STREAM ECOSYSTEM RESPONSE TO URBANIZATION IN THE UPPER OCONEE WATERSHED, GEORGIA, USA

by

JESSICA LYNN STERLING

(Under the Direction of Amy D. Rosemond)

ABSTRACT

Watershed urbanization has detrimental effects on stream ecosystems due to increases in watershed impervious cover and associated stressors. During a two-year study, I investigated a wide range of biotic groups exhibiting different taxonomic and life history characteristics and ecosystem functional responses to explore urbanization effects on streams in the upper Oconee watershed, Georgia, USA. I found decreased macroinvertebrate biomass and increased dominance of tolerant taxa as watershed impervious cover and streamwater pollutants increased and stream organic matter decreased. I identified reduced overall nutrient storage in benthic biofilms indicating lower nutrient retention as watershed impervious cover increased, and faster wood breakdown in urban streams, suggesting lower carbon storage. Overall, results from this thesis highlight the importance of carbon in urban streams. Management goals for urban streams in the upper Oconee watershed should include strategies that promote the storage and retention of carbon resources.

INDEX WORDS: Urbanization, Macroinvertebrates, Biomass, Biofilms, Algae, AFDM, C:N, C:P, Wood breakdown, Ecosystem function, Nutrients

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Preface

Urban populations are expanding throughout the United States and the rest of the world (Cohen 2003). For example, in 1900 only 10% of the U.S. population lived in cities; currently, more than 50% of the population lives in urban areas, and the U.S. population is projected to increase by another 10% in the next 50 years (Grimm et al. 2008). At the same time, urban and suburban land use is increasing. This growth has and will continue to alter the number of streams impacted by humans though buffer degradation (Wenger 1999), stream burial (Elmore and Kaushal 2008), and increases in the amount of impervious surface cover in watersheds (Paul and Meyer 2001). Most of the streams impacted by land use change are small streams which are hotspots for both biodiversity and ecosystem function (Freeman et al. 2007, Meyer et al. 2007). Small streams are potentially active sites for sediment retention, carbon processing (Cole et al. 2007) and assimilation and transformation of nutrients (Mulholland et al. 2008), all contributing to the improvement of overall downstream water quality.

It is essential to further our understanding of how urbanization is impacting stream ecosystem quality. In order to make informed urban planning and watershed management decisions, it is important to conduct studies that examine the ecosystem-level responses to urbanization in small streams (Walsh et al. 2005, Wenger et al. 2009). When examining the effects of land use on the ecosystem, it is critical to consider changes to both structure (e.g. macroinvertebrates, algae, fishes, organic matter) and function (e.g. nutrient uptake, carbon breakdown, primary production, secondary production, respiration) (Gessner and Chauvet 2002, Young et al. 2008, Wenger et al. 2009).

In most conceptual models of in-stream effects on watershed urbanization, there are two broadly defined environmental variables that drive alterations in stream structure and function: modified hydrology and non-point source pollutants (Paul and Meyer 2001, Walsh et al. 2005, Wenger et al. 2009). Effects of urbanization on ecological structure and function are difficult to predict because (1) there are often multiple stressors that act concurrently (Walsh et al. 2005), and (2) different stressors can have opposing effects on any one variable (Chadwick et al. 2006). Thus, challenges in identifying how stream structure and function respond to urbanization include comparing established patterns in variables to a reference state and attempting to isolate important stressors.

Project overview

This thesis is part of a larger partnership between the Unified Government of Athens-Clarke County, Georgia, (ACC), the nonprofit Upper Oconee Watershed Network (UOWN) and the University of Georgia River Basin Center (UGA-RBC) funded by the U.S. Environmental Protection Agency (US EPA) 319(h) program. The overall objectives of the ACC-led project were to: (1) put into effect Total Maximum Daily Load (TMDL) implementation plans for the impaired watersheds in this study, (2) develop Watershed Management Plans according to US EPA Watershed Planning Guidelines, (3) develop a public engagement strategy to provide community support for watershed management, and (4) establish a watershed monitoring system to further scholarly research on urban streams and identify appropriate best management practices (BMPs) for urban streams. The research in this thesis was part of objective 4.

