

Impacts of Land-Use on Leaf Breakdown and Macroinvertebrate Assemblages in Southern
Appalachian Streams

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ABSTRACT

Land-use practices have long been associated with alterations in stream ecosystem structure and function, however, 'exurbanization' and its impact on streams is poorly understood. This study compares the ecosystem structure and function of 9 southern Appalachian streams of differing land-use (forested, exurban, agricultural).

Impacts of land-use on leaf breakdown are examined in Chapter 1. Leaf breakdown rates were significantly related to land-use. Forested streams exhibited the slowest breakdown rates, followed by exurban streams, with agricultural streams having the fastest rates. Leaf breakdown was most strongly related to discharge (white oak) and some fine sediment metrics (red maple). Our results suggest that the altered hydrological regimes in agricultural streams, as well as the influx of fine sediments into streams from exurban development, can play a role in altering in-stream organic matter processing. The taxa and number of shredders present may play a role to a lesser extent.

Impacts of land-use on macroinvertebrate assemblages are examined in Chapter 2. Shannon diversity, %EPT, and NCBI were significantly related to land-use regime. There were significant negative relationships between macroinvertebrate diversity and conductivity and temperature. In addition, biotic integrity had a significant negative relationship with conductivity. Canonical Correspondence Analysis (CCA) showed that agricultural streams were characterized by temperature and flow, forested streams by MPS and standing stock coarse particulate organic matter (SSCPOM), and two of three exurban streams by conductivity and temperature. Principal Coordinates Analysis (PCoA) revealed that while macroinvertebrate communities overlapped, some differences in community assemblage could be seen between land-use types.

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General Introduction

Stream ecosystems are highly influenced by the surrounding terrestrial landscape, depending on the neighboring environment for exchange of materials and nutrients (Vannote et al. 1980, Allan 2004). Anthropogenic disturbance of the riparian landscape has long been associated with alterations in both structural and functional stream integrity (Hynes 1975, Frissell et al. 1986). As natural riparian zones are cleared to make way for pastureland or development, changes in hydrology, geomorphology, ecosystem processes, and faunal community composition are likely to occur (Harding et al. 1998, Burcher et al. 2007, Sheldon et al. 2012). Alterations often observed include increased bank erosion and sedimentation, loss of organic matter retention structures, and a decrease in faunal biodiversity coupled with an increase in abundance of tolerant organisms (Sponseller and Benfield 2001, Maloney and Weller 2011).

Influence of agriculture practices on streams has been widely studied. Denudation of the riparian zone greatly reduces energy import in the form of allochthonous leaf litter, disrupting natural ecosystem processes (DeLong and Brusven 1994). In addition, light penetration and stream temperature increase (Hagen et al. 2010). Removal of rooted, climax vegetation leads to bank instability and erosion, which may be compounded by livestock trampling (Strand and Merritt 1999). This can lead to altered hydrology regimes and increased amounts of fine sediment input into the stream (DeLong and Brusven 1994, Allan 2004, Burcher and Benfield 2006). Elevated concentrations of nutrients and contaminants such as insecticides, as well as low dissolved oxygen concentrations, are often associated with agriculturally impacted streams (Hagen et al. 2006, Quinn and Stroud 2013). All of these factors contribute to the alteration of community structure and stream function by homogenizing habitat and limiting food resources, such as periphyton and organic matter (Strand and Merritt 1999, Bertrab et al. 2013). These changes, in turn, can modify faunal trophic structure and interactions (Greenwood et al. 2007).

The effects of exurbanization on stream ecosystems are not as well understood. The term 'exurbanization', as it applies to land-use, remains relatively ambiguous and liberally defined. However, most definitions of exurbanization describe the phenomenon as a movement of people from urban centers to rural areas (Egan and Luloff 2000), attracted by the low cost of living, outdoor recreation opportunities, and a slower pace of life (Gragson and Bolstad 2006, Vercoe et

al. 2014). Often times, retirees with seasonal homes in rural areas are also mentioned alongside permanent exurban transplants (Egan and Luloff 2000, Vercoe et al. 2014). With an increase in population comes an increase in land clearance, more infrastructure, and greater impervious surface cover (Egan and Luloff 2000). Urbanized watersheds have shown altered geomorphology and hydrology as a result of stormwater runoff (Walters et al. 2009) from impervious surfaces such as rooftops, parking lots, and roadways (Allan 2004, Burcher and Benfield 2006). Impervious surface runoff also carries toxins, sewage wastewater, and metals that act as pollutants in streams, impairing water quality and stream ecological processes and biota (Gardiner et al. 2008, Walters et al. 2009). In addition to residential development, historically, many suburban and exurban streams were once a part of agricultural land. These streams contain legacy sediments and other characteristics of previously impacted, pastureland streams (Allan 2004, Burcher and Benfield 2006, Walters et al. 2009, Maloney and Weller 2011). Because of the wide array of land-use impacts that can influence exurban streams, the structural and functional ecosystem responses are varied and signal the need for more studies.

One of the most vulnerable stream systems lies in the heart of the southern Appalachian Mountains. This ancient stretch of mountains serves as a water tower (Viviroli et al. 2007) to the southeastern portion of the United States. Due to the unique topography, geology, and age of the southern Appalachians, the mountain range serves as a biodiversity hotspot and provides many ecosystem services to mankind. The mountains and their valleys have undergone many anthropogenic land-use transitions throughout their history. Between 800 and 1500 A.D., the land was settled, cleared, cultivated, and burned by the residing Native Americans. Forests returned when the native peoples were decimated by disease introduced by European settlers. The settlers began to use agriculture extensively, introducing livestock and pastureland as well. Logging and mining followed in the early 20th century. By the mid-1900s, most row-crop agricultural practices had been abandoned (Gragson and Bolstad 2006) and were replaced by residential areas, forested lands, and a few “plasticulture” operations.

Appalachian streams, particularly in the Southern Blue Ridge Province, are relatively unbuffered and are sensitive to pollutants and sediments (Baker et al. 1990, Sullivan et al. 2011). These streams require attention because of the recent influx of development and urban sprawl into the southern Appalachians (Gragson and Bolstad 2006). The acceleration of exurbanization

in the southern Appalachians over the last 20 years (Egan and Luloff 2000) has been recognized as a key factor in transforming rural landscapes, leading to abrupt shifts in hydrological regimes that have altered stream biota assemblages and in-stream processes (Gardiner et al. 2008). Exurbanization has resulted in unique patchwork of land-use regimes including forested land, mountainside residential zones, and traditional valley agriculture. Although agricultural gradients, rural-urban gradients, and spatial scale have been studied extensively (Wear et al. 1998, Sponseller et al. 2001, Sponseller and Benfield 2001, Hagen et al. 2006, Burcher et al. 2007, Walters et al. 2009, Woodward et al. 2012) this unique mosaic of land-use types, and their effects on stream ecosystem processes and community structure, have been studied very little.

When assessing the integrity or quality of streams, it is important to use structural as well as functional indicators of stream health (Gessner and Chauvet 2002, Woodward et al. 2012). This thesis contains two chapters, wherein I examine the impacts of land-use on organic matter processing and macroinvertebrate assemblages in southern Appalachian streams. In the first chapter, I investigate how different land-use regimes alter in-stream ecosystem processes, using a measure of leaf breakdown to assess function. I also investigate the physical impacts of land use on streams by assessing sediment load and looking for relationships between leaf breakdown and sedimentation. In the second chapter, I examine the effects of different types of land-use on physio-chemical variables and macroinvertebrate community composition to assess stream ecosystem structure.

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Chapter 1

Impacts of land-use on leaf breakdown in southern Appalachian streams

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Abstract

Land-use practices have long been associated with alterations in stream ecosystem structure and function, namely in-stream organic matter processing. Impacts of agriculture on stream integrity have been widely studied, however, the relatively new phenomenon of exurbanization and its impact on streams is poorly understood. This study compared the ecosystem function of 9 Southern Appalachian streams of different land-use (forested, exurban, agricultural). Leaf breakdown rate was used as an indicator of stream integrity. Breakdown rates for both Red Maple (*Acer rubrum*) and White Oak (*Quercus alba*) leaves were significantly related to land-use. Forested streams exhibited the slowest breakdown rates, followed by exurban streams, with agricultural streams having the fastest rates. Forested and agricultural streams' breakdown rates varied significantly. Leaf breakdown was most strongly related to discharge (white oak) and some fine sediment metrics (red maple). In addition, shifts in shredder assemblages and the type of feeding habits they displayed seemed to play a role in leaf breakdown rates. Our results suggest that the altered hydrological regimes in agricultural streams, as well as the influx of fine sediments into streams from exurban development, can play a role in altering in-stream organic matter processing. The taxa and number of shredders present may play a role to a lesser extent.

Introduction

Stream ecosystems are strongly influenced by the surrounding terrestrial landscape, depending on the neighboring environment for exchanges of materials and nutrients (Allan 2004). Allochthonous input, primarily in the form of leaf litter, is an essential energy source for streams with detritus-based food webs (Vannote et al. 1980, Webster and Waide 1982). Leaf breakdown is a complex ecological process that incorporates many biological, chemical, and physical factors. Leaves that enter a stream become trapped in debris or substrate, where they lose initial mass through the leaching of soluble compounds (Hagen et al. 2006). Microbial colonization and growth (Webster and Waide 1982, Imberger et al. 2008), or ‘conditioning’, follows and begins the breakdown process (Petersen and Cummins 1974, Bärlocher and Kendrick 1975). The leaves are then fed on by shredding macroinvertebrates, which can greatly influence breakdown rates (Benfield and Webster 1985, Stout et al. 1993). Physical and chemical factors such as high flow rates, substrate abrasion, and sedimentation can also impact leaf breakdown (Webster and Waide 1982, Benfield et al. 1991, Paul et al. 2006, Fonseca et al. 2013), as can temperature, water chemistry, and nutrient concentrations (Webster and Waide 1982, Sponseller and Benfield 2001, Hagen et al. 2006, Greenwood et al. 2007).

Due its complex nature, the leaf breakdown process may be heavily influenced by anthropogenic alterations to the riparian landscape (Webster and Benfield 1986, Benfield et al. 1991). This has led to the proposal of leaf breakdown as a functional indicator of stream integrity (Webster and Benfield 1986, Gessner and Chauvet 2002, Greenwood et al. 2007, Woodward et al. 2012, Fonseca et al. 2013). Alterations in hydrologic regimes, such as the addition of impoundments or channelization and incision of stream banks, have been shown to increase leaf breakdown through physical abrasion and loss of organic matter retention structures (Webster and Benfield 1986, Paul et al. 2006, Fonseca et al. 2013). Suberkropp and Chauvet (1995) found that elevated concentrations of nitrate from agricultural runoff accelerated leaf breakdown rates through increased fungal growth and conditioning. Greenwood et al. (2007) achieved similar results through nutrient enrichment. Burial via sedimentation has also been shown to reduce leaf breakdown rates (Webster and Waide 1982, Benfield et al. 1991).

Denudation or modification of the riparian area may also alter leaf breakdown rates by causing a shift in allochthonous input and shredder community composition (Gessner and Chauvet 2002).

The use of leaf breakdown as a functional indicator, however, has been called into question due to conflicting results in breakdown rates in similar stream systems, as well as similar breakdown rates in impacted and non-impacted streams. Gessner and Chauvet (2002) suggested that simple coefficients of leaf breakdown alone may not be sufficient in accurately assessing stream integrity. For example, a lack shredder feeding activity, caused by high nutrient concentrations, could result in a slower leaf breakdown rate. However, high nutrient levels could also cause an increase in fungal growth and leaf consumption, resulting in a faster leaf breakdown rate. Indeed, additional studies have found supporting evidence of this. When comparing woodland streams to pastureland streams, Hladyz et al. (2010) found that breakdown rates did not differ among sites. In addition, breakdown attributed to shredders was greater in the forested streams, whereas breakdown by fungi was greater in pastureland streams. Studies along forested-agricultural gradients have expanded on those findings, showing that an extensive nutrient gradient resulted in a hump-shaped leaf breakdown curve across sites (Hagen et al. 2006, Woodward et al. 2012). While urbanized streams have been shown to lose leaf mass rapidly because of stormwater runoff, channelization, and dams (Webster and Benfield 1986, Paul et al. 2006), they also contain a heavy fine sediment load and chemical toxins from roadways, which could slow leaf breakdown (Webster and Waide 1982, Gessner and Chauvet 2002). Clearly, leaf breakdown rate can be easily altered by anthropogenic land-use and is affected by many different drivers.

In this study, we used leaf breakdown as a functional indicator of stream integrity to assess land-use impacts on southern Appalachian streams. Over the years, the southern Appalachian Mountains have been subjected to various types of transitional land-use regimes (Gragson and Bolstad 2006). At present, a patchwork of forested, mountainside residential, and traditional valley agricultural lands map out a mosaic of terrestrial cover in the area (Webster et al. 2012). First, we aimed to determine if leaf breakdown rate differed in streams that flow through forested, exurban, and agricultural lands. Second, because of the intricacies and multiple factors involved in organic matter processing, we aimed to tease out the main drivers of leaf breakdown in streams draining different land-use categories. We incorporated a modified

“flashiness” index calculated from stage heights (McMahon et al. 2003, Phillips and Scatena 2013), fine sediment measurements, a pebble count, and leaf-shredding macroinvertebrate (shredder) metrics in order to acquire a better understanding of why leaf breakdown rates may differ across land-use types.

Materials and Methods

Site Description

This study was performed in the Upper Little Tennessee River Basin (ULTRB) in southwestern North Carolina, USA. This region of North Carolina lies in the southern end of the Blue Ridge Physiographic province of the Appalachian Mountains, which stretches from the New River divide in Virginia to northern Georgia. This area is geographically unique, having lower temperatures and high rainfall in the higher altitudes which gradually shift to more typical climatic conditions in the lower altitudes. Because of this climatic gradient, the Appalachians in this region are home to an enormous biodiversity of flora and fauna, supporting ‘northern’ species left over from the last glaciation at high altitudes and more standard fauna at the low altitudes (Crespi et al. 2003, Gragson and Bolstad 2006).

The Little Tennessee River is a medium-sized, northward-flowing river that drains part of the southern Blue Ridge physiographic province. The headwaters originate in northeastern Georgia and flow through western North Carolina (Price and Leigh 2006). It is part of the Tennessee/Ohio/Mississippi River system. The Little Tennessee and its tributaries contain a mosaic of land-use types, both past and present. The valleys were farmed extensively, home to both pasturelands and intensive, large scale row-crop operations, but extensive agriculture waned in the 1950s (Gragson and Bolstad 2006). A few exceptions persist today as a scattering of commercial “plasticulture” operations on the floodplains of the Little Tennessee and some larger tributaries. The decline of extensive logging operations in the early 1920s, coupled with federal acquisition of Appalachian lands for national forests, allowed forests to regrow (Yarnell 1998, Price and Leigh 2006). However, in the 1990s, a population influx led to deforestation of unprotected lands for residential developments (Gragson and Bolstad 2006). The mosaic of land use patterns present in the ULTRB are representative of the socio-ecological patterns seen across all of southern Appalachia (Vercoe et al. 2014), making the basin an ideal study location.

