopods and water velocity. Day was set as the covariate and p-values $\leq 0.05$ are in bold.

<table>
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<th>d.f.</th>
<th>F-ratio</th>
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<tr>
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<td><strong>Velocity experiment</strong></td>
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<td>Treatment</td>
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<td>48.062</td>
<td>$&lt;0.001$</td>
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Discussion

Isopods increase leaf litter breakdown

Macroinvertebrates are important drivers of leaf breakdown rate in forested streams (Flores et al., 2013; Wallace et al., 1982; Wallace et al., 1996), but the effect of shredding macroinvertebrates on leaf litter breakdown in urban streams has been considered less important than the effects of water velocity and nutrient enrichment (Pascoal et al., 2005; Paul et al., 2006; Imberger et al., 2008). This is attributed to lower macroinvertebrate richness and diversity, and the lack of specialized shredders in urban streams relative to forested streams (Paul and Meyer, 2001). However, generalist macroinvertebrate taxa, which are tolerant of urban conditions, may fill the niche of shredders when leaf litter is available. For example, Chadwick et al. (2006) found reduced diversity of macroinvertebrates in urban streams in Jacksonville, Florida, but that snails, which are typically considered grazers, functionally replaced shredders in the streams. Our laboratory study was designed to isolate the role of aquatic isopods on leaf litter breakdown in experimental streams, which has not previously been quantified and is potentially important for ecosystem function in urban streams.

Leaf breakdown rate was approximately 40% faster in streams containing isopods than control streams, which is consistent with our predictions. Although considered generalists, isopods consume organic matter in aquatic environments when available (Marchant, 1981), and isopod abundance was positively related to leaf breakdown among 5 study sites in the urbanized North Branch of the Chicago River watershed (see Chapter 2). In addition to breakdown rate, the role of isopods in the shredding of leaves was
confirmed by visual evidence. For example, by day 84 of the study, leaves in the isopod-containing streams were ‘skeletonized’, where the remaining leaf tissue consisted of unpalatable stems and leaves. In contrast, leaves from the control streams were nearly intact (Figure 11).

*Role of isopods in stream ecosystem function*

Given their generalist diet and abundance in streams with human impacts (e.g., urban and agricultural watersheds), isopods can be important for stream ecosystem processes and food webs, especially at sites with reduced macroinvertebrate diversity (Voshell, 2002). Isopods are considered ‘tolerant’ organisms, which can thrive in polluted habitats (Maltby, 1991). Isopods feed on detritus, dead and live animals, and live and decaying plants (Voshell, 2002). However, rate of organic matter consumption by stream isopods has only been individually quantified in a few studies. For example, Swan and Palmer (2006) studied leaf breakdown in plastic chambers and found that isopods prefer certain species of leaves, and therefore the isopods contribution to breakdown rate was dependent on leaf species composition. In agricultural streams in Indiana, Michigan, and Ohio, shredders (which consisted mainly of isopods) and water temperature explained variations in breakdown rates (Griffiths et al., 2012). Our results from field and laboratory analyses add to this modest data set. We show isopods can have a significant role in leaf breakdown, and therefore they may be an important component of ecosystem processes in urban streams, where more specialized feeding guilds are absent.
Isopod abundance was variable in artificial streams

We maintained isopod abundance near our target density, however, the number of isopods was variable among the replicate artificial streams at the end of the experiment. The final isopod population sizes in the 3 replicate streams were 2,399, 601, and 208 individuals per stream. This was unexpected because conditions among the 3 streams were identical. Differences in food availability, growth, and reproduction apparently occurred, despite our attempt to control all potential variables. Variation among the 3 streams could be due to predation, demographics, and sex ratios. Isopods may prey on one another (Voshell, 2002), and variable incidences of cannibalism among streams could have affected population growth patterns. In addition, an isopod lifespan is approximately 1 year (Ellis, 1961), and we may have unintentionally selected isopods at different life stages at the beginning of the experiment, which could also affect population growth. Finally, we did not identify the sex of the isopods when beginning the experiment. Variation in sex ratios among replicate streams is another likely explanation for the difference in population sizes. Although we did not maintain the same isopod abundance among the 3 streams, the unintentional variation among replicates provided further evidence that isopods increased leaf breakdown rate, as breakdown rate was positively correlated with isopod density (correlation r=0.866).

Water velocity increased leaf litter breakdown

The high water velocity treatment (0.07 m s\(^{-1}\)) increased leaf breakdown rate by approximately 33% relative to streams with the low water velocity treatment (0.02 m s\(^{-1}\)), most likely by increasing leaf fragmentation. Our laboratory and field studies show
complementary results, where leaf breakdown was positively correlated with both water velocity (laboratory study) and stream discharge (field study; Chapter 2). Other studies have shown velocity to be an important driver of leaf fragmentation. For example, Chadwick et al. (2006) found that mechanical abrasion due to increased stream flow was one of the main drivers of leaf breakdown among 18 streams across an urban gradient near Jacksonville, Florida. Dos Santos Fonseca et al. (2013) concluded that water velocity was the main driver of decomposition of leaves and twigs in artificial channels in a laboratory. The water velocity settings in dos Santos Fonseca et al. (2013) were 0.00, 0.05, and 0.10 m s\(^{-1}\), which are similar to the values in our study.