The watersheds chosen for the project and studied in the subsequent chapters in this thesis are tributaries of the North and Middle Oconee rivers within Athens-Clarke County, Georgia, USA Three of the watersheds, Brooklyn Creek (urban), Hunnicutt Creek (suburban) and Trail Creek (mixed-use) are all on the State of Georgia 303(d) list as impaired due to fecal coliform pollution and are required to have TMDL implementation plans to address pollution issues outlined on the 303(d) list. While the Brooklyn, Hunnicutt and Trail Creek watersheds are listed for fecal coliform pollution, other impairments likely exist beyond those identified on the 303(d) list in these streams. These additional impairments are likely due to the relatively high values of watershed impervious surface cover and associated stressors. In order to compare watershed conditions in these urbanized and urbanizing watersheds, we chose three additional watersheds in the upper Oconee River basin that are predominantly forested as reference sites – Bear Creek, Big Creek and Shoal Creek.

The overall ACC-led project had innovative aspects that have extended its scope beyond the monitoring of local streams that is mandated by the US EPA in order to obtain a National Pollutant Discharge Elimination System (NPDES) permit for stormwater discharge. It has aimed not only to address TMDL implementation, but also to define a process for effective watershed management in Athens-Clarke County through the evaluation of stressors and planning of BMPs. Goals of this project that were not required as a part of the NPDES permitting process include addressing impairments not outlined in existing TMDLs through additional research and a community education campaign. The project has benefited from collaborative effort between a local government, a university and a local citizen group.

Overview of thesis chapters

The objectives of this thesis were to establish patterns in structural and functional components in streams subject to urbanization. Response variables that were chosen included a wide range of biotic groups exhibiting different taxonomic and life history characteristics and ecosystem functional responses. The response variables include macroinvertebrate biomass and functional feeding group composition, stream biofilm biomass, nutrient content and carbon processing rates.

The first objective, addressed in Chapter 2, examined macroinvertebrate feeding traits and biomass to understand effects on resource-consumer pathways in urbanized streams. Potential shifts in energy flow may be reflected by shifts in functional macroinvertebrate community composition and changes in basal food resources with stream urbanization. I hypothesized that the biomass of macroinvertebrates would decline as catchments become more urbanized and that effects would differ based on the feeding ecology of macroinvertebrate taxa.

In Chapter 3, I assessed watershed urbanization effects on biofilms and their role in nutrient storage. I quantified mass and nutrient content (carbon, nitrogen and phosphorus) of biofilms along a gradient of watershed impervious surface cover (% ISC). I examined whether biofilm nutrient content was related to streamwater nutrient concentrations and estimated whole-stream carbon and nitrogen storage in biofilms. To determine whether nitrogen in biofilms was derived from anthropogenic sources, I also tested whether there was a positive relationship between streamwater nitrogen concentrations and the $\delta^{15}N$ of biofilms. I hypothesized that biofilm nutrient content would increase with streamwater nutrient concentrations, but whole stream nutrient storage would decline along the imperviousness gradient due to decreased retention of biofilm mass.

I examined how carbon processing (the rate of wood breakdown) was affected by watershed urbanization in Chapter 4. Allocthonous carbon sources, such as leaves and wood, are important basal food resources in streams. Alterations in the rates of carbon processing are known to be an indicator of stream impairment, and might increase or decrease due to urbanization (Walsh et al. 2005). I determined the relative contribution of specific drivers (nutrients, physical abrasion, microbial activity) to wood breakdown rates across sites. I also investigated variability in wood breakdown rates among sites within land use classes (urban, suburban, mixed-use or forested).

Lastly, Chapter 5 summarizes the results of the previous three chapters and provides management recommendations. Overall, I expect each response variable addressed in Chapters 2, 3 and 4 to respond to environmental stressors in different ways, thus yielding a comprehensive view of urbanization in Georgia Piedmont streams.

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CHAPTER 2

WATERSHED LAND USE AFFECTS MACROINVERTEBRATE BIOMASS AND TROPHIC STRUCTURE IN SOUTHEASTERN U.S. PIEDMONT STREAMS [1]

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[1] Sterling, J.L., A.D. Rosemond, and S.J. Wenger. To be submitted to *Freshwater Science*.

Abstract

Macroinvertebrate assemblages are used in bioassessment of stream ecosystems, largely based on species composition and the presence of taxa that are tolerant or sensitive to pollution. Stressed ecosystems are characterized not only by loss of sensitive taxa, but also by loss of overall macroinvertebrate biomass and production, which are important ecosystem functions. These losses likely occur through reduced basal food production and inputs of resources to streams. In this study, we determined trends in macroinvertebrate biomass and functional feeding group composition across a gradient of percent impervious surface cover (% ISC). We collected macroinvertebrates in 12 sites in urban, suburban, mixed-use and forested watersheds in the upper Oconee River basin, Georgia, USA. We identified to genus, measured, and assigned them to functional feeding groups (FFGs). Model results indicate that for every 1% increase in % ISC, macroinvertebrate biomass declined 7%. Biomass of all functional feeding groups declined, with the exception of collector-gatherers and filterers. Proportionally, macroinvertebrate communities shifted from a community that represented all FFGs to one that was dominated by collector-gatherers and filterers as watershed % ISC increased, indicating reduced functional diversity. Physical/chemical drivers associated with declines in macroinvertebrate biomass included streamwater dissolved inorganic nitrogen (DIN) and conductivity. Food resources, particularly benthic ash-free dry mass (AFDM), were positively related to total macroinvertebrate biomass and biomass of predators, scrapers and collectors. Our results indicate that reductions in macroinvertebrate biomass were associated with both measures of pollution (e.g., conductivity) and reductions in basal resources (AFDM). This suggests that management to improve conditions for aquatic life in urban streams should focus on reducing