Site Selection

To assess a hillslope gradient of land use, three broad categories were chosen – mountaintop forested, mountainside residential (exurban), and developed valley agriculture. Potential study sites were chosen by the Coweeta LTER using topographic maps, land cover, stream size, watershed scale, and previous as well as ongoing research conducted in the area. These sites were synoptically sampled for stream water N and sediment loads. Sites were further reduced to three sampling locations per land use category, giving a total of 9 study sites, by gaining permission from landowners.

Falls Branch, Willis Cove, and Hugh White Creek (all forested) contain a riparian area of dense rhododendron cover as well as a thick, hardwood upper canopy. Agricultural streams Mica City and Skeenah Creek both contain a partially-shaded riparian area on one side of the stream and are open on the other. Mica City is actively used as cattle pastureland, while Skeenah Creek flows through a large field. Bates Branch, the third agricultural stream, flows through a hay field and is deeply incised, containing virtually no riparian cover. Ammons Branch and Crawford Branch, both exurban streams, contain a scrub/shrub riparian area with minimal hardwood cover. The third exurban stream, Stillhouse Branch, contains a dense cover of rhododendron, plus a hardwood upper canopy. It is much larger than the other two exurban streams, both in stream width and depth (Table 1). All sites are located within Macon County, North Carolina, with one of the forested reference sites within the original Coweeta LTER study basin.

Leaf Breakdown

Breakdown rates of red maple (*Acer rubrum*) and white oak (*Quercus alba*) leaves were measured over a period of five months and eight months, respectively, following the leaf bag method (Benfield and Webster 1985). Red Maple and White Oak were chosen because both species are characteristic of the southern Appalachians and serve as allochthonous energy sources for forested streams (Hutchens and Wallace 2002). Red maple is also present in the riparian area of anthropogenically impacted streams (Hagen et al. 2006). Red Maple is classified as having an intermediate breakdown rate (Petersen and Cummins 1974, Ardon et al. 2009),

while White Oak exhibits a slow breakdown rate (Petersen and Cummins 1974, Webster and Waide 1982).

Senescent leaves were collected in autumn 2012 in Blacksburg, Virginia and were allowed to air-dry. Once the leaves had reached a constant weight, 5 grams of the leaves were placed in coarse-mesh leaf bags (mesh size = 5mm). Red maple packs were zip-tied to individual nylon strings in groupings of five and white oak packs were tied to string in groupings of eight. The leaf packs were deployed in the streams in November 2012. Three strings of each species were taken to each of the nine stream sites, making a total of six strings per each ~50m reach. Nylon strings and bags were secured in place with wiring on the streambank and large cobble on the streambed. Ten leaf packs were constructed, transported to the sites and brought back to the laboratory to account for handling loss. One maple leaf bag was clipped from each string after 23, 63, 98, 126, and 167 days. The same was done for oak bags after 23, 63, 98, 126, 167, 203, 234, and 261 days. Leaf packs were immediately placed in plastic bags and transported to the laboratory on ice. Leaves were rinsed over a stack of sieves (mesh size 1mm, 250 μ m, 63 μ m) to remove and collect macroinvertebrates and sediments, then placed in a drying oven at 60°C for at least 24 hours to reach a constant weight. Individual packs were weighed for dry mass, ground with a Wiley-Thomas mill, subsampled (250 mg), and ashed in a muffle furnace at 550°C for 1 hour to determine ash-free dry mass (AFDM). Breakdown rates (k , d^{-1}) were calculated as the slope of $\ln(\% \text{ AFDM remaining})$ vs. time using a negative exponential decay model (Petersen and Cummins 1974, Webster and Waide 1982). Breakdown rates per cumulative degree day (k , dd^{-1}) were calculated as well by summing the average daily water temperature over each incubation period (time between leaf pack deployment and subsequent retrievals) and substituting degree day for day (Petersen and Cummins 1974, Benfield 1996).

Macroinvertebrate Assemblages

Macroinvertebrates were rinsed from each individual leaf pack and collected on sieves (mesh size = 1mm and 250 μ m), sorted from the debris, and preserved in 80% ethanol. Insects were identified to the lowest practical taxonomic level, typically genus, following Merritt et al (2008). Chironomid larvae were identified to subfamily. Other invertebrates were identified to the lowest practical taxonomic level. Leaf pack invertebrates were quantified in terms of

shredder richness, shredder taxa distribution per each site, and the proportion of shredders to total macroinvertebrate abundance.

Physio-chemical Measurements

Fine and very fine sediments present in the leaf bags were separated according to sieve mesh size (1mm, 250 μm , 63 μm) and left to air dry. Sediments were quantified by dry weight per each leaf bag. Proportions of class sizes in each bag were also calculated. Sediments from each leaf bag were also classified as % organic and % inorganic in composition by ashing in a muffle furnace at 550°C for 1 hour to obtain ash mass and AFDM.

A pebble count was conducted at each study site using a standard gravelometer and the Wolman Pebble Count Procedure to better identify benthic habitat types. A 50-meter reach was measured within the vicinity of the leaf bags. The reach was walked heel-to-toe in transects and a pebble was blindly selected from underneath the toe of the boot at each step. The pebble was measured along the intermediate axis (width, b) by dropping it through the openings in the gravelometer (Wolman 1954). The gravelometer measures stones from a range of <2 mm to 300 mm, with openings increasing by powers of $2^{0.5}$, or half phi grain classes (Krumbein 1934). Pebble sizes were recorded as smaller than the next largest sieve size, i.e., a stone with a 64 mm intermediate axis would be recorded as falling into the 52 mm size class (Kondolf 1997). Particles were tallied in each sampling site until the total count reached 100. Particle size frequencies and mean substrate size were calculated per reach. Particles were placed into size class based on the USGS Wentworth Grain Size Chart.

Specific conductance and temperature were measured once a month and averaged for each study site, with the exception of Skeenah Creek. Discharge was also calculated each month and averaged for each site using the slug injection method with NaCl as the tracer. Specific conductance and temperature were measured with a YSI Model 30/50 conductivity meter. A modification of the FSC Flashiness Index (McMahon et al. 2003, Phillips and Scatena 2013) was calculated from stage heights taken by Teledyne Isco automatic water samplers at each site. Median height variation per hour for each stream stage was found from continuous hourly measurements of stage heights. The percentage of hourly measurements above that median was then calculated. Values were standardized by multiplying the percentage by the median to

account for water sampler malfunctions, missing data, and differing median stage heights among sites.

Data Analyses

Leaf breakdown rates at each site were analyzed via regression to test the null hypothesis ($k = 0$) and an analysis of covariance (ANCOVA) was used to compare breakdown rates among all sites. Leaf breakdown rates, mean substrate size, fine sediment metrics, physio-chemical variables, and shredding macroinvertebrate metrics were compared among the three land use categories using one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. All ANCOVA, ANOVA, and post hoc analyses were performed in SAS JMP Pro 10.0 (Cary, NC).

Regression analysis was used to explore relationships between leaf pack sediment metrics (average % organic and % inorganic matter, average weight organic and inorganic matter(g), and average weight of size classes (1mm, 250 μm , 63 μm)) and leaf breakdown rates. Relationships between leaf breakdown rates and shredding macroinvertebrate metrics were also explored through regression. Regression analyses were performed in SAS JMP Pro 10.0 (Cary, NC).

Results

Leaf Breakdown

Red maple leaf breakdown rates ranged from 0.0105 d^{-1} at exurban site Stillhouse Br. to 0.0404 d^{-1} at agricultural site Bates Br. White oak breakdown rates ranged from 0.0071 d^{-1} at forested site Willis Cove to 0.0225 d^{-1} at agricultural site Mica City (Table 2). Average breakdown rates per land-use category for both species showed that leaf breakdown was slowest in the forested streams, intermediate in the exurban streams, and fastest in the agricultural streams. Red maple breakdown rates were significantly different among land-use categories ($p = 0.034$, $\alpha = 0.10$; Figure 1A), as were white oak breakdown rates ($p = 0.055$, $\alpha = 0.10$; Figure 1B). A Tukey's multiple comparison test found significant differences in breakdown rates in both species in the forested and agricultural streams, but no differences between the exurban streams and the other two land-use regimes. Leaf breakdown rates, both per day and per degree day were

also compared among all 9 individual streams and were found to be significantly different ($p < 0.0001$; Table 2).

Red maple breakdown rates showed a positive relationship to % organic fine sediments and were negatively related to % inorganic fine sediments accumulated in the leaf packs. Breakdown rates were also negatively related to the average weight (g) of the 1mm size class sediments. Although not significant, leaf breakdown rates showed a slightly negative relationship to total inorganic fine sediment weight. Breakdown rates of white oak leaves had no significant relationships with any of the fine sediment metrics. However, rates showed a slightly negative relationship to the average weight of the 1mm size class sediments (Table 3). Neither red maple or white oak breakdown rates responded significantly to mean substrate size at the 50-m reach scale.

Shredders

The percentage of shredders, as compared to total number of insects collected from red maple leaf packs, differed significantly among land-use types ($p = 0.018$). Shredder percent was highest in the forested streams, intermediate in the exurban streams, and lowest in the agricultural streams. A Tukey's multiple comparison test showed that while the forested and agricultural shredder proportions differed, the exurban shredder proportions were not significantly different from either. The total number of shredders found in the maple leaf packs over the duration of the experiment did not differ significantly among land-use categories but followed the same trend as the percentage of shredders (Table 4). Both the percentage and the total number of shredders collected from the oak leaf packs differed significantly among land-use types ($p = 0.043$; $p = 0.018$). Forested streams contained the highest total number and percentage, followed by exurban streams, and then agricultural streams. Again, a significant difference was found between the forested and agricultural streams (Table 5). Shredder richness at the genus level was consistent throughout all streams and land-use types (Table 4, Table 5). No significant differences were found .

There was no relationship between breakdown rates for either leaf species and the percent shredders in the leaf packs. There was also no relationship between total number of shredders collected and red maple breakdown rates. However, there was a strong negative relationship

between white oak breakdown rates and total number of shredders collected in those leaf packs ($r^2 = 0.751, p = 0.003$).

Fine Sediments

The average weight of accumulated fine sediments in each red maple leaf pack was significantly higher in the forested and exurban streams than the agricultural streams. There was a nearly 4-fold increase in the amount of sediment accumulated in the leaf packs from agricultural to exurban streams. When the sediment weights were broken down into size class, exurban sites had significantly more 1mm sediments than agricultural sites. Forested streams contained an intermediate weight of the 1mm sediments but did not differ significantly from either of the other two types of streams. The 250 μm size class of sediments was present in the largest amounts in exurban red maple leaf packs, significantly more than both the forested and agricultural streams. The smallest of the fine sediments, the 63 μm size class, did not differ significantly among streams of differing land-use, although there was a definite trend of decreasing weight from exurban to forested to agricultural streams. Sediments were also broken down into % organic and % inorganic components. All three land-use categories contained very similar ratios of organic and inorganic sediment material, respectively a 1:10 ratio (Table 6).

Although none of the calculated sediment metrics for the white oak leaf packs were significantly different among streams of different land-use, there were still some observable trends. Forested streams contained the highest total sediment, as well as the highest amounts of sediment in each size class. Exurban white oak leaf packs contained the second highest amounts, with the exception of the 63 μm size class, in which agricultural leaf packs contained the second highest amount of sediment. The ratio of organic to inorganic sediment in the agricultural white oak packs was slightly higher than 1:10, although forested and exurban streams still had a constant 1:10 organic to inorganic ratio (Table 7).

Physio-chemical Measurements

Physio-chemical variables were loosely related to land-use, although none were significant with the exception of mean particle size (MPS). Results from the pebble count indicated that agricultural streams had the largest bed substrate and exurban streams had the smallest ($p = 0.017$) with forested streams having an intermediate and not significantly different

pebble size from the other two categories (Table 8). Discharge and frequency of flashy flows tended to be highest in the agricultural streams, followed by exurban streams and then forested streams. Conductivity and especially stream temperature, averaged among land-use types, were fairly constant but highest in exurban streams, intermediate in agricultural streams, and lowest in forested streams (Table 9). Physio-chemical variables in each individual stream varied quite a lot, even within the same land-use category (Table 1). A significant positive relationship was found between discharge and white oak leaf breakdown rates. In addition, white oak breakdown rates had weak positive relationships with flashy flows and temperature, although these were not significant. Other than the aforementioned factors, leaf breakdown rates could not be strongly predicted by other variables (Table 10).

Discussion

Leaf Breakdown

The mean breakdown rates of white oak (0.0124 d^{-1}) and red maple (0.0209 d^{-1}) across all sites were slightly faster than previously published (Ardon et al. 1999), with both species falling under the ‘fast’ decaying category as defined by Petersen and Cummins (1974). However, oak leaves decayed at a slower rate than maple, agreeing with previously published work (Petersen and Cummins 1974, Wallace et al. 1982). Land-use impacts were clear, as the average leaf breakdown rate was faster in anthropogenically impacted streams than the forested reference streams. While average breakdown rates for both species were significantly different between forested reference and agricultural streams, rates in the exurban streams were intermediate and not significantly different from those in the forested or agricultural streams. In addition, there was more variance in breakdown rates within land-use category as rates increased from forested, to exurban, to agricultural streams. ANCOVA analysis and Tukey’s post-hoc revealed that while all forested streams grouped together, agricultural and urban streams tended to have a lot of overlap in breakdown rates. For example, exurban stream Ammons Br. had a higher White Oak breakdown rate than two of the three agricultural streams, whereas the average breakdown rate for the three land-use categories indicated that agricultural streams have non-significant, yet higher, breakdown rates than exurban streams. In addition, when breakdown rates were corrected for differences in thermal regimes using cumulative degree day analysis, ANCOVA and Tukey’s post-hoc revealed an even greater overlap in breakdown rates. There

were no evident groupings among land-use regimes and only Hugh White Cr. (forested) and Mica City (agricultural) seemed to respond to temperature differences. While the average decay rates in each land-use category showed significance, further analysis comparing each stream indicated a much more confounded and complex collection of breakdown rates, particularly in the agricultural and exurban streams.