Discharge and velocity represent slightly different aspects of water movement, and each may have positive and negative correlations with leaf breakdown. Both factors can increase fragmentation of leaves, as more water volume (discharge) and faster moving water (velocity) can break apart leaf fibers (dos Santos Fonseca et al., 2013; Entrekin et al., 2008; González et al., 2013). However, discharge and velocity could reduce leaf decomposition by scouring biofilm organisms off the leaf surface. In addition, both factors could increase export of leaf litter from a stream reach, especially in urban streams with reduced channel complexity and retention structures (Paul and Meyer, 2001). In our field and laboratory studies, the effects of velocity and discharge on leaf litter export was mitigated by using leaf decomposition bags, which likely reduced the effect of biofilm scour. In addition, leaf litter standing stock was unrelated to discharge among the 5 sites in the field study. The effects of discharge and velocity on biofilm
scouring appeared to be minimal, and both factors affected breakdown rates most likely through physical effects on fragmentation.

In urban streams, flooding is common due to high impervious surface cover, which increases variability in discharge (Paul and Meyer, 2001). Our measurements from both field and laboratory studies suggest that elevated discharge and velocity increase breakdown rate via leaf fragmentation. However, we note that in the laboratory experiment, velocity was constant. Few other studies have quantified how variation in hydrology affects leaf breakdown. Rueda-Delgado et al. (2006) found the hydrological fluctuations in Amazon River tributaries increased leaf breakdown rate. Measuring the effect of variation in discharge on leaf breakdown in a laboratory setting is possible, and would simply require the artificial stream velocities to be altered over the course of the breakdown measurements. To our knowledge, these experiments have not yet been completed.

The effect of water movement on leaf litter breakdown is important to study because discharge and velocity are variable throughout a watershed (i.e., from headwater tributaries to mainstem) and in urban watersheds compared to those without human development. However, measuring leaf breakdown rate at multiple sites within the same watershed is not a common analytical approach. It is more conventional to compare among sites of approximately the same size (Young et al., 2008), which span gradients of urban development (Carter et al., 2009). The within-watershed approach is advantageous because variations in temperature and weather patterns, which change among different watersheds, are removed. Thus, results reflect the influence of environmental drivers
within the watershed only. This approach has precedence in the literature. For example, Pascoal et al. (2001) measured leaf breakdown rate in sites with varying levels of nutrients within the same watershed, and found that nutrients were stimulating the leaf breakdown process. Tiegs et al. (2009), measured litter decomposition at different sites throughout stream networks including streams from orders 1-4. Results showed that variability within streams was attributable to reach-specific factors such as riffles, which sped up decomposition rate. Advances in watershed-scale approaches for stream management or restoration will require more studies, which examine leaf litter breakdown across multiple sites within watersheds. These studies will also benefit from paired laboratory explorations of factors that are correlated with leaf breakdown at the watershed scale. Relationships documented in laboratory studies could be used to parameterize models of stream ecosystem function in urban watersheds.

The use of artificial streams to measure leaf breakdown

There are many outstanding questions for research on ecosystem processes in urban streams (Wenger et al., 2009), and artificial streams represent a useful and potentially overlooked technique for measuring environmental factors that drive leaf breakdown in conditions typical of urban streams. Benefits of artificial streams include control over the environment, replication, and the ability to create experimental designs compatible with inferential statistics (Lamberti and Steinman, 1993). However, measurements of leaf litter decomposition from artificial streams have some caveats that affect data interpretation. Because our laboratory experiment lasted 5 months, algal growth was variable and generally increased over time. Algae provided the isopods with
additional feeding options, and algal growth was difficult to control or match to in situ conditions. Although, we note that isopods fed on leaf litter despite (or in addition to) the presence of algal food resources. In addition, the artificial streams have a constant velocity that does not match flow variable patterns in natural urban streams.

The majority of leaf breakdown studies are conducted in a field setting, and relatively few measurements have been completed using laboratory approaches with artificial streams (i.e., not chambers or mesocosms). Studies with artificial streams have examined the effects of macroinvertebrates, fungi, and water velocity on breakdown. For example, Short and Maslin (1977) used artificial streams to show that a shredding stoneflies (Plecoptera) increased leaf litter processing by 20%. Duarte et al. (2006) showed that higher hyphomycete diversity increased mass loss of Alnus glutinosa leaves in laboratory microcosms. Dos Santos Fonseca et al. (2013) showed increasing water velocity enhanced breakdown of leaves and twigs in glass flumes. Our results add to this set of laboratory-based decomposition measurements by showing isopods and velocity both increase leaf breakdown.
Figure 11. On day 84 of the laboratory study, there was strong visual evidence of isopod consumption of leaf soft tissue.
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