negative watershed inputs and also in promoting retention of food resources for higher trophic levels.

Introduction

Stream consumers are key integrators of aquatic ecosystems by connecting basal resource flow to emergent ecosystem properties. Urban streams typically have degraded or altered macroinvertebrate consumer assemblages and altered ecosystem functioning (Wenger et al. 2009). These two phenomena are closely tied together: consumer assemblages are likely to shift in urban streams with changes in resource availability and/or water quality, thus affecting processing rates of resources. Evidence for water quality effects on biota include altered assemblage structure associated with increased total suspended solids (Freeman and Schorr 2004), conductivity (Roy et al. 2003), water column nitrogen and phosphorus concentration (Yuan 2010), and flood magnitude and frequency (Dewson et al. 2007). Macroinvertebrate assemblages may also shift due to a reduction of critical levels of resources such as primary producers and detrital organic matter (Cummins et al. 2005). Changes in macroinvertebrate communities with urbanization might also be evidenced in the abundance or biomass of macroinvertebrate functional feeding groups (FFGs) due to changes in resource availability, but these patterns have not been previously examined.

Measuring the functional role of stream consumers can provide insights into alterations in energy and material flow through stream food webs. Structural measures such as macroinvertebrate biomass may correlate with measures of energy flow (secondary production) (Woodcock and Huryn 2008) and can be used as surrogate measures of such, with appropriate caveats and context. Benthic macroinvertebrate biomass may be a better measure of ecological integrity than abundance metrics alone. Stephenson and Morin (2009) found that catchment forest cover explained more variation in invertebrate and fish biomass than in fish and invertebrate community metrics. Macroinvertebrate biomass and functional feeding group biomass may be associated with other stream ecosystem functions and can be important links between community trends and ecosystem processes in river systems. It has been suggested that the dominance of taxa in different functional feeding groups will shift based on the resources available to consumers (Cummins and Klug 1979), thus providing a coarse assessment of resources available across streams. Conversely, changes in the biomass of taxa in different functional feeding groups can reflect altered demand on, and consumption rates of, resources.

By examining both FFG traits and biomass together, we attempt to understand some of the mechanisms driving alterations in streams with urbanization and to infer how energy flow in systems might shift with increases in watershed impervious cover. Few studies have examined the patterns in biomass of aquatic macroinvertebrates with changes in land use (Chadwick et al. 2006, Compin and Cereghino 2007, Stephenson and Morin 2009) and little research has investigated whether documented changes in macroinvertebrate community structure occur in biomass as well. We hypothesized that the biomass of macroinvertebrates would decline as catchments become more urbanized and that effects would differ based on the feeding ecology of macroinvertebrate taxa. We collected macroinvertebrates from streams with different levels of urbanization (forested, mixed-use, suburban, urban), identified them, and calculated biomass based on published length-mass regressions (Benke et al. 1999). We aimed to determine whether there were predictive relationships between total macroinvertebrate biomass and FFG biomass with differences in watershed impervious surface cover and mechanistic drivers. Specifically, we assessed whether trends in biomass changed with (1) land use (% ISC and watershed area), (2) water column nutrient concentrations (nitrogen and phosphorus), (3) physical and chemical drivers (conductivity, pH, total suspended solids), or (4) basal resources (ash-free dry mass in depositional areas and algal biomass). Our goal was to gain additional insight into mechanisms driving macroinvertebrate biomass changes beyond what could be discerned from abundance and diversity metrics alone.