Agricultural Land-use Effects on Leaf Breakdown

Both the red maple and white oak leaf packs in the agricultural streams, despite having the highest average breakdown rate, contained a significantly lower percentage of shredders. In addition, white oak leaf packs also contained significantly fewer shredders. While the decline of shredders is to be expected in a disturbed system (Wallace et al. 1982, Gessner and Chauvet 2002, Paul et al. 2006), a low proportion/abundance of shredders should result in a slower leaf breakdown rate (Wallace et al. 1982). This discrepancy could possibly be due to the different shredder taxa present. Leaf packs in forested reference streams were dominated (> 50% of total shredders collected) by the stonefly *Tallaperla*, whereas agricultural sites had a more evenly distributed abundance of several taxa, including caddisflies *Pycnopsyche* and *Micrasema*, dipteran *Tipula*, and a variety of other stoneflies such as *Taeniopteryx*, *Pteronarcys*, *Leuctra*, and *Amphinemura*. *Tallaperla* nymphs and other small stoneflies shred leaves by eating softer tissue and leaving the tough vascular system intact, essentially ‘skeletonizing’ the leaf (Hynes 1941, Wallace et al. 1970), whereas larger shredders such as *Tipula* tear and eat bigger chunks of leaves (Gray and Ward 1979). Perhaps due to differences in feeding habits, as well as the observed tendency for larger shredders to enter, eat, grow, and get stuck inside leaf packs, essentially trapped within an abundant food resource, can partially explain the relationship between fewer shredders and a faster leaf breakdown rate.

Organic matter, in the form of fine benthic organic matter (FBOM), also appeared to play a role in leaf breakdown rates. Red maple breakdown rates exhibited a significant negative relationship to % inorganic matter and a significant positive relationship to % organic matter. While not significant, leaf packs in agricultural streams tended to have the highest organic to inorganic fine sediment ratio of the 3 stream types. On a watershed scale, the greater amount of fine organic material could have resulted from in-stream processes upstream (Vannote et al. 1980) or, perhaps more likely, could have come from the local riparian area. For example, an

influx of cow manure (e.g., Mica City) or grass and hay cuttings from pastureland and lawns (e.g., Bates Branch and Skeenah Creek) could have contributed to the high organic particle ratio. Our site on Skeenah Creek was also the location of a household compost pile that had spilled into the stream. An influx of organic material into agricultural streams has been shown to increase nutrient concentrations (Hagen et al. 2006), which in turn usually hastens leaf breakdown rates (Webster and Benfield 1986, Robinson and Gessner 2000, Suberkropp and Chauvet 2005) by way of a microbe/fungal colonization and feeding pathway (Gessner and Chauvet 2002, Hladysz et al. 2010, Woodward et al. 2012). Considering that breakdown rate and organic particle content were positively correlated, nutrient concentrations were not considered in this study, so we can only speculate the magnitude of effect and causation of increased nutrient input.

Agricultural streams consistently had a lower mass of accumulated fine sediments present in leaf packs, significantly so in red maple packs. In addition, these streams also tended to have higher discharge and more flashy flows than the other two types of streams. There was a significant relationship with stream discharge and white oak breakdown rates, and white oak breakdown had a weak positive relationship with the flashy flow index. These results support previous findings from Fonseca et al. (2013), who concluded that flow velocity greatly influenced the weight loss of leaves. An earlier experiment by Heard et al. (1999) showed that leaf packs tethered to a longer chain were able to flow freely in the current and became more fragmented as a result when compared to leaf packs fastened tightly to bricks. Indeed, our analysis and field observations support this, as we used the tether method and collected leaf packs a few times during or immediately after stormflow. The leaf packs in agricultural streams were uprooted from the streambed and were flowing freely on their tethers in the rapid flow, whereas most leaf packs in forested and exurban sites were still anchored to the streambed or held in place by a debris dam.

Exurban Land-use Effects on Leaf Breakdown

Exurban streams did not differ significantly from forested or agricultural streams with regard to most variables measured in the study, usually falling somewhere between the two other stream categories. There were a few exceptions, notably sediment content in red maple leaf packs. Exurban leaf packs, along with forested leaf packs, had a significantly higher total

sediment content than agricultural packs. Exurban leaf packs also had a significantly higher 250 μ m size class sediment content than forested and agricultural streams and a significantly higher 1mm size class content than agricultural streams. While not significant, sediment parameters showed many of the same trends in white oak leaf packs. In addition, results from the pebble count indicated that exurban streams contained significantly smaller bed substrate than agricultural streams, with Ammons Br. and Crawford Br. having a much higher % sand than all other streams. During field retrieval, many leaf packs in all three exurban streams were buried in sediment and sometimes digging was necessary to pull them out. Webster and Waide (1982) saw a similar phenomenon when investigating the effects of forest clearcutting on leaf litter processing. They attributed the slower breakdown rates during clearcutting to an influx of sediment that resulted in leaf burial. Other studies have led researchers to similar conclusions, that leaf burial in sediment reduces oxygen and nutrient exchange, limiting microbial and macroinvertebrate colonization, and leads to slower breakdown rates (Cornut et al. 2010, Navel et al. 2013). Indeed, many of the leaves in buried packs appeared to be virtually untouched by shredders, were not fragmented, and had a black coloration and a sulfuric odor, indicative of anoxia. Perhaps this explains the overall slower exurban breakdown rates when compared to agricultural streams. However, exurban streams had a faster average leaf breakdown rates than forested streams. Leaf packs in exurban streams tended to contain a higher abundance of *Tipula* larvae than the other two stream types and an intermediate abundance of total shredders. An abundance of shredders that rip large chunks as opposed to skeletonizing could have influenced breakdown rates. In addition, exurban streams had a greater average discharge than forested streams, which explained white oak breakdown rates in part. Other factors that have been shown to influence breakdown rates such as temperature (Webster and Benfield 1986, Chauvet and Superkropp 1998) and conductivity (Pascoal et al. 2003) were not significantly different among stream types and did not significantly predict breakdown rates.

On average, the environmental variables in exurban streams usually fell between those of forested and agricultural streams. However, much like the relatively ambiguous definition of the land-use category, individual streams within the exurban group did not fit neatly into their category and exhibited much variance in all measured variables. When leaf breakdown rates were compared by individual streams, Stillhouse Br. had a slow red maple rate that grouped with the forested streams, and Ammons Br. had an identical breakdown rate to Skeenah Cr., an

agricultural stream. When comparing white oak breakdown rates, again Ammons Br. had a faster rate than two of the three agricultural streams. Mean particle size (MPS) ranged from about 23 mm to 67.48 mm across all sites. Two of the exurban streams, Crawford Br. and Ammons Br., had the smallest MPS while the third exurban stream, Stillhouse Br., had the largest MPS. Stillhouse Br. also had fewer shredder taxa than the other exurban sites, as well as the forested and agricultural sites. When comparing other physio-chemical variables, the standard error for the averaged exurban streams was usually the highest. Visually, the local riparian habitat for the three exurban streams was very different. Crawford Br. consisted of both scrub and patchy to moderate hardwood tree cover. Stillhouse Br. was both a larger stream and had a canopy layer of rhododendron on at least one side of the stream, as well as moderate hardwood tree cover. Ammons Br. was dominated by scrub, honey locust, and invasive privet shrubs. Both honey locust and privet have small, compound leaflets as foliage. Perhaps the lack of allochthonous input in the form of native, large hardwood tree leaves in Ammons Br. caused our leaf packs to be used as resource islands for shredders, explaining the faster breakdown rate of both leaf species in this stream despite the low percentage of shredders and high proportion of fine sediment. This type of patch effect has been documented before in disturbed streams (Webster and Waide 1982, Benfield et al. 1991).

Conclusions

Differences in leaf breakdown rates could be explained by land-use regimes, but only to a point. Forested streams exhibited less flashy flows, heterogeneous bed substrate, and ample allochthonous inputs as well as an abundance of pollution intolerant, cool-water shredders (e.g. *Tallaperla*) usually found in forested, headwater streams. Agricultural streams, on the other hand, lacked a riparian buffer on at least one side of the stream, experienced high discharge and flashy flows, and supported a community of more tolerant shredders (e.g. *Tipula*, *Taeniopteryx*, *Amphinemura*). Exurban streams tended to have larger accumulations of fine sediments, although other variables did not differ significantly from either forested or agricultural land-use. Discharge (white oak) and some fine sediment metrics (red maple) significantly predicted breakdown rates, but other possible causative variables proved inconclusive.

Agricultural streams experienced a significantly faster breakdown rate than forested streams, as has been seen in other studies (Suberkropp and Chauvet 1995, Paul et al. 2006, Greenwood et al. 2007). Many researchers have found that along a gradient of agricultural intensity (Hagen et al. 2006) or nutrient influx (Woodward et al. 2012), leaf breakdown rates will exhibit a hump-shaped curve. Of the limited water chemistry variables measured, none of the agricultural streams were significantly different from the forested streams. In addition, the agricultural sites were used primarily for small farm pastureland or hay production, not extensive row-crop agriculture. Our streams would most likely fall somewhere within light to moderate agriculture, the land-use intensities shown to have the highest breakdown rates in a previous study (Hagen et al. 2006). Our findings for leaf breakdown rates in forested and agricultural streams are consistent with much of the literature. Leaf breakdown rates, however, were highly variable in the exurban streams and did not differ significantly from forested or agricultural breakdown rates. Little work has been done on understanding functional impacts of exurbanization. Paul et al. (2006) studied leaf breakdown in forested, agricultural, urban, and suburban streams and found that leaf breakdown increased as land-use moved from forested, to suburban, to urban, to agricultural. Generally, exurban lands fall between forested and suburban anthropogenic alteration intensity (Egan and Luloff 2000, Vercoe et al. 2014). Our findings support this, as leaf breakdown rates in exurban streams were intermediate between forested and agricultural streams. However, our findings are far from conclusive. Great care needs to be taken when classifying this type of land use, as our exurban 'replicates' displayed the most variance in almost every variable measured. Rigorous water chemistry measurement, as well as detailed land delineation on multiple spatial scales, could possibly narrow down the criteria for exurban stream classification. In addition, leaf breakdown could be a suitable measure for assessing stream function, if used with caution, many replicate streams and leaf packs, and supplemented by a slew of causative variables associated with land-use.

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Table 1.1. Physio-chemical parameter measurements taken at each study site, excluding Skeenah Creek. Data are means \pm 1 SE taken once monthly during the leaf breakdown experiment (n=5), except temperature (n=3) and the FSC Index value.

Physio-chemical variable	Forested			Exurban			Agriculture	
	Falls Br.	Willis Cove	Hugh White Cr.	Stillhouse Br.	Crawford Br.	Ammons Br.	Bates Br.	Mica City
Discharge (L/s)	46.62 \pm 35.35	6.31 \pm 1.90	8.10 \pm 1.65	83.09 \pm 30.16	8.45 \pm 2.74	26.49 \pm 8.94	48.35 \pm 20.12	125.73 \pm 41.96
Conductivity (μ S)	14.08 \pm 0.36	25.52 \pm 1.05	10.70 \pm 0.50	15.62 \pm 0.66	22.10 \pm 0.59	42.88 \pm 1.21	27.38 \pm 1.53	15.06 \pm 0.74
Temperature ($^{\circ}$ C)	16.03 \pm 0.44	15.50 \pm 0.42	14.23 \pm 0.18	16.40 \pm 0.46	16.60 \pm 0.55	18.87 \pm 0.34	16.43 \pm 0.27	16.33 \pm 0.73
FSC (Flashiness Index)	0.14	0.22	0.16	0.37	0.11	0.30	0.22	0.36

Table 1.2. Breakdown rates per day (k , d^{-1}) and per cumulative degree day (k , dd^{-1}) of red maple (*Acer rubrum*) and white oak (*Quercus alba*) leaves in the study streams. Coefficients of determination (r^2) were derived from significant ($p < 0.05$) regressions of change in leaf mass over time. Breakdown rates were compared among all sites using an ANCOVA. Rates sharing a common superscript are not significantly different.

Site	k (day^{-1})	r^2	k (dd^{-1})	r^2
Red Maple				
Falls Br.	0.0121 ^A	0.956	0.0020 ^A	0.939
Willis Cove	0.0147 ^A	0.975	0.0020 ^A	0.988
Hugh White Cr.	0.0136 ^A	0.950	0.0015 ^A	0.964
Stillhouse Br.	0.0105 ^A	0.835	0.0012 ^A	0.78
Crawford Br.	0.0211 ^{ABC}	0.974	0.0023 ^{AB}	0.975
Ammons Br.	0.0226 ^{AB}	0.966	0.0029 ^{ABC}	0.958
Skeenah Cr.	0.0226 ^{ABC}	0.898	-	-
Bates Br.	0.0404 ^C	0.865	0.0049 ^C	0.861
Mica City	0.0309 ^{BC}	0.966	0.0041 ^{BC}	0.954
White Oak				
Falls Br.	0.0096 ^A	0.867	0.0012 ^{ABC}	0.950
Willis Cove	0.0071 ^A	0.786	0.0007 ^{AB}	0.680
Hugh White Cr.	0.0076 ^A	0.909	0.0007 ^A	0.920
Stillhouse Br.	0.0109 ^{AB}	0.823	0.0011 ^{ABC}	0.897
Crawford Br.	0.0111 ^{AB}	0.915	0.0011 ^{ABC}	0.971
Ammons Br.	0.0153 ^{BC}	0.981	0.0015 ^{BC}	0.952
Skeenah Cr.	0.0131 ^{ABC}	0.948	-	-
Bates Br.	0.0144 ^{ABC}	0.991	0.0016 ^{BCD}	0.984
Mica City	0.0225 ^C	0.976	0.0030 ^D	0.972

Table 1.3. Coefficients of determination (r^2) derived from simple linear regressions of leaf breakdown rates and fine sediment metrics at the study streams. Significant relationships are denoted with an asterisk (*).

Fine Sediment Metrics	Red Maple		White Oak	
	r^2	P	r^2	P
Average % organic matter	0.509	0.031*	0.077	0.469
Average organic sediments (g)	0.277	0.146	0.017	0.741
Average inorganic sediments (g)	0.405	0.065	0.333	0.104
Average 1mm size class(g)	0.486	0.037*	0.404	0.066
Average 250 μm size class (g)	0.228	0.194	0.21	0.214
Average 63 μm size class (g)	0.249	0.171	0.147	0.309

Table 1.4. Red Maple leaf bag shredder composition and distribution among the study sites. Values are percentages of the total number of shredders represented by each genus found over the duration of the leaf breakdown experiment. % shredders represents the proportion of shredders to the total number of macroinvertebrates found in the leaf bags.