Methods

Study sites were located in the upper Oconee River Basin in Athens-Clarke County, in northeast Georgia, USA (Figure 2.1). The research area is located in the Piedmont physiographic province, which is characterized by red clay soils and granitic-gneiss bedrock. We chose twelve sites in six watersheds with a range of land uses across a gradient of impervious surface cover (<5%–35%). We collected macroinvertebrates at three tributaries in an urban watershed (URB1, URB2, URB3), three tributaries in a suburban watershed (SUB1, SUB2, SUB3) and three tributaries in a mixed-use watershed (called ‘mixed’; MIX1, MIX2, MIX3). The mixed watershed had less impervious cover than the suburban watershed and included light industrial, agricultural and residential land uses and some forest cover. We also sampled three separate, predominantly forested watersheds (called ‘forest’; FOR1, FOR2, FOR3). All streams were 2nd to 3rd order. Urban, suburban and mixed sites were nested, with sites on two tributaries and a downstream site in the same watershed.

We derived percentage impervious surface cover (% ISC) and watershed area from digital aerial images of Athens-Clarke County with a 15.2 cm resolution collected in 2008, and delineated catchments using a GIS (ArcMap 10). We estimated % ISC in each catchment by overlaying each spatial zone on a land cover map.

Macroinvertebrate sampling methods

We collected benthic macroinvertebrates from four riffles and four pools at each site on April 4–6, 2008 during baseflow conditions. We sampled riffles using a Surber sampler (0.09 m^2, 250 µm mesh) by scrubbing the rocks with a brush for three minutes. We sampled pools with a core sampler (0.04 m^2) by removing the top 10 cm of sediment from the core, transferring the sediment to a bucket and elutriating through a 250 µm mesh sieve in the field. Before sampling, we mapped 50 m reaches at each site and determined the percentage of each habitat type (riffle, pool and woody debris) at each 5 m subreach to determine any major differences in available habitat between sites.

In the laboratory, we washed samples through stacked 1 mm and 250 µm sieves to separate into size class categories of >1 mm and ≤1 mm, and stored them in 70% ethanol. We separated macroinvertebrates from organic matter and sediment under a dissecting scope at 10X magnification. If necessary, we subsampled small invertebrates (250µm–1 mm) using a wheel sampler (Waters 1969). We counted, measured (to the nearest 1 mm) and identified macroinvertebrates to the lowest taxonomic order possible using standard keys (Merritt and Cummins 2007). We identified non-insects to order and Chironomids [Diptera] as Tanypodinae or non-Tanypodinae. We assigned each invertebrate to a functional feeding group (FFG; collector-gatherer, collector-filterer, predator, scraper or shredder) based on published information about the mode of feeding (Merritt and Cummins 2007). We calculated biomass (mg/m^2) for each individual invertebrate using the measured length (to the nearest 1 mm) and published genus-specific length-mass regressions (Benke et al. 1999). If the length-mass regression was not available, we used the regression for the closest related taxon. We assigned tolerance values to each taxon using the North Carolina Biotic Index (NCBI) (Lenat 1993). We used averaged species-level tolerance values to obtain a value for each genus (Roy et al. 2003).

We added a constant of 0.2 to NCBI tolerance values for each genus to correct for winter/ spring collection (Lenat and Crawford 1994).

Water Chemistry

We collected samples for water chemistry monthly at baseflow from June 2009 to May 2010. We field-filtered samples for NH_4–N, NO_3–N, and PO_4–P through 0.45 µm Whatman nylon-membrane filters into acid-washed polypropylene bottles, returned them to the laboratory on ice and froze them until analysis in the University of Georgia Odum School of Ecology Analytical Chemistry Laboratory. Samples for NH_4–N, NO_3–N and soluble reactive phosphorus (SRP) were analyzed using continuous flow colorimetry (APHA 1998). We used mean values for each nutrient to characterize each stream reach. Total suspended solids were quantified by filtration (APHA 1998). We measured conductivity and stream temperature continuously in each reach from February 2009 to May 2010 with a data sonde (Eureka Manta X2, Austin, TX). A summary of physical and chemical characteristics is listed in Table 2.1.

Basal resources

We collected algae from rocks with a modified Loeb sampler (4.9 cm^2 area) (Loeb 1981) and ash-free dry mass (AFDM) from depositional pools with a modified PVC core (9.8 cm^3 area) bimonthly from May 2008–April 2010. We filtered samples onto two pre-weighed 0.7µm GFF (Fisher) filters to obtain algal biomass (as chlorophyll *a*) and ash-free dry mass (AFDM). Chlorophyll *a* was extracted in the dark in 90% acetone and determined spectrophotometrically (Wetzel and Likens 2000). Samples for AFDM were dried at 55°C for 48h and weighed, then ashed in a muffle oven at 500°C. Average chlorophyll *a* and AFDM for each site were used in subsequent analyses.

Data Analysis

We analyzed the response of log-transformed total macroinvertebrate biomass in samples (n=96) to (1) land-use parameters (% ISC, watershed area), (2) nutrients (DIN and SRP), (3) physical and chemical parameters (total suspended solids, pH, conductivity) and (4) basal resources (AFDM and algal biomass) using a multilevel modeling approach (Gelman and Hill 2007). Multilevel modeling uses random effects to account for unexplained spatial (or temporal) dependence; in our case, we had multiple samples from each site, so we included a random intercept to represent site-level variance and distinguish it from sample-level variance. Because we predicted the biomass of FFGs would differ between pool and riffle habitat, we included a factor for habitat. If habitat did not improve model fit over the null model, it was not included in subsequent candidate models. Therefore, models containing scrapers and shredders did not include habitat (Appendix 2.1). We ranked the resulting models using Akaike's Information Criterion (AIC) (Burnham and Anderson 2002). We repeated this process using the log-transformed biomass of each of the FFGs for the models containing land-use parameters and primary resources as the predictor variables. To analyze how the relative compositions of FFGs in each watershed differed, we averaged biomass estimates from each sample and then calculated percent contribution of each FFG to mean total biomass. We used simple linear regression to predict how the relative contribution of each FFG to mean total biomass changed with % ISC.

To combine elements of the bioassessment approach and measures of biomass, we tested for shifts in the biomass of tolerant versus sensitive macroinvertebrate taxa with % ISC. The biomass of taxa was summed based on each taxa's individual NCBI tolerance value (Lenat 1993). A taxon was determined to be sensitive if it had an NCBI value less than or equal to 5.7 and tolerant if the value was greater than 5.7. A score of 5.7 indicates sites with good to fair water quality according to the NCBI index (Lenat 1993). Linear regression was used to determine whether the biomass of tolerant and sensitive taxa changed across a gradient of % ISC. All calculations were computed using the statistical package R 2.12.1 (R Development Core Team 2004).

Results

We identified 32,900 individual invertebrates across 12 sampling sites. The most common taxa we collected at all sites were Chironomidae [Diptera], Oligochaeta and Copepoda. Other common taxa found at most sites were *Ephemerella* sp. [Ephemeroptera]*, Antocha* sp. [Diptera] and adults and larvae in the family Elmidae [Coleoptera]. The most common FFG was collector-gatherers, dominated by Chironomidae and Oligochaeta. Shredders were the least common FFG found. Benthic macroinvertebrate biomass estimates ranged from < 1 mg/m^2 (SUB3) to 8,109 mg/m^2 (FOR2), and density ranged from 89 individuals per m^2 (SUB3, 28.5% ISC) to 26,675 individuals per m^2 (FOR3, 3.9% ISC). Density (no./m^2) and biomass (mg/m^2) in each sample were weakly correlated ($r^2 = 0.06$, $p > 0.05$ $n = 93$).

Land use and patterns in total and FFG biomass

Total macroinvertebrate biomass decreased sharply as the amount of impervious surface cover in the watershed increased (Figure 2.2). Model estimates indicated that there was a 7.3% decrease in total biomass for each 1% increase in ISC (Table 2.2) and 46.3% lower biomass in riffles compared to pools, indicating that pool habitat made a relatively larger contribution to overall biomass (Table 2.2). Predators, scrapers and shredders all declined with increasing % ISC (Figure 2.2, Table 2.2). Results indicated that there was a decrease in predator biomass for each 1-km2 increase in watershed size (watershed sizes range from 1 km^2 to 32 km^2). At sites with 0.1–5% ISC, we collected fairly tolerant but large predators in the family Odonata such as *Progomphus sp.* and *Cordulagaster sp*. Plecoptera such as *Suwilla sp.* and *Perlesta sp.* were also a dominant portion of predator biomass at these sites, in addition to Ceratopogonidae and tanypode Chironomidae [Diptera]. At urban sites with > 28% ISC (URB1, URB2, URB3), the only predators collected were Ceratopogonids and predatory Chironomids.

For each 1% increase in ISC, scraper biomass declined by almost 10% (Figure 2.2A, Table 2.2). Forested sites were dominated by scraper taxa in the order Ephemeroptera such as *Baetis*, *Centroptillum*, *Ephemerella*, *Drunella* and *Choroterpes.* Macroinvertebrates in the family Elmidae were also present. Notably, in all forested sites we collected both adult and larval Elmidae. At the urban sites (URB1, URB2, URB3) there were very few scraper taxa represented. Elmidae larvae were collected at URB1 and URB3, but no adult Elmidae were collected at any of the urban sites. At URB1 and URB2, we collected a small number of *Ephemerella sp.* that contributed to only a negligible proportion of total biomass. Results indicated the biomass of collector-gatherers and filterers did not change with increasing % ISC, but filterers exhibited an 11.71% increase in biomass with an increase in watershed area of 1 km^2 (Table 2.2).