<i>Taxa</i>	Forested			Stillhouse Br.	Exurban		Agriculture		
	Falls Br.	Willis Cove	Hugh White Cr.		Crawford Br.	Ammons Br.	Skeenah Cr.	Bates Br.	Mica City
Plecoptera									
<i>Leuctra</i>	20.9	2.1	1.6	12.9	12.0	3.3	11.5	26.9	4.2
<i>Amphinemura</i>	4.5	8.3	3.1	22.6			15.4	7.7	8.3
<i>Tallaperla</i>	50.7	61.2	79.7	54.8	24.0	6.7	7.7	15.4	4.2
<i>Taeniopteryx</i>	1.5	3.1	3.4	6.5			46.2	3.8	29.2
<i>Pteronarcys</i>							7.7	19.2	4.2
Trichoptera									
<i>Micrasema</i>	7.5	2.1		3.2	25.3	20.0	3.8	7.7	16.7
<i>Hydatophylax</i>		4.1						3.8	
<i>Pycnopsyche</i>	10.4	17.2	9.4		6.7	46.7		7.7	25.0
Diptera									
<i>Tipula</i>	1.5	1.4	3.1		25.3	13.3	7.7	7.7	8.3
Coleoptera									
<i>Anchytarsus</i>	3.0				6.7	10.0			
Total Number	67	145	64	31	75	30	26	26	24
Shredder Richness	8	8	6	5	6	6	7	9	8
% Shredders	37.5 ^A	45.5 ^A	24.8 ^A	15.0 ^{AB}	23.6 ^{AB}	19.8 ^{AB}	9.0 ^B	8.0 ^B	18.4 ^B

Table 1.5. White Oak leaf bag shredder composition and distribution throughout the study sites. Values are percentages of the total number of shredders represented by each genus found over the duration of the leaf breakdown experiment. % shredders represents the proportion shredders to the total number of macroinvertebrates found in the leaf bags.

<i>Taxa</i>	Forested			Exurban			Agriculture		
	Falls Br.	Willis Cove	Hugh White Cr.	Stillhouse Br.	Crawford Br.	Ammons Br.	Skeenah Cr.	Bates Br.	Mica City
Plecoptera									
<i>Leuctra</i>	30.0	2.8	8.8	47.2	59.9	26.3		21.1	4.2
<i>Amphinemura</i>	1.0	7.5	7.1	7.0	4.1	5.3	3.0		5.9
<i>Tallaperla</i>	67.9	74.5	52.4	28.6	9.6		48.5	18.4	35.3
<i>Taeniopteryx</i>	2.0	1.4	1.8	9.0				2.6	11.8
<i>Pteronarcys</i>								5.3	
Trichoptera									
<i>Micrasema</i>	4.6	2.8	5.9	4.5	5.1	8.8	21.2		20.6
<i>Hydatophylax</i>	1.0				0.5	3.5	18.2	2.6	
<i>Pycnopsyche</i>	5.6	11.7	2.4		4.6	29.8	3.0	13.2	8.8
Diptera									
<i>Tipula</i>	3.6	2.3	0.6	3.5	11.7	17.5	6.1	13.2	8.8
Coleoptera									
<i>Anchytarsus</i>	0.5				4.6	8.8		23.7	
Total Number	196 ^A	214 ^A	170 ^A	199 ^{AB}	197 ^{AB}	57 ^{AB}	33 ^B	38 ^B	34 ^B
Shredder Richness	9	7	7	6	8	7	6	8	7
% Shredders	44.2 ^A	37.6 ^A	31.4 ^A	39.0 ^{AB}	31.7 ^{AB}	23.5 ^{AB}	12.5 ^B	16.9 ^B	26.5 ^B

Table 1.6. Fine sediment metrics calculated from the averaged sediment weights in Red Maple leaf bags collected from 10 December 2012 to 3 May 2013 in the study streams. Significant differences in sediment content among land-use types are denoted with an asterisk (*). Values sharing a common superscript are not significantly different.

Red Maple Fine Sediment Metrics	Land-use			<i>P</i>
	Forested	Exurban	Agriculture	
Average sediments present (g)	5.42 ± 0.86 ^A	8.69 ± 0.86 ^A	1.09 ± 0.86 ^B	0.002*
% organic matter	11.38 ± 1.14	10.01 ± 1.23	16.72 ± 2.43	0.069
% inorganic matter	88.62 ± 1.14	89.99 ± 1.23	83.28 ± 2.43	0.069
1mm size class (g)	7.56 ± 1.83 ^{AB}	11.02 ± 2.12 ^A	1.23 ± 0.96 ^B	0.018*
250 µm size class (g)	3.30 ± 0.28 ^B	7.30 ± 0.98 ^A	0.85 ± 0.39 ^B	0.001*
63 µm size class (g)	4.12 ± 1.99	6.78 ± 1.38	0.84 ± 0.35	0.066

Table 1.7. Fine sediment metrics calculated from the averaged sediment weights in White Oak leaf bags collected from 10 December 2012 to 5 August 2013 in the study streams. Significant differences in sediment content among land-use types are denoted with an asterisk (*). Values sharing a common superscript are not significantly different.

White Oak Fine Sediment Metrics	Land-use			<i>P</i>
	Forested	Exurban	Agriculture	
Average sediments present (g)	9.81 ± 2.16	7.10 ± 2.16	6.16 ± 2.16	0.506
% organic matter	9.90 ± 1.53	11.76 ± 3.01	15.73 ± 4.35	0.460
% inorganic matter	90.13 ± 1.53	88.24 ± 3.01	84.27 ± 4.35	0.460
1 mm size class (g)	11.48 ± 2.91	8.09 ± 0.80	4.41 ± 1.76	0.120
250 µm size class (g)	5.85 ± 0.42	4.53 ± 2.26	3.08 ± 2.03	0.571
63 µm size class (g)	9.95 ± 2.69	6.79 ± 1.90	7.86 ± 5.61	0.837

Table 1.8 Mean particle size (MPS, ± 1 SE) of bed substrate in study streams calculated from pebble count. Size class distribution is also included. Values sharing a common superscript are not significant.

Parameter	Forested			Exurban			Agriculture		
	Falls Br.	Willis Cove	Hugh White Cr.	Stillhouse Br.	Crawford Br.	Ammons Br.	Skeenah Cr.	Bates Br.	Mica City
MPS (mm)	41.91 ± 4.54 ^{AB}	47.74 ± 5.07 ^{AB}	51.70 ± 5.37 ^{AB}	67.48 ± 8.02 ^B	23.66 ± 3.93 ^B	22.32 ± 3.05 ^B	55.50 ± 4.92 ^A	38.27 ± 4.68 ^A	56.37 ± 5.33 ^A
% Sand	8	11	9	6	18	20	8	5	3
% Pebble	61	52	55	62	70	66	52	68	55
% Cobble	31	37	36	32	12	14	40	27	42
% Boulder				4			1		

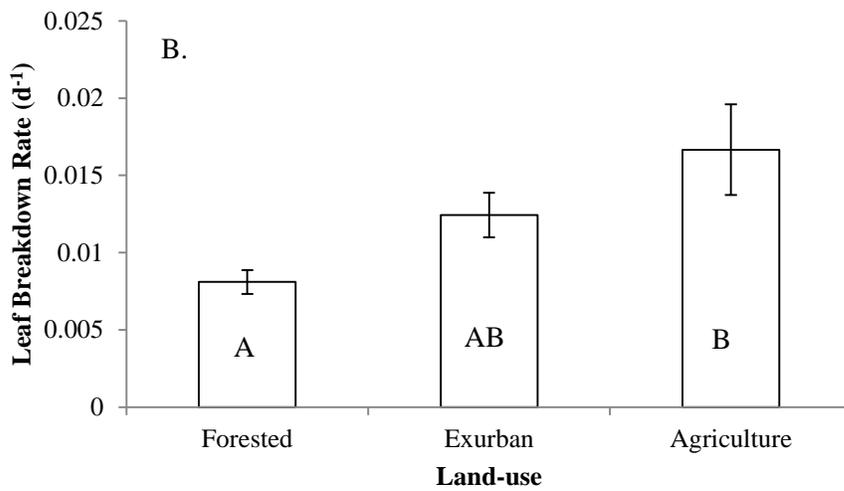
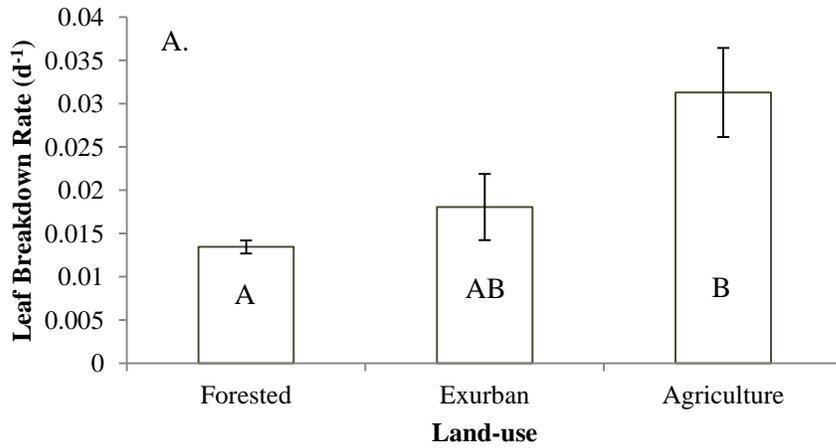
Table 1.9 Physio-chemical parameter measurements averaged among land –use types. Data are means \pm 1 SE (n=3), except agricultural site measurements (n=2, Skeenah Creek excluded). *P* denotes differences in physio-chemical variables among land-use types.

Physio-chemical variable	Land -use			<i>P</i>
	Forested	Exurban	Agriculture	
Discharge (L/s)	20.34 \pm 13.15	39.34 \pm 22.49	87.04 \pm 31.59	0.239
Conductivity (μ S)	16.77 \pm 4.48	26.87 \pm 8.22	21.22 \pm 5.03	0.567
Temperature ($^{\circ}$ C)	15.26 \pm 0.53	17.29 \pm 0.79	16.38 \pm 0.04	0.150
FSC (Flashiness Index)	0.17 \pm 0.02	0.26 \pm 0.08	0.29 \pm 0.06	0.438

Table 1.10 Coefficients of determination (r^2) derived from simple linear regressions of leaf breakdown rates and physio-chemical parameters at the study streams. Significant relationships are denoted with an asterisk (*).

Physio-chemical variable	Red Maple		White Oak	
	r^2	<i>P</i>	r^2	<i>P</i>
Discharge (L/s)	0.091	0.467	0.582	0.028*
Conductivity (μ S)	0.124	0.391	0.035	0.659
Temperature ($^{\circ}$ C)	0.093	0.463	0.242	0.215
FSC (Flashiness Index)	0.020	0.740	0.356	0.118

Figure 1.1 A and B. Red Maple (A) and White Oak (B) leaf breakdown rates averaged from the three study streams in each land-use category. Connected letters denote no significant differences.



Chapter 2

Impacts of land-use on macroinvertebrate assemblages in southern Appalachian streams

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Abstract

Land-use practices have long been associated with alterations in stream ecosystem structure and biota assemblages, however, exurbanization and its impact on streams is poorly understood. This study compared the macroinvertebrate assemblages of 9 Southern Appalachian streams of different land-use (forested, exurban, agricultural). Shannon diversity, %EPT, and NCBI values were significantly related to land-use regime. There were significant negative relationships between macroinvertebrate diversity and conductivity and temperature, as well as biotic integrity and conductivity. Canonical Correspondence Analysis (CCA) showed that agricultural streams were best characterized by temperature and flow, forested streams by MPS and standing stock coarse particulate organic matter (SSCPOM), and two of three exurban streams by conductivity and temperature. One exurban stream grouped with the forested streams. Some macroinvertebrate taxa were predicted by environmental variables indicative of their feeding or life habits. Principal Coordinates Analysis (PCoA) revealed that while macroinvertebrate communities overlapped, some differences in community could be seen with regards to different land-use regimes. Confounding factors mainly came from the ambiguity as well as variance in all measurements taken in the exurban streams.

Introduction

Impacts of land-use on macroinvertebrate assemblages have been well studied, especially recently, as a premium has been put on preserving biodiversity and managing ecosystems in a constantly changing landscape (Hynes 1975, Harding et al. 1998, Allan 2004, Moore and Palmer 2005). Variation in the geology and vegetation surrounding a catchment can alter the geomorphology, water chemistry, ecosystem processes, and the macroinvertebrate community structure of streams (Harding et al. 1998, Burcher et al. 2007, Sheldon et al. 2012). Removal of the riparian zone results in increased light penetration, elevated stream temperature, and loss of allochthonous inputs (Hagen et al. 2010). This, in turn, can result in an increase in primary production, altering trophic interactions and food webs (Noel et al. 1986, Greenwood et al. 2007, Chessman et al. 2009). Food sources in such streams are limited for both shredders (Benfield and Webster 1985) and macroinvertebrates downstream (Vannote et al. 1980). In addition, elimination of riparian cover can raise the ambient stream temperature above safe levels for sensitive macroinvertebrate taxa (Howell and Voshell 1982, Kraft and Mundahl 1984, Voelz et al. 1994). Removal of rooted vegetation cover has been shown to increase bank instability, sedimentation, and homogenization of benthic habitat (Allan 2004, Sutherland et al. 2012), altering or diminishing stream biota (Wood and Armitage 1999). Pollutants such as nutrient runoff from agricultural operations (Hagen et al. 2006), and metals, toxins, and salt from roadways and impervious surfaces (Burcher and Benfield 2006, Gardiner et al. 2008, Walters et al. 2009) also have detrimental effects on stream biota. All of these stressors lead anthropogenically-impacted streams to exhibit a decline in biodiversity coupled with an increase in abundance of tolerant organisms (Sponseller and Benfield 2001, Maloney and Weller 2011).

Streams impacted by agriculture exhibit many of the impairments described above. Pastureland streams are especially susceptible to bank erosion, which is compounded by cattle trampling (Strand and Meritt 1999). In addition, Braccia and Voshell (2007) found that most macroinvertebrate metrics responded negatively to increasing density of grazing cattle. Agricultural streams can also experience flashier flows because of altered hydrology regimes and incised or eroded banks (Baker et al. 2004), and the sudden increase in discharge may lead to catastrophic drift by macroinvertebrates (Anderson and Lehmkuhl 1968). An influx of nutrients, coupled with an increase in net primary production, may occur due to agricultural runoff in the

form of animal waste and fertilizers. This has been shown to shift organic matter resources from allochthonous-based to autochthonous-based, altering functional feeding group distributions (DeLong and Brusven 1998). A large influx of nutrients may also result in lower dissolved oxygen levels, leading to an increase in tolerant taxa such as dipterans and coleopterans with open respiratory systems (Davis et al. 2013).