Habitat was an important factor in predicting biomass of predators, collector-gatherers and filterers, but not of scrapers or shredders. Model estimates predicted that there was 70% less predator biomass and 46.5% less collector-gatherer biomass in riffles than pools. The best model for filterer biomass included habitat (Table 2.4), but the confidence interval around the parameter estimate were very wide, indicating uncertainty about the magnitude and even the direction of the relationship.

Shifts in relative proportion of functional feeding group biomass

To infer functional assemblage shifts, we examined linear relationships between % ISC and the proportions of FFGs across a gradient of % ISC. The proportion of collector-gatherers increased with greater % ISC, and the proportion of scrapers declined with % ISC (Figure 2.3).

The proportions of predator biomass, filterer biomass, and shredder biomass were not significantly correlated with % ISC (Figure 2.3).

Total biomass and water column nutrients, and stream physical/chemical characteristics

We ran separate models for nutrients and other physical/chemical characteristics because some predictor variables were correlated. The best model relating water column nutrient concentrations (habitat, DIN and SRP) based on AIC (Appendix 2.1) contained both habitat type and water column DIN concentrations. Total macroinvertebrate biomass decreased as water column DIN increased (Table 2.4). Similarly, the best model relating stream physical/chemical characteristics (habitat, TSS, conductivity, pH; Appendix 2.1) to total macroinvertebrate biomass contained only habitat and conductivity; total macroinvertebrate biomass declined as conductivity increased (Table 2.4).

Basal resources and shifts in macroinvertebrate biomass

There were positive relationships between total biomass and biofilm AFDM, and between total biomass and biofilm chlorophyll *a* (Table 2.4). There were positive relationships between shredder biomass and AFDM and between predator biomass and AFDM, but no relationship between filterers and AFDM (Table 2.4). Generally, there was a positive relationship between the biomass of scrapers and the biomass of algae, but the amount of chlorophyll *a* was not included in the best model (Appendix 2.1).

Tolerant versus sensitive macroinvertebrates

We tested the relationship of tolerant taxa biomass and sensitive taxa biomass at each site to % ISC. Results predicted that the biomass of both tolerant and intolerant organisms declined with increasing % ISC (Table 2.5), but the biomass of sensitive organisms declined more rapidly. Between 5% and 25% ISC, the model predicted a loss of 1,444 mg/m^2 of sensitive macroinvertebrate biomass, while over the same gradient, the model predicted a loss of 978 mg/m^2 of tolerant macroinvertebrate biomass.

Discussion

Trends in macroinvertebrate biomass and functional feeding group composition

Watershed urbanization was associated in this study with dramatic declines in macroinvertebrate biomass, which were related to both higher streamwater conductivity and lower basal food resources. These results are consistent with presumed mechanisms of degradation of stream health due to urbanization that occur via increased runoff and reduced retention of organic matter (Wenger et al. 2009). Other studies that have quantified macroinvertebrate biomass have found both increased and decreased macroinvertebrate biomass along an urban land use gradient. As in our study, Woodcock and Huyrn (2007) found decreased macroinvertebrate biomass and production with urbanization, attributing the declines to higher sediment metal concentrations and less stored organic matter. Sudduth and Meyer (2006) also reported a decrease in biomass in urban streams as compared to forested streams, with significantly lower macroinvertebrate biomass in bank habitats in urban streams. In contrast, Stephenson and Morin (2009) found greater macroinvertebrate biomass in streams with less forest cover, but proportionally lower biomass of that taxa belonged to the orders Ephemeroptera, Plecoptera and Tricoptera (EPT). This suggested that the greater biomass was composed of less sensitive taxa. Helms et al. (2009) also found increasing biomass with % ISC in streams in southwest Georgia, but a greater proportion of those taxa were also found to be tolerant to pollution. Systems in which diversity is reduced but biomass is elevated due to the presence of tolerant taxa (i.e. Oligochetes and Chironomids) may still maintain a significant capacity to retain nutrients and/or organic matter and support higher-level organisms.