The impacts of exurbanization on streams, and the subsequent alterations in benthic macroinvertebrate communities, have been far less studied than agricultural impacts. Although exurban lands are not as densely populated as suburban or urban areas, their streams still share common maladies with the aforementioned. Shields et al. (2008) found that streams classified as suburban/exurban carried the greatest annual nitrogen loads when compared to other types of land-use due to a combination of stream channelization, altered hydrological regimes, and increased impervious surface runoff and sewer leakage. Cunningham et al. (2009) reported similar findings when agricultural inputs alone did not explain the amount of nitrogen and salt influx into streams. In addition, Roy et al. (2003) found that macroinvertebrate indices started showing a downward trend – a decrease in diversity coupled with an increased abundance of tolerant organisms – at only 15% urbanized land cover. They attributed this to a combination of increased sediment transport, reduced streambed particle size, and increased dissolved particle content.

Macroinvertebrates are highly sensitive to both riparian and in-stream alterations. As such, macroinvertebrate metrics and indices have long been used as reliable indicators of stream integrity by scientists and management agencies (Roy et al. 2003, Hagen et al. 2006, Woodward et al. 2012, Sutherland et al. 2012). In this study, we used macroinvertebrate community composition as an indicator of stream integrity to assess land-use impacts on southern Appalachian streams. Over the years, the southern Appalachians have been subjected to varying types of land-use regimes (Gragson and Bolstad 2006). At present, a patchwork of forested, mountainside residential, and traditional valley agricultural lands constitute a mosaic of terrestrial cover in the area (Webster et al. 2012). First, we aimed to determine if macroinvertebrate assemblages differed in streams that flow through forested, exurban, and agricultural lands. We incorporated traditional metrics such as Shannon diversity, density, richness, and %EPT. Second, because of the multiple stressors involved in altering

macroinvertebrate community composition, we aimed to tease out the main drivers of macroinvertebrate distribution in streams of different land-use categories. We incorporated physio-chemical parameters, a pebble count, and standing stock organic matter in order to seek a better understanding of why macroinvertebrate communities may differ across land-use types.

Materials and Methods

Site Description

This study was performed in the Upper Little Tennessee River Basin (ULTRB) in southwestern North Carolina, USA. This region of North Carolina lies in the southern end of the Blue Ridge Physiographic province of the Appalachian mountains, which stretches from the New River divide in Virginia to northern Georgia. This area is geographically unique, having lower temperatures and high rainfall in the higher altitudes, which gradually shift to more typical climatic conditions in the lower altitudes. Because of this climatic gradient, the Appalachians in this region are home to an enormous biodiversity of flora and fauna, supporting ‘northern’ species left over from the last glaciation at high altitudes and more typical, low-elevation fauna at the low altitudes (Crespi et al. 2003, Gragson and Bolstad 2006).

The Little Tennessee River is a medium-sized, northward-flowing river that drains part of the southern Blue Ridge physiographic province. The headwaters originate in northeastern Georgia and flow through western North Carolina (Price and Leigh 2006). It is part of the Tennessee/Ohio/Mississippi River system. The Little Tennessee and its tributaries contain a mosaic of land-use types, both past and present. The valleys were farmed extensively, home to both pasturelands and intensive, large scale row-crop operations, but extensive agriculture waned in the 1950s (Gragson and Bolstad 2006). A few exceptions persist today as a scattering of commercial “plasticulture” operations on the floodplains of the Little Tennessee and some larger tributaries. The decline of extensive logging operations in the early 1920s, coupled with federal acquisition of Appalachian lands for national forests, allowed forests to regrow (Yarnell 1998, Price and Leigh 2006). However, in the 1990s, a population influx led to deforestation of unprotected lands for residential developments (Gragson and Bolstad 2006). The mosaic of land use patterns present in the ULTRB are representative of the socio-ecological patterns seen across all of southern Appalachia (Vercoe et al. 2014), making the basin an ideal study location.

Site Selection

To assess a hillslope gradient of land use, three broad categories were chosen – mountaintop forested, mountainside residential (exurban), and developed valley agriculture. Potential study sites were chosen by the Coweeta LTER using topographic maps, land cover, stream size, watershed scale, and previous as well as ongoing research conducted in the area. These sites were synoptically sampled for stream water N and sediment loads. Sites were further reduced to three sampling locations per land use category, giving a total of 9 study sites, by gaining permission from landowners.

Falls Branch, Willis Cove, and Hugh White Creek (all forested) contain a riparian area of dense rhododendron cover as well as a thick, hardwood upper canopy. Agricultural streams Mica City and Skeenah Creek both contain a partially-shaded riparian area on one side of the stream and are open on the other. Mica City is actively used as cattle pastureland, while Skeenah Creek flows through a large field. Bates Branch, the third agricultural stream, flows through a hay field and is deeply incised, containing virtually no riparian cover. Ammons Branch and Crawford Branch, both exurban streams, contain a scrub/shrub riparian area with minimal hardwood cover. The third exurban stream, Stillhouse Branch, contains a dense cover of rhododendron, plus a hardwood upper canopy. It is much larger than the other two exurban streams, both in stream width and depth (Table 1). All sites are located within Macon County, North Carolina, with one of the forested reference sites within the original Coweeta LTER study basin in Otto, North Carolina.

Physio-chemical Measurements

A pebble count was conducted at each study site using a standard gravelometer and the Wolman Pebble Count Procedure to better identify benthic habitat types. A 50 meter reach was measured within the vicinity of the sample sites. The reach was walked heel-to-toe in transects and a pebble was blindly selected from underneath the toe of the boot at each step. The pebble was measured along the intermediate axis (width, b) by dropping it through the openings in the gravelometer (Wolman 1954). The gravelometer measures stones from a range of <2 mm to 300 mm, with openings increasing by powers of $2^{0.5}$, or half phi grain classes (Krumbein 1934). Pebble sizes were recorded as smaller than the next largest sieve size, e.g., a stone with a 64 mm

intermediate axis would be recorded as falling into the 52 mm size class (Kondolf 1997). Pebbles were tallied in each sampling site until the total count reached 100. Frequencies of pebble sizes were calculated, as well as mean substrate size per reach.

Specific conductance and temperature were measured once a month and averaged for each study site, with the exception of Skeenah Creek. Discharge was also calculated each month and averaged for each site using the slug injection method with NaCl as the tracer. Specific conductance and temperature were measured with a YSI Model 30/50 conductivity meter. A modification of the FSC Flashiness Index (McMahon et al. 2003, Phillips and Scatena 2013) was calculated from stage heights taken by Teledyne Isco automatic water samplers at each site. Median height variation per hour for each stream stage was found from continuous hourly measurements of stage heights. The percentage of hourly measurements above that median was then calculated. Values were standardized by multiplying the percentage by the median to account for water sampler malfunctions, missing data, and differing median stage heights among sites.

Standing stock coarse particulate organic matter (SSCPOM) measurements were also taken once a month at each study site, with the exception of Skeenah Creek. SSCPOM was measured by placing a 0.41m² frame into the stream bed and collecting all organic matter present within the frame. Samples were taken back to the lab, dried in the oven at 60°C until constant weight was reached, then weighed.

Macroinvertebrate Assemblages

Macroinvertebrates were quantitatively sampled following the standard procedure of our laboratory (Harding et al. 1998). A 0.41m² metal frame was placed in a riffle habitat in the streambed. A modified quantitative kick net (mesh size = 250 µm) was placed inside the frame and the area within was comprehensively disturbed for 2 minutes. This procedure was executed five times at each sampling site. Samples were charged with 80% ethanol in the field and taken back to the laboratory, where they were sorted from debris and preserved in 80% ethanol and subsequently identified to lowest taxonomic resolution practical, typically to genus (Merritt et al. 2008) or subfamily (Chironomidae).

To address macroinvertebrate community composition, we employed traditional measures of density, richness, evenness, diversity, and two indices used to assess environmental sensitivity. Macroinvertebrate density was calculated by dividing the sum of individuals in each sample by the sampling area (0.41m²). Taxa number (S) and the Shannon-Wiener Diversity Index were used to assess richness and evenness, given by:

$$H' = -\sum p_i \ln p_i, \text{ from } [1, S]$$

Where p_i represents the proportion of individuals from the i th taxon, with values summed across all taxa present (S) (Pielou 1969, Hill 1973). The EPT index for disturbance-intolerant taxa (Ephemeroptera + Plecoptera + Trichoptera) was also used because it is a very simple, yet often effective way to assess the impacts of disturbance on stream macroinvertebrate communities (Crawford and Lenat 1989, Wallace et al. 1996). The North Carolina Biotic Index (NCBI), developed for southeastern U.S. streams, also assesses the impacts of disturbance on sensitive stream macroinvertebrates, given by:

$$\text{NCBI} = \sum ((TV_i N_i) / N_1), \text{ from } [1, S]$$

Where TV_i is the tolerance value of the i th taxon, N_i is the density of the i th taxon as either abundance (number per square meter) or biomass (milligrams ash-free dry mass per square meter), and N_1 is the total abundance (or biomass) of macroinvertebrates in the sample, with values summed across all taxa present (S). Tolerance values range from 0 (highly intolerant taxa) to 10 (highly tolerant taxa). The lower the NCBI score, the higher the biotic integrity of the stream is (Lenat 1993, Wallace et al. 1996). We used an abundance-based NCBI. Tolerance values were provided by Lenat (1993) and Barbour et al. (1999).

Data Analyses

ANOVAs and Tukey's post hoc multiple comparison tests were used to compare macroinvertebrate metrics (density, richness, Shannon diversity, %EPT, NCBI) among the study sites of different land-use regimes. Regression analyses were used to identify relationships between macroinvertebrate metrics and physio-chemical variables. All analyses were performed in SAS JMP Pro 10 (Cary, NC).

Canonical Correspondence Analysis (CCA) was used to explore the both response of macroinvertebrates, as well as the distribution of the sites, to environmental variables. Environmental variables incorporated into the analysis included standing stock coarse particulate organic matter (g/m^2), mean substrate size (mm), discharge (L/s), specific conductance ($\mu\text{S/cm}$), and temperature ($^{\circ}\text{C}$). The Monte Carlo permutation test (500 permutations, $p < 0.05$) was used to confirm significance of the CCA axes. To determine if any of the environmental variables exhibited multicollinearity, variance inflation factors were calculated ($\text{VIF} < 20$). Rare species were eliminated from the analysis if their summed abundance over all 45 samples was less than 15 individuals. The cut-off point was selected to preserve the presence of all functional feeding groups while eliminating noise in the data. Only 8 of the 9 sites were included in the analysis. Skeenah Creek, an agricultural site, was added to the project after physio-chemical variable sampling had taken place. The CCA and all related analyses were conducted in R 3.0.2 using the *vegan* package.

Differences in macroinvertebrate community composition between sites were determined by Principal Coordinates Analysis (PCoA). Bray-Curtis and Jaccard dissimilarity distance metrics were compared to see if macroinvertebrate communities were abundance or presence-absence driven. Correlations between the original macroinvertebrate abundance data and the PCoA results were calculated to find which taxa were strongly correlated with the PCoA axes. We conducted the PCoAs and related analyses in R 3.0.2 using the *vegan* and *labdsv* packages.

Results

Physical Parameters

Mean particle size (MPS) of bed substrate ranged from 22.3 mm at Ammons Br. to 67.5 mm at Stillhouse Br., both exurban streams. Exurban streams tended to have a greater percentage of the sand size class and a lower percentage of the cobble size class when compared to forested and agricultural sites (Table 2). Averaged, the agricultural streams had the highest MPS at 50.07 mm, followed by forested streams at 47.12 mm, then exurban streams at 38.11 mm. Differences in MPS among land-use types were significant ($p = 0.017$, Table 2). Discharge and frequency of flashy flows tended to be highest in the agricultural streams, followed by exurban streams; forested streams had the lowest discharge. Conductivity and especially

stream temperature, averaged among land use types, were fairly constant but highest in exurban streams, intermediate in agricultural streams, and lowest in forested streams (Table 3).

Macroinvertebrates

Macroinvertebrate density ranged from 153 m⁻² at Ammons Br. to 537 m⁻² at Willis Cove, an exurban and a forested stream, respectively. Although not statistically significant, agricultural streams had the highest average macroinvertebrate density followed closely by forested streams; exurban streams had the lowest density (Table 4). Taxa richness was again lowest at Ammons Br. and highest at Willis Cove, 31 and 48 taxa, respectively. When richness of study sites of the same land-use category were averaged, no significant differences were found among land-use types. Forested and agricultural streams contained about the same number of taxa and exurban streams had slightly fewer. Percent EPT was significantly different among streams of land-use, with the highest percentage of EPT taxa occurring in agricultural streams and the lowest percentage occurring in forested streams. Exurban streams were intermediate (Table 4). The unusually high percent EPT in agricultural streams was driven by the presence and abundance of tolerant EPT genera (Table 6). Shannon diversity was also significantly different among land-use types. Forested streams contained the highest diversity of macroinvertebrates while agricultural streams contained the lowest. Exurban streams, again, were intermediate. NCBI scores were significantly lower in forested streams than in exurban streams, with agriculture streams having an intermediate score (Table 4). It should be noted that one exurban stream, Stillhouse Br., had an NCBI score comparable to the forested streams. However, average NCBI values for the exurban category were still significantly different than average forested NCBIs, even with Stillhouse Branch's inclusion in the exurban category.

Most macroinvertebrate metrics were not related to physio-chemical variables with a few noted exceptions. Shannon diversity exhibited a significantly negative relationship with both stream temperature and conductivity. NCBI values exhibited a significantly positive relationship with conductivity, as well as a significantly negative relationship with MPS. Weaker, although noticed, trends included a slightly positive relationship between MPS and Shannon diversity, as well as a positive relationship between MPS and taxa richness. Taxa richness also had a slight negative relationship to temperature. Slightly positive relationships were also seen between

percent EPT and stream temperature, conductivity and flashiness of flow, as well as NCBI and temperature (Table 5).

Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) was used to examine macroinvertebrate and study site responses to environmental predictor variables. CCA axes 1 and 2 explained the most variance, with 16.5% and 9%, respectively. With all five axes accounted for, the entire constrained portion of the CCA explained a total of 36.3 percent of the variance (Table 7). Standing stock coarse particulate organic matter (SSCPOM) was most negatively correlated with axis 1 while temperature was the most positively correlated. Flow and mean particle size (MPS) were the most negatively correlated variables on axis 2 while conductivity was the most positively correlated (Table 8). Forested sites were better predicted by SSCPOM and MPS, agricultural sites were best predicted by temperature and flow, and exurban sites were best predicted by conductivity and temperature, with the exception of Stillhouse Branch (Figure 1). Most macroinvertebrate taxa were centered closely around the origin of the CCA biplot, therefore did not respond strongly to the environmental predictors that were included in the analysis. However, *Dixa*, *Tipula*, and many genera of Elmidae larvae tended to be predicted by the same variables associated with agricultural and exurban land-use. Shredding stoneflies such as *Tallaperla* and *Amphinemura*, as well as collecting detritivores *Diplectrona* and chironomid sub-family Chironominae were more associated with SSCPOM and MPS, the strongest predictors of the forested sites (Figure 1).

Principal Coordinates Analysis

Principal Coordinates Analysis (PCoA) was used to assess differences in macroinvertebrate community composition among the land-use categories. Two dissimilarity metrics, Bray-Curtis and Jaccard, were used in separate analyses to investigate whether community composition was abundance-driven (Bray-Curtis) or presence/absence driven (Jaccard). The Bray-Curtis distance metric explained more of the variance than did the Jaccard metric (Table 9). In addition, the two ordinations mapped the sites in species space in different ways (Figure 2, Figure 3), indicating that while presence/absence and rare taxa contributed to the distribution, community composition among sites was more abundance-driven.

Although taxa distributions exhibited quite a bit of overlap among land-use categories in the Bray-Curtis ordination, some trends could be seen. Agricultural sites tended to be positively correlated with PCO 1 and negatively correlated with PCO 2. Exurban sites did not have strong correlations with PCO 1 and tended to have both positive and negative correlations with PCO 2. Forested sites also tended to span PCO 2 and were slightly negatively correlated with PCO 1 (Figure 2). Because no distinct site groups arose in either PCoA analyses, I calculated correlations between the results of the PCoA and the original abundance data in order to assess which macroinvertebrates were strongly related to which axis. Ephemeroptera genera *Tricorythodes* and *Stenonema*, as well as elmid larvae *Macronychus* and *Optioservus*, were positively correlated with PCO 1 in the Bray-Curtis analysis. Caddisfly genus *Chimarra* and dipteran *Brachyremna* were negatively correlated with PCO 1. PCO 2 in the Bray-Curtis ordination had the strongest positive correlations with mayfly *Leptophlebia*, stonefly *Leuctra*, Collembola, and numerous Diptera larvae, including *Hexatoma* and two sub-families of chironomids. The riffle beetle *Promoresia*, both adult and larva, were negatively correlated with PCO 2, as were the dipteran genus *Antocha* and mayfly *Stenonema* (Table 10).

In the Jaccard PCoA analysis, land-use categories overlapped so much that few trends could be seen with regard to presence/absence community composition. However, the agricultural streams, with the exception of Mica City, tended to be negatively correlated with PCO 1 (Figure 3). Macroinvertebrates that also had a strong negative correlation with PCO 1 include Ephemeroptera genera *Epeorus* and *Tricorythodes*, stonefly *Pteronarcys*, multiple genera of riffle beetles, both adult and larvae, and water penny *Psephenus* (Table 11).

Discussion

Physical Parameters and Macroinvertebrate Metrics

Shannon diversity and %EPT were indicative of land-use type, decreasing and increasing as land use shifted from forested, to exurban, to agricultural, respectively. While the decrease in diversity was expected, the increase in %EPT was not. However, %EPT applies to a very coarse taxonomic scale (order-level) and does not take into account the differences in pollution intolerance within families or genera. In fact, our high %EPT in the agricultural streams was driven by the presence/abundance of more tolerant Ephemeroptera such as *Callibaetis*, multiple

genera of Family Heptageniidae, and very large numbers of *Tricorythodes*. In addition, net-spinning caddisfly *Hydropsyche* was present. I also found that % EPT was also slightly higher in the exurban streams than in forested streams. This was driven primarily by mayflies *Leptophlebia* and, again, *Tricorythodes*, as well as a few relatively tolerant plecopterans. The EPT index should be used with caution as it does not work on such fine taxonomic resolution, or it should be coupled with other biotic indices. Indeed, the NCBI resulted in forested streams scoring significantly lower than exurban streams, meaning that forested streams had a higher biotic integrity and contained more pollution-intolerant macroinvertebrates. Although not significant, forested streams also had a slightly higher biotic integrity than agricultural streams as well. Macroinvertebrate density and richness did not differ significantly among land-use categories, however, both metrics exhibited similar values in the forested and agricultural streams, with values in the exurban streams proving slightly lower.

Mean particle size was the only physio-chemical variable that was significantly related to land-use type, decreasing from agricultural, to forested, to exurban land-use. Macroinvertebrate diversity and taxa richness showed a slight positive response to increasing mean particle size. This type of response has previously been identified by others and attributed to the fact that, other than burrowing insects and silk case spinners such as chironomids, macroinvertebrates need the interstitial spaces of large particles for habitat and protection from predators (Minshall 1984, Roy et al. 2003). In addition, fine sediment deposition has been shown to exclude insects that do not possess the essential morphological and physical adaptations to live in a low oxygen environment (Wood and Armitage 1997, 1999). While discharge, conductivity, temperature, and flashy flow frequency did not differ significantly across the different types of land use, there were significant negative relationships between macroinvertebrate diversity and conductivity and temperature. In addition, the NCBI showed that as conductivity increased, the biotic integrity of the stream declined significantly. Alterations in macroinvertebrate community structure have been shown to be negatively influenced by conductivity (Tate and Heiny 1995, Roy et al. 2003) and at least altered by temperature via shifts in canopy cover and allochthonous and autochthonous food inputs (Noel et al. 1986, Chessmen et al. 2009, Hagen et al. 2010). Temperature (~14.2-18.8 °C) and conductivity (~10.7-42.9 µS), however, were not beyond the bounds of biological tolerance for most macroinvertebrates. Perhaps these predictors, conductivity in particular, are indicative of underlying, unmeasured variables that cause a more

serious response in declining diversity. Indeed, Roy et al. (2003) found consistently strong negative relationships between biotic indices and specific conductance. They proposed conductivity to be a surrogate ‘chemical signal’, as it measures dissolved ion content of non-point source pollution such as fertilizers, pesticides, salts, and contaminated sediments. Perhaps we are seeing the same effect, but we can only speculate as other water chemistry variables were not measured. It is interesting, however, that the land-use category with the highest temperature and conductivity, the smallest MPS coupled with the largest %sand size class, and the lowest taxa richness, density, and biotic integrity was exurbanization. These kinds of relationships have been found previously, but in agricultural streams (e.g., Sutherland et al. 2012). It is important to note that while the three streams are presently categorized as exurban, at least one, possibly two, were historically dominated by agriculture (Ammons Br. and Crawford Br.). The ambiguity of what constitutes an ‘exurban’ stream is further confounded by agricultural land-use legacies, which may still be driving these stream systems (Harding et al. 1998, Jackson et al. 2005, Maloney and Weller 2011).

Canonical Correspondence Analysis

SSCPOM and temperature were the predictor variables most strongly correlated with CCA axis 1. Flow, MPS, and to a lesser extent, temperature, were the driving variables on CCA axis 2. All forested sites were best characterized by SSCPOM and MPS. These results were to be expected, as the forested streams in this area are detritus-based streams, receiving most of their energy inputs from allochthonous sources (Webster et al. 1992) and had the most heterogeneous bed substrate makeup, especially when compared to exurban sites. The macroinvertebrate taxa predicted by the same variables included Chironominae, Clitellata (aquatic earthworms), *Ectopria*, *Rhyacophila*, Ceratopogonidae, *Tallaperla*, *Amphinemura*, and *Ameletus*. The Chironominae and the earthworms, while not shredders or not generally thought of as living in water with high integrity, were in fact found in greater abundance at the forested sites. This can be attributed to the fact that both are collecting detritivores and can feed on smaller, processed particles of organic matter present in allochthonous-based, forested streams. Robinson et al. (1998) had also noticed a number of chironomids in their artificial leaf packs. In addition, Sylvestre and Bailey (2005) saw similar results in their macroinvertebrate ordination and suggested that these macroinvertebrates were not using leaf packs for food, but for habitat.

Predators such as Ceratopogonidae and the caddisfly *Rhyacophila* were most likely best predicted by SSCPOM because their prey (e.g. small mayflies, *Gammarus*, and chironomids) use CPOM and debris dams for suitable habitat (Martin and Mackay 1982, Elliott 2005). Shredding plecopterans *Tallaperla* and *Amphinemura*, most likely due to their feeding habits, were predicted by both SSCPOM and MPS. *Ameletus* was very strongly predicted by MPS, and unusually so since these mayfly nymphs tend to be found in riffle and pool, not boulder/outcrop habitats (Huryn and Wallace 2008).

The agricultural sites grouped together as well and were best characterized by temperature, flow, and to a lesser extent, conductivity. This was to be expected because the agricultural streams lacked a proper riparian area and were unshaded, resulting in higher stream temperatures. In addition, the agricultural streams experienced both the highest flows and the flashiest flows. Macroinvertebrates that tended to group with the agricultural streams were tolerant/semi-tolerant mayflies *Tricorythodes* and multiple Heptageniidae genera. Also predicted were a few genera of elmids larva, the odonate *Lanthus* and dipteran *Tipula*. Many of the more tolerant mayfly taxa are collector-gatherers or scrapers, feeding on periphyton that grows on unshaded bed substrate (Lenat 1984) or fine particulate organic matter (FPOM). Riffle beetles are very common in mildly to moderately impacted agricultural streams, living in zones of high flow (Braccia and Voshell 2007, Brown 2008). *Lanthus*, a predator, eats many kinds of macroinvertebrates but it has been shown that it favors plecopterans and chironomids (Wallace et al. 1987), both of which were present in the agricultural streams. *Tipula* is a common tolerant shredder that is often found in agricultural streams (Hagen et al. 2006).

Two of the exurban streams, Ammons Br. and Crawford Br., grouped together and were predicted by conductivity and temperature. These results were expected as temperatures in these two exurban streams was slightly higher than in forested streams due to a smaller riparian buffer made of primarily scrub. In addition, urbanized streams experience more runoff from impervious surfaces (Allan 2004), introducing road salts and other chemicals into the stream (Gardiner et al. 2008, Walters et al. 2009), causing an increase in conductivity (Halstead et al. 2014). While exurban streams do not experience as much of this runoff, a low-density road and housing infrastructure still exists. Macroinvertebrates also predicted by conductivity and temperature were *Maccaffertium*, *Callibaetis*, some Elmids larvae, and *Dixa*. The mayflies,

Callibaetis in particular, are pollution tolerant and act as scrapers and collector-gatherers, eating FPOM (Vannote et al. 1980). *Dixa* also belong to the collector-gatherer functional feeding group. Although measurements of FPOM or FBOM were not taken, visual identification of the stream habitat during fieldwork confirmed that Ammons Br. and Crawford Br. contained murky water, a high amount of TSS, and very fine particles of leaf litter and organic matter both in debris dams and in the water column. Stillhouse Br., the third exurban site, grouped with the forested sites and was predicted by SSCPOM and MPS. Stillhouse Br. did indeed have the greatest individual MPS out of all the sites, while its two other replicate streams had the smallest. In addition, Stillhouse Br. had a dense canopy of rhododendron and an upper riparian layer of hardwood forest, much like the forested sites. Again, much like the meaning of the term itself, the exurban ‘replicate’ sites exhibited quite a bit of variance.

Overall, the entire CCA, or our five predictor environmental variables, explained 36.3% of the variance in our sites and macroinvertebrate taxa. Many taxa were centered closely to the origin of the biplot, meaning they were not predicted by any variable measured. Additional measurements, such as nutrient concentrations, dissolved oxygen, and spatial data, would have been of great benefit. Not only do our three land-use categories differ by classification of land use, but spatially they vary longitudinally as well as in watershed size, some streams even occupying the same watershed. Macroinvertebrates are also sensitive to nutrient concentrations, as well as dissolved oxygen levels (Hagen et al. 2006). Perhaps we would have seen a better explanation of variance if other environmental variables had been included.

Principal Coordinates Analysis

The Bray-Curtis and Jaccard PCoA analyses explained 43.43% and 25.48% of the variance in macroinvertebrate abundance and distribution among the study sites. A Bray-Curtis distance metric incorporates both presence/absence and abundance of macroinvertebrates, while Jaccard incorporates only the presence/absence of macroinvertebrate taxa (McCune and Grace 2002). Since the data were arranged differently in the ordination space for the two analyses and the Bray-Curtis metric explained more variance, the macroinvertebrate communities in these streams were more abundance-driven, with rare taxa playing only a small role.

The Bray-Curtis PCoA did not contain very distinctive groups based on land-use category, but despite much overlap, land-use categories were slightly separated in the ordination. Ephemeroptera genera *Tricorythodes* and *Stenonema*, Elmidae larvae *Macronychus*, *Promoresia*, and *Optioservus*, and Diptera genus *Antocha* were the most present taxa in the agricultural streams. These genera are frequently found in higher order agricultural streams (Sylvestre and Bailey 2005). Also, many of these insects are either scrapers or collector-gatherers. Forested streams had the greatest abundance of caddisfly genus *Chimarra*, mayfly *Leptophlebia*, stoneflies *Leuctra* and *Tallaperla*, Collembola, and numerous Diptera larvae, including *Hexatoma*, *Brachypremna*, and two sub-families of Chironomids. The functional feeding groups present in the forested streams included predators, collectors, shredders. Also, habitat requirements range from leaf packs and debris dams to semi-aquatic and sandy stream margins, further indicating good, heterogeneous habitat. Exurban sites tended to span the Bray-Curtis PCoA, having some similarities with both forested and agricultural macroinvertebrate communities.

In the Jaccard PCoA analysis, land-use categories overlapped so much so that few trends could be seen with regard to presence/absence community composition. Perhaps rare taxa were distributed among all the sites. However, the agricultural streams, with the exception of Mica City, tended to be negatively correlated with PCO 1. Macroinvertebrates that also had a strong negative correlation with PCO 1 include Ephemeroptera genera *Epeorus* and *Tricorythodes*, stonefly *Pteronarcys*, multiple genera of riffle beetles, both adults and larvae, and the water penny *Psephenus*. All of these organisms, with the exception of *Pteronarcys*, are considered high order agricultural stream dwellers (Sylvestre and Bailey 2005). However, *Pteronarcys* is commonly found in relatively clean, high order streams and has been shown to be resilient to stormflow events (Angradi 1997).

Conclusion

Overall, macroinvertebrate assemblages were indicative of land use, especially in the forested and agricultural land-use categories. Shannon diversity was significantly higher in forested streams than in agricultural streams, and was significantly negatively related to conductivity and temperature. The unexpected, yet significant % EPT values were easily explained by the presence and abundance of tolerant EPT genera in agricultural and exurban streams.