Macroinvertebrate functional diversity was compressed in streams with greater % ISC in this study, as we observed increased dominance of collector-gatherer biomass, comprising approximately 60–90% of macroinvertebrate biomass at highly urbanized sites. Other studies that have quantified the shifts in relative abundance (not biomass) of these FFGs have also found a significant increase in the proportion of collector-gatherers with urbanization (Stepenuck et al. 2002, Compin and Cereghino 2007). The greatest declines in macroinvertebrate biomass with % ISC were in predator and scraper biomass. The absolute amount of predator biomass declined with % ISC in the watershed (Table 2.4), but notably the proportional amount of predator biomass did not change with % ISC (Figure 2.3). Essentially, the predator biomass decline tracked the decline in total biomass along the urban gradient. The biomass of predator taxa was reduced in disturbed streams and was dominated by small, tolerant predatory Chironomids. The presence of small predators resulted in an insignificant decline in the proportion of predator biomass with an increase in % ISC (Figure 2.3), but the decline in total biomass of predators (Table 2.4) can likely be attributed to the absence of larger predatory Odonata and Plecoptera. The decline in scraper biomass was dramatic both in the absolute biomass and the proportional biomass. The biomass of scrapers declined faster than overall biomass, as evidenced by the significant relationship between the proportion of scraper biomass in the assemblage at each site and % ISC in the watershed (Figure 2.3), suggesting that scrapers are almost absent from the assemblage at the most urban sites.

What factors were identified as drivers of changes in macroinvertebrate biomass?

Changes in FFG composition could be due to alterations in food availability or, conversely, could occur as a function of chemical stressors. Our evidence for these pathways (food vs. pollutants) is mixed, and both are likely important. Our measure of AFDM was not a true measure of food availability, but a measure of organic matter retention in the stream. The pattern of greater organic matter retention with lower % ISC in streams may indicate capability to support the biomass of all macroinvertebrate functional groups, particularly collector-gatherers. Sheih et al. (2002) found that non-tanypode Chironomids (a collector-gatherer) were the major trophic pathway in urban and forested sites in Colorado, USA and that amorphous detritus supported this resource base. Their results showed that at urban sites, lower predation pressure resulted in higher production of non-tanypode Chironomids. A relatively comparable amount of collector-gatherer biomass in streams across the impervious cover gradient in our study might suggest that detritus is likely supporting the biomass of collector-gatherers in urban streams, but our evidence that AFDM is lower as watershed % ISC increases does not support that notion. In anthropogenically altered streams, detritus often remains an important basal resource although nutrient loading (Gulis et al. 2004, Imberger et al. 2008) and altered flow regimes (Chadwick et al. 2006, Paul et al. 2006) may speed up organic matter processing, thus altering the availability of terrestrially derived coarse particulate organic matter (CPOM) as a primary food source for benthic organisms (Kominoski and Rosemond 2012). The positive relationship of AFDM to both total biomass and the biomass of predators supports the view that external inputs to streams (in the form of higher storm flows or greater nutrient concentrations) may be driving reductions in basal resource availability, thus shifting the overall assemblage (Miserendino and Masi 2010).

In addition, the decline in more sensitive taxa with higher watershed % ISC points to stream pollutants as a primary driver in the decline in macroinvertebrate biomass. We saw a decline in total biomass as conductivity and nutrients (as DIN) increased. The decline in total biomass with % ISC is mostly attributed to scrapers and predators. The taxa in these functional groups are usually larger-bodied and typically more sensitive to pollution (Lenat 1993). Several studies have demonstrated a decline in sensitive macroinveretbrate taxa with increased stream pollutants (Stepenuck et al. 2002, Roy et al. 2003, Helms et al. 2009), so pollutants that increase conductivity are likely an additional driver of reduced biomass in this study.

We predicted that the amount of algal biomass would also be a driver of patterns in the biomass of functional groups, particularly scraper biomass. In our study, we observed a greater standing crop of algal biomass in urban and suburban streams, especially in the spring months (JLS., unpublished data), but those sites had significantly reduced scraper biomass. In our analysis of basal resources, we did not find a predictive relationship between algal biomass and the biomass of scrapers, although the relationship was negative overall. Several factors may be causing this decrease in scraper biomass. At a relatively low level of impervious cover (7%), Stepenuk et al. (2002) showed a marked decline in the proportion of scrapers (as density) in the assemblage and attributed the decline to a dominance of filamentous algae in streams with higher % ISC. Filamentous algae are known to be less palatable to scrapers than other algal taxa such as diatoms (Cummins and Klug 1979). Riseng et al. (2004) found that high flow disturbance reduced scraper biomass due to increased bed mobility in riffle habitats. This resulted in a positive, indirect effect on algal biomass. In streams with low hydrologic disturbance, herbivore biomass was adequate to control the biomass of algae. Lacking more information on algal species composition, we suggest that hydrology, not algal availability, was potentially driving decreased scraper biomass in urban sites in this study.