Furthermore, an index that works on a finer taxonomic resolution, the NCBI, indicated that forested streams had the highest biotic integrity and boasted the most sensitive organisms. Exurban streams contained the most tolerant organisms, with the biotic integrity of agricultural streams proving intermediate. Forested streams were best predicted by SSCPOM and MPS and contained many pollution-intolerant organisms, as well as a good representation of varying functional feeding groups. Agricultural streams were best predicted by flow and temperature and contained a large proportion of scrapers and collector-gatherers, as well as many tolerant genera. Exurban streams were not as easy to interpret. Two of the three streams, Ammons Br. and Crawford Br., were unsurprisingly predicted by temperature and conductivity. Stillhouse Br., however, was grouped with the forested sites in the CCA. All three exurban streams did not contain many distinct taxa in the PCoAs, instead sharing many taxa with both forested and agricultural sites. This was reflected in the macroinvertebrate metrics as well, as exurban sites were not significantly different from the other two categories with regard to diversity and % EPT. However, it should be noted that exurban streams, on average, contained the highest number of tolerant organisms and the lowest biotic integrity. Again, Stillhouse Branch proved to be an outlier with regard to the NCBI scores, but its low score did not alter significance in the analysis. Interestingly, although not significant, was that exurban streams had the lowest average taxa richness and density. This requires more study, perhaps incorporating nutrient data, spatial data, and a closer look at potential toxins and pollutants that come from impervious surface. In addition, perhaps streams that are classified as 'exurban' in the future should be examined in situational context, as our exurban replicate streams, particularly Stillhouse Br., were very different in almost every measured variable.

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Table 2.1. Physio-chemical parameter measurements taken at each study site, excluding Skeenah Creek. Data are means \pm 1 SE taken once monthly during the leaf breakdown experiment (n=5), except temperature (n=3) and the FSC Index value.

Physio-chemical variable	Forested			Exurban			Agriculture	
	Falls Br.	Willis Cove	Hugh White Cr.	Stillhouse Br.	Crawford Br.	Ammons Br.	Bates Br.	Mica City
Discharge (L/s)	46.62 \pm 35.35	6.31 \pm 1.90	8.10 \pm 1.65	83.09 \pm 30.16	8.45 \pm 2.74	26.49 \pm 8.94	48.35 \pm 20.12	125.73 \pm 41.96
Conductivity (μ S)	14.08 \pm 0.36	25.52 \pm 1.05	10.70 \pm 0.50	15.62 \pm 0.66	22.10 \pm 0.59	42.88 \pm 1.21	27.38 \pm 1.53	15.06 \pm 0.74
Temperature ($^{\circ}$ C)	16.03 \pm 0.44	15.50 \pm 0.42	14.23 \pm 0.18	16.40 \pm 0.46	16.60 \pm 0.55	18.87 \pm 0.34	16.43 \pm 0.27	16.33 \pm 0.73
FSC (Flashiness Index)	0.14	0.22	0.16	0.37	0.11	0.30	0.22	0.36

Table 2.2. Mean particle size (MPS, \pm 1 SE) of bed substrate in study streams calculated from pebble count. Size class distribution is also included. Values sharing a common superscript are not significant.

Parameter	Forested			Exurban			Agriculture		
	Falls Br.	Willis Cove	Hugh White Cr.	Stillhouse Br.	Crawford Br.	Ammons Br.	Skeenah Cr.	Bates Br.	Mica City
MPS (mm)	41.91 \pm 4.54 ^{AB}	47.74 \pm 5.07 ^{AB}	51.70 \pm 5.37 ^{AB}	67.48 \pm 8.02 ^B	23.66 \pm 3.93 ^B	22.32 \pm 3.05 ^B	55.50 \pm 4.92 ^A	38.27 \pm 4.68 ^A	56.37 \pm 5.33 ^A
% Sand	8	11	9	6	18	20	8	5	3
% Pebble	61	52	55	62	70	66	52	68	55
% Cobble	31	37	36	32	12	14	40	27	42
% Boulder				4			1		

Table 2.3. Physio-chemical variables averaged for each land-use type. Data are means \pm 1 SE (n=3), except agricultural site measurements (n=2, Skeenah Creek excluded). *P* denotes differences in physio-chemical variables among land-use types.

Physio-chemical variable	Land -use			<i>P</i>
	Forested	Exurban	Agriculture	
Discharge (L/s)	20.34 \pm 13.15	39.34 \pm 22.49	87.04 \pm 31.59	0.239
Conductivity (μ S)	16.77 \pm 4.48	26.87 \pm 8.22	21.22 \pm 5.03	0.567
Temperature ($^{\circ}$ C)	15.26 \pm 0.53	17.29 \pm 0.79	16.38 \pm 0.04	0.150
FSC (Flashiness Index)	0.17 \pm 0.02	0.26 \pm 0.08	0.29 \pm 0.06	0.438

Table 2.4. Macroinvertebrate metrics calculated from quantitative samples (n=5) taken on 24 March 2013 and averaged by land-use category. Significance is denoted by an asterisk (*). Values sharing a common superscript are not significant.

Metrics	Land-use			<i>P</i>
	Forested	Exurban	Agriculture	
Density (# ind. /m ²)	346 \pm 98	291 \pm 77	356 \pm 99	0.889
Taxa Richness (S)	42 \pm 4	37 \pm 4	43 \pm 2	0.503
% EPT	41.1 \pm 5.2 ^B	58.4 \pm 7.2 ^{AB}	65.8 \pm 2.3 ^A	0.040*
Shannon Diversity (H')	3.83 \pm 0.05 ^A	3.58 \pm 0.18 ^{AB}	3.45 \pm 0.04 ^B	0.014*
NCBI	4.06 \pm 0.11 ^B	4.54 \pm 0.11 ^A	4.43 \pm 0.11 ^{AB}	0.012*

Table 2.5. Coefficients of determination (r^2) derived from simple linear regressions of macroinvertebrate metrics and physio-chemical variables for the study streams. Significant relationships are denoted with an asterisk (*).

Physio-chemical variable	Density (# ind./m ²)		Richness (<i>S</i>)		%EPT		Shannon Diversity (<i>H'</i>)		NCBI	
	r^2	<i>p</i>	r^2	<i>p</i>	r^2	<i>p</i>	r^2	<i>p</i>	r^2	<i>p</i>
Discharge (L/s)	0.059	0.599	0.011	0.807	0.171	0.309	0.041	0.632	0.171	0.309
Conductivity (μ S)	0.000	0.975	0.049	0.598	0.191	0.230	0.641	0.017*	0.504	0.048*
Temperature ($^{\circ}$ C)	0.124	0.393	0.327	0.139	0.247	0.211	0.686	0.011*	0.272	0.185
FSC (Flashiness Index)	0.008	0.830	0.003	0.906	0.431	0.077	0.115	0.412	0.038	0.645
MPS (mm)	0.055	0.576	0.180	0.295	0.000	0.969	0.304	0.157	0.569	0.031*

Table 2.6. Tolerance values (Lenat 1993, Barbour et al. 1999) and percentages of each EPT taxon present in the three stream types. Percentages were calculated by dividing the taxon abundance in the sample by the total density, then averaging within land-use type.

Tolerance value	Taxa	Land-use			Tolerance value	Taxa	Land-use		
		Forested	Exurban	Agriculture			Forested	Exurban	Agriculture
	Ephemeroptera					Trichoptera			
2.1	<i>Ameletus</i>	0.2	0.8	0.0	–	<i>Beraea</i>	0.0	0.1	0.0
9.3	<i>Callibaetis</i>	1.5	1.5	3.7	–	<i>Austrotinodes</i>	0.5	0.0	0.1
4	<i>Ephemerella</i>	0.1	1.5	1.6	1.5	<i>Micrasema</i>	1.0	1.1	0.9
3.9	<i>Danella</i>	0.0	0.5	0.0	1.5	<i>Glossosoma</i>	0.0	0.0	0.2
4.7	<i>Hexagenia</i>	0.2	0.2	0.1	0	<i>Arctopsyche</i>	0.3	0.0	0.1
1.2	<i>Epeorus</i>	1.1	1.2	1.7	2.2	<i>Diplectrona</i>	7.7	2.8	1.7
3	<i>Stenacron</i>	0.3	0.0	0.0	6	<i>Hydropsyche</i>	0.0	0.0	0.2
5.8	<i>Stenonema</i>	0.0	0.2	1.1	2	<i>Stactobiella</i>	0.0	0.1	0.0
–	<i>Maccaffertium</i>	1.0	2.7	1.5	1	<i>Pseudostenophylax</i>	0.0	0.0	0.1
0	<i>Habrophlebia</i>	0.2	0.3	0.0	2.5	<i>Pycnopsyche</i>	0.4	0.4	0.2
6.4	<i>Leptophlebia</i>	3.7	5.6	1.6	2.8	<i>Chimarra</i>	0.3	0.0	0.0
5.4	<i>Tricorythodes</i>	6.3	13.7	28.9	0	<i>Pseudogoera</i>	0.0	0.1	0.0
	Plecoptera				0	<i>Psilotreta</i>	0.0	0.1	0.1
–	<i>Utaperla</i>	0.8	3.9	2.2	4.4	<i>Neureclipsis</i>	0.4	0.0	0.0
0.7	<i>Leuctra</i>	3.1	3.4	0.5	2	<i>Rhyacophila</i>	1.4	0.9	0.6
3.4	<i>Amphinemura</i>	5.9	5.8	0.9					
1.4	<i>Tallaperla</i>	8.6	6.5	1.7					
2.2	<i>Acroneuria</i>	0.3	0.2	0.2					
1.6	<i>Neoperla</i>	0.2	0.5	0.3					
2	<i>Archoptryx</i>	0.3	0.7	0.4					
2.2	<i>Isoperla</i>	1.3	1.3	1.6					
6.3	<i>Taeniopteryx</i>	0.0	0.1	0.0					
1.8	<i>Pteronarcys</i>	0.0	0.1	0.5					

Table 2.7. Canonical correspondence analysis axes summary for macroinvertebrate data constrained by environmental variables in the 8 streams, March 2013. Bold values are axes plotted in ordination.

Statistic	CCA 1	CCA 2	CCA 3	CCA 4	CCA 5	<i>P</i> value*
Eigenvalue	0.1940	0.10586	0.05919	0.04070	0.02752	0.002
% variance explained	16.5	9.0	5.0	3.5	2.3	
Cumulative % variance Explained	16.5	25.5	30.5	34.0	36.3	

*The *P* value is the result of the Monte Carlo permutation test (permutations = 500) of significance on all CCA axes

Table 2.8. Biplot scores and variance inflation factors for the environmental variables in the CCA. VIFs indicated no multicollinearity among predictors (VIF < 20). Bolded values are the strongest correlations between noted axis and the environmental variable.

	CCA 1	CCA 2	Variance inflation factor (VIF)
Flow	0.5118	-0.7331	5.05
Standing stock CPOM	-0.877	0.3498	8.0
Temperature	0.8739	-0.0067	14.7
Conductivity	0.6339	0.4619	5.53
Mean substrate size	-0.4601	-0.6871	3.48

Table 2.9. Percent variance explained by the two Principal Coordinates Analyses (dimensions = 2), one using a Bray-Curtis dissimilarity metric and the other using a Jaccard dissimilarity metric.

Distance metric	Axes	% variance explained	Cumulative % variance explained
Bray-Curtis	PCO 1	24.77	43.43
	PCO 2	18.66	
Jaccard	PCO 1	15.60	25.48
	PCO 2	9.88	

Table 2.10. Correlations of macroinvertebrate taxa and PCO axes 1 and 2 in the Bray-Curtis PCoA. Weak correlations with an r lower than 0.20 were not included in the table.

<i>Taxa</i>	PCO 1	PCO 2	<i>Taxa</i>	PCO 1	PCO 2
<i>Callibaetis</i>	0.65029038		<i>Chimarra</i>	-0.23079038	
<i>Ephemerella</i>	0.50095707		<i>Dolophilodes</i>		0.31311525
<i>Hexagenia</i>		0.21893418	<i>Neureclipsis</i>		0.50305707
<i>Epeorus</i>	0.52564194		<i>Rhyacophila</i>	0.53578345	0.48667784
<i>Stenocron</i>		0.43272370	<i>Blepharicera</i>	0.27638349	
<i>Stenonema</i>	0.68096984	-0.36241640	<i>Maruina</i>		0.32372342
<i>Maccaffertium</i>	0.29136746		<i>Caloparyphus</i>		0.31035121
<i>Habrophlebia</i>		0.41392853	<i>Hexatoma</i>	0.31639528	0.64078386
<i>Leptophlebia</i>		0.69497108	<i>Tipula</i>	0.22495708	0.34208876
<i>Tricorythodes</i>	0.82482335	-0.24416015	<i>Atherix</i>	0.52396532	0.29585287
<i>Utaperla</i>	0.40549440	0.29963683	<i>Antocha</i>	0.53206367	-0.30725881
<i>Perlomyia</i>	0.30723035	0.67409765	<i>Hemerodromia</i>	0.21639460	0.39658175
<i>Paranemoura</i>		0.50455214	Ceratapogonidae	0.20854027	0.59455619
<i>Tallaperla</i>		0.56398998	Chironominae	0.27162137	0.78089681
<i>Acroneuria</i>	0.22994853		Orthoclaudiinae	0.39533476	0.67998664
<i>Eccopectera</i>	0.26366520		Tanypodinae		0.49458190
<i>Paragnetina</i>		-0.20965490	Simuliidae	0.33651189	0.31051092
<i>Neoperla</i>	0.43659543		<i>Thaumalea</i>		0.27535195
<i>Archoptryx</i>	0.45412608	0.25846636	<i>Dixa</i>	0.45507293	-0.26376826
<i>Isoperla</i>	0.38092709	0.24015243	<i>Anchytarsus</i>	0.47494414	
<i>Taeniopteryx</i>		0.24622210	<i>Macronychus.adult.</i>	0.20907310	0.36931642
<i>Pteronarcys</i>	0.52305171		<i>Macronychus.larvae.</i>	0.68947889	
<i>Corduligaster</i>		0.29262817	<i>Promoresia.adult.</i>	0.55083382	-0.29805515
<i>Erpetogomphus</i>		0.34877349	<i>Promoresia.larvae.</i>	0.32774907	-0.26508899
<i>Lanthus</i>		0.21941570	<i>Optioservus.larvae.</i>	0.71434911	
<i>Beraea</i>		0.22849035	<i>Ectopria</i>		0.44303244
<i>Micrasema</i>	0.23812246		<i>Psephenus</i>	0.56659653	-0.24739869
<i>Glossosoma</i>	0.47649362	-0.24415215	<i>Nigronia</i>		0.26660333
<i>Ceratopsyche</i>	0.20647475		Decapoda		0.28940854
<i>Diplectrona</i>	0.20875402	0.62333328	Hydrachnidia	0.28822008	
<i>Hydropsyche</i>	0.33851745		Clitellata		0.40698290
<i>Pseudostenophylax</i>	0.37580866	-0.20625347	Turbellaria	0.21778740	
<i>Pycnopsyche</i>		0.54667460	Collembola	0.20751795	0.67804747

Table 2.11. Correlations of macroinvertebrate taxa and PCO axes 1 and 2 in the Jaccard PCoA. Weak correlations with an r lower than 0.20 were not included in the table.