Implications for stream ecosystem function and management recommendations

In this study, we demonstrated that urban streams have lower macroinvertebrate biomass and simplified trophic structure, suggesting that energy flow may be altered in these streams

(Woodcock and Huryn 2007). Increased and decreased macroinvertebrate production have been documented with urban land use (Shieh et al. 2002, Carlisle and Clements 2003, Woodcock and Huryn 2007, 2008). Increased production has been attributed to increased dominance of short-lived tanypode Chironomids (Shieh et al. 2002), and decreases have been attributed to physical and chemical stresses (Woodcock and Huyrn 2007). Whether production increased or decreased in this study, the loss of predator and scraper taxa and increased dominance of tolerant collector-gatherer taxa with increasing % ISC indicates altered community trophic dynamics. We suggest that these alterations in urban streams are driven by modifications to basal resources via decreased amounts of organic matter and increased delivery of pollutants to the stream.

To promote biomass and functional diversity of macroinvertebrates in urban streams, we recommend restoration projects that promote the storage of organic matter and reduce the delivery of conductivity-increasing pollutants to streams. Creative stormwater management solutions that reduce stormflow inputs and increase stormwater filtration, especially during smaller, more frequent storm events (Walsh et al. 2005), have the potential to promote organic matter storage and the flux of pollutants to the stream.

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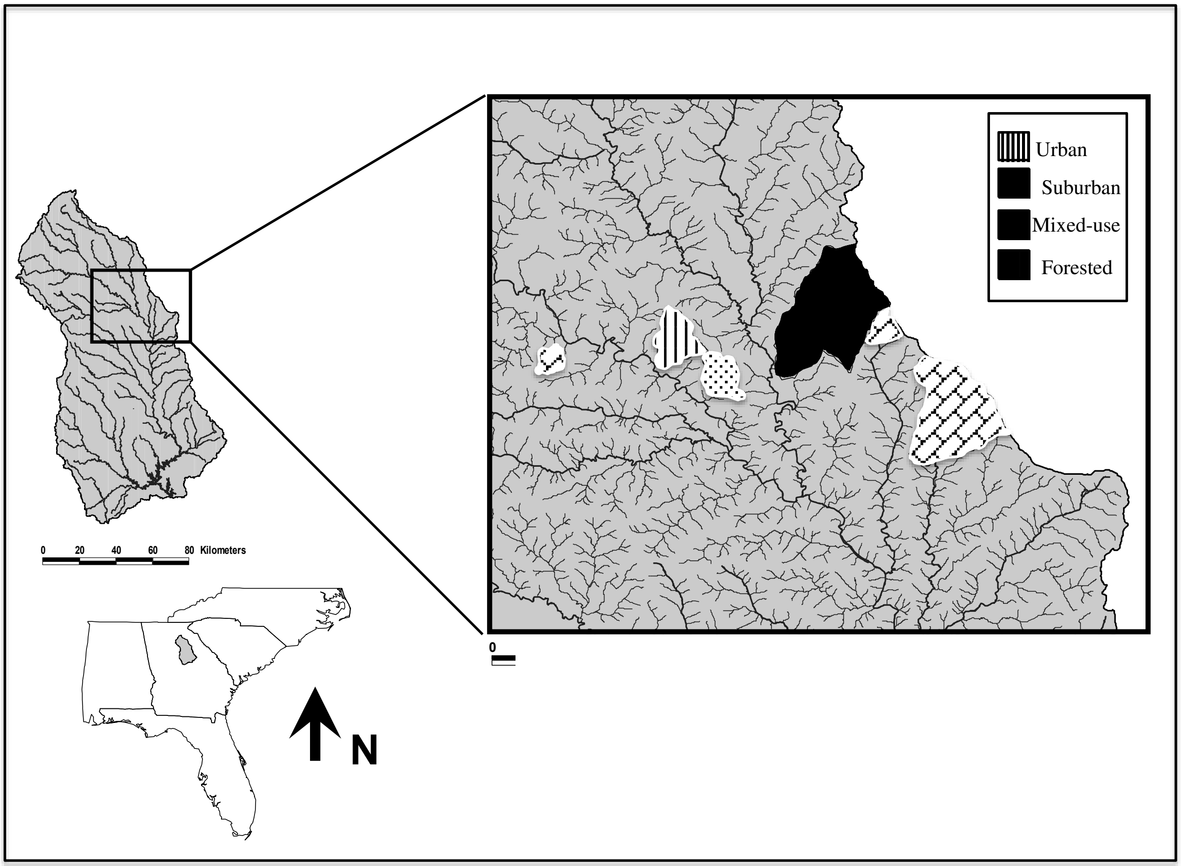