<i>Taxa</i>	PCO 1	PCO 2	<i>Taxa</i>	PCO 1	PCO 2
<i>Ameletus</i>	-0.214119261		<i>Chimarra</i>	0.258452509	
<i>Callibaetis</i>	-0.574395424	-0.061603128	<i>Neureclipsis</i>		-0.340259093
<i>Ephemerella</i>	-0.561349099	-0.208455228	<i>Rhyacophila</i>	-0.350200682	
<i>Hexagenia</i>	0.256002781	-0.295913192	<i>Blepharicera</i>	-0.298463481	
<i>Epeorus</i>	-0.584877359		<i>Nymphomyia</i>	-0.201617302	
<i>Stenocron</i>	0.270807525	-0.365974950	<i>Maruina</i>		-0.257784576
<i>Stenonema</i>	-0.700170390	0.281369379	<i>Brachypremna</i>		-0.211259403
<i>Maccaffertium</i>		-0.373304924	<i>Hexatoma</i>		-0.533946672
<i>Leptophlebia</i>		-0.277822034	<i>Tipula</i>		-0.225470765
<i>Tricorythodes</i>	-0.801877952		<i>Atherix</i>	-0.359392713	
<i>Utaperla</i>	-0.281441367		<i>Antocha</i>	-0.552507718	0.229877624
<i>Perlomyia</i>		-0.488546224	<i>Hemerodromia</i>		-0.381482257
<i>Paranemoura</i>		-0.341294118	<i>Ceratopogonidae</i>		-0.370037557
<i>Tallaperla</i>		-0.286399435	<i>Chironominae</i>		-0.463248587
<i>Eccopectera</i>	-0.334765448		<i>Orthocladiinae</i>		-0.382637882
<i>Neoperla</i>	-0.382867789		<i>Simuliidae</i>		-0.249996637
<i>Archoptryx</i>	-0.364829968		<i>Dixa</i>	-0.501990113	-0.221861395
<i>Isoperla</i>	-0.233872524	0.258008599	<i>Anchytarsus</i>	-0.548133423	
<i>Pteronarcys</i>	-0.585026686		<i>Macronychus.adult.</i>		-0.339942629
<i>Corduligaster</i>	0.328039574	-0.358695044	<i>Macronychus.larvae.</i>	-0.643186494	
<i>Erpetogomphus</i>	0.227833436	-0.452064764	<i>Promoresia.adult.</i>	-0.640918390	
<i>Lanthus</i>	0.200578918	-0.262658536	<i>Promoresia.larvae.</i>	-0.393166064	
<i>Austrotinodes</i>		0.398758905	<i>Optioservus.larvae.</i>	-0.645192014	
<i>Glossosoma</i>	-0.567048058		<i>Ectopria</i>		-0.421842799
<i>Cheumatopsyche</i>	-0.200654406		<i>Psephenus</i>	-0.576358088	0.219716047
<i>Diplectrona</i>	0.045979235	-0.251628627	<i>Nigrionia</i>		-0.281748446
<i>Hydropsyche</i>	-0.416300590		<i>Hydrachnidae</i>	-0.262007209	-0.218837415
<i>Pseudostenophylax</i>	-0.478053823		<i>Collembola</i>		-0.472900450

Figure 2.1 CCA biplot of macroinvertebrate taxa (text) and study sites (circles) as predicted by environmental variables (arrows). Environmental variable codes: TEMP = temperature (°C), COND = specific conductance (µS), FLOW = discharge (L/s), MPS = mean particle size (mm), SSCPOM = standing stock coarse particulate organic matter (g/m²). Genera abbreviation codes: *Acro* = *Acroneuria*, *Amel* = *Ameletus*, *Amph* = *Amphinemura*, *Arch* = *Archoptryx*, *Athe* = *Atherix*, *Call* = *Callibaetis*, *Ceto* = *Ceratopogonidae*, *Chir* = *Chironominae*, *Clit* = *Clitellata*, *Dipl* = *Diplectrona*, *Dixa* = *Dixa*, *Ecto* = *Ectopria*, *Epeo* = *Epeorus*, *Ephe* = *Ephemerella*, *Heto* = *Hexatoma*, *Isop* = *Isoperla*, *Lant* = *Lanthus*, *Lept* = *Leptophlebia*, *Leuc* = *Leuctra*, *Macc* = *Maccaffertium*, *MacL* = *Macronychus* (larvae), *Micr* = *Microsema*, *Neop* = *Neoperla*, *OptL* = *Optioservus* (larvae), *Orth* = *Orthocladiinae*, *ProL* = *Promoresia* (larvae), *Pycn* = *Pycnopsyche*, *Rhya* = *Rhyacophila*, *Simu* = *Simuliidae*, *Sten* = *Stenonema*, *Tall* = *Tallaperla*, *Tany* = *Tanytopodinae*, *Tipu* = *Tipula*, *Tric* = *Tricorythodes*, *Utap* = *Utaperla*.

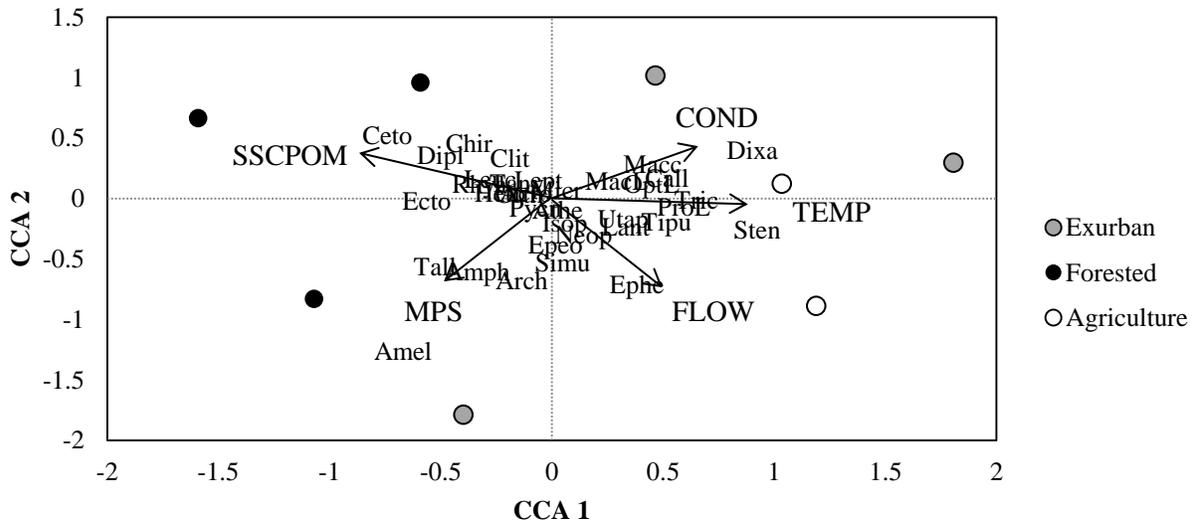
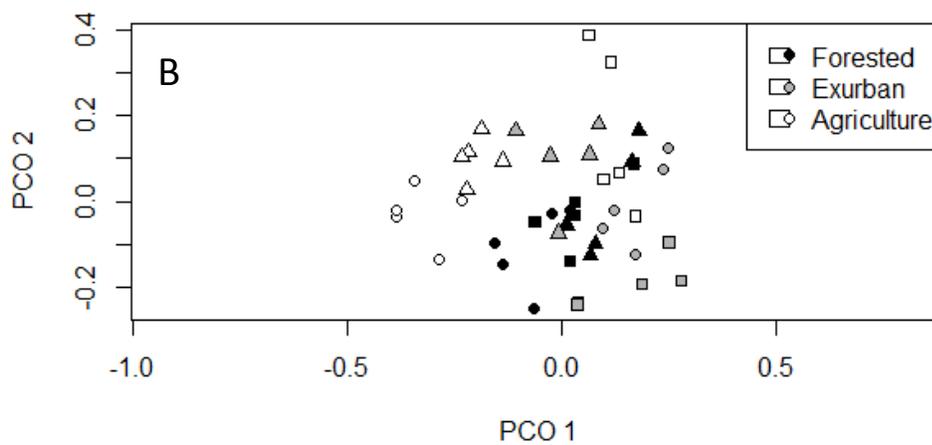
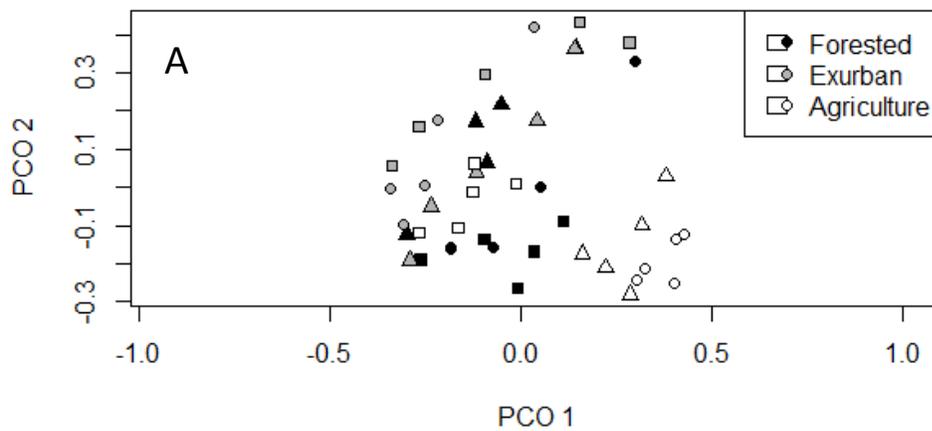


Figure 2.2 A and B Principal coordinates analyses (PCoA) results from quantitative macroinvertebrate samples taken at the 9 study sites in March 2013. Bray-Curtis (A) and Jaccard (B) distance metrics were used. Individual sites are: Falls Branch (●), Willis Cove (■), Hugh White Creek (▲), Stillhouse Branch (◎), Ammons Branch (◻), Crawford Branch (△), Bates Branch (○), Mica City (◻), Skeenah Creek (△).



Summary

This study addressed the impacts of land-use on leaf breakdown and macroinvertebrate assemblages in southern Appalachian streams. Results from both chapters show that differences in leaf breakdown rates and macroinvertebrate assemblages could be explained by land-use regime, but only to a point. Leaf breakdown rates were significantly related to land-use. Forested streams exhibited the slowest average breakdown rates, followed by exurban streams, with agricultural streams having the fastest rates. Forested and agricultural breakdown rates were significantly different. Leaf breakdown was most strongly related to discharge (white oak) and some fine sediment metrics (red maple). Our results suggest that the altered hydrological regimes in agricultural streams, as well as the influx of fine sediments into streams from exurban development, can play a role in altering in-stream organic matter processing. The taxa and number of shredders present may play a role to a lesser extent.

Shannon diversity, %EPT, and NCBI were significantly related to land-use regime. There were significant negative relationships between macroinvertebrate diversity and conductivity and temperature. In addition, biotic integrity had a significant negative relationship with conductivity. Canonical Correspondence Analysis (CCA) showed that agricultural streams were characterized by temperature and flow, forested streams by MPS and standing stock coarse particulate organic matter (SSCPOM), and two of three exurban streams by conductivity and temperature. Principal Coordinates Analysis (PCoA) revealed that while macroinvertebrate communities overlapped, some differences in community assemblage could be seen between land-use types, particularly forested and agricultural streams.

Despite seeing some significant results, our findings are far from conclusive. Of the few physio-chemical variables measured, only mean particle size (MPS) was significantly different with regard to land-use regime. In addition, exurban streams, much like their relatively ambiguous land-use category, exhibited much variance in almost every variable measured. Stillhouse Branch frequently grouped with forested sites while Ammons Branch and Crawford Branch had the smallest MPS coupled with the highest percentage of sand/silt, the highest conductivity and water temperature, and the lowest taxa richness and poorest biotic integrity out of the 9 study streams. Interestingly, these responses have been seen before but in agricultural streams. Although currently classified as exurban, both Ammons Br. and Crawford Br. flow

through lands that were historically dominated by agriculture. Great care should be taken when classifying a stream as 'exurban'. Rigorous water chemistry measurement, as well as detailed land delineation on multiple spatial scales, could possibly narrow down the criteria for exurban stream classification. Past land-use, both recent and historical, should also be considered, as it appears that two of our exurban sites, Ammons Br. and Crawford Br., exhibit legacy agricultural impairments.

APPENDIX A – Annotated List of Figures

Fig. 1.1 A and B: Red Maple (A) and White Oak (B) leaf breakdown rates averaged from the three study streams in each land-use category. Connected letters denote no significant differences.

Fig. 2.1: CCA biplot of macroinvertebrate taxa (text) and study sites (circles) as predicted by environmental variables (arrows). Environmental variable codes: TEMP = temperature (°C), COND = specific conductance (µS), FLOW = discharge (L/s), MPS = mean particle size (mm), SSCPOM = standing stock coarse particulate organic matter (g/m²). Genera abbreviation codes: *Acro* = *Acroneuria*, *Amel* = *Ameletus*, *Amph* = *Amphinemura*, *Arch* = *Archoptryx*, *Athe* = *Atherix*, *Call* = *Callibaetis*, *Ceto* = *Ceratopogonidae*, *Chir* = *Chironominae*, *Clit* = *Clitellata*, *Dipl* = *Diplectrona*, *Dixa* = *Dixa*, *Ecto* = *Ectopria*, *Epeo* = *Epeorus*, *Ephe* = *Ephemerella*, *Heto* = *Hexatoma*, *Isop* = *Isoperla*, *Lant* = *Lanthus*, *Lept* = *Leptophlebia*, *Leuc* = *Leuctra*, *Macc* = *Maccaffertium*, *MacL* = *Macronychus* (larvae), *Micr* = *Microsema*, *Neop* = *Neoperla*, *OptL* = *Optioservus* (larvae), *Orth* = *Orthoclaudiinae*, *ProL* = *Promoresia* (larvae), *Pycn* = *Pycnopsyche*, *Rhya* = *Rhyacophila*, *Simu* = *Simuliidae*, *Sten* = *Stenonema*, *Tall* = *Tallaperla*, *Tany* = *Tanytodinae*, *Tipu* = *Tipula*, *Tric* = *Tricorythodes*, *Utap* = *Utaperla*.

Fig. 2.2 A and B: Principal coordinates analyses (PCoA) results from quantitative macroinvertebrate samples taken at the 9 study sites in March 2013. Bray-Curtis (A) and Jaccard (B) distance metrics were used. Individual sites are: Falls Branch (●), Willis Cove (■), Hugh White Creek (▲), Stillhouse Branch (◎), Ammons Branch (▣), Crawford Branch (△), Bates Branch (○), Mica City (□), Skeenah Creek (Δ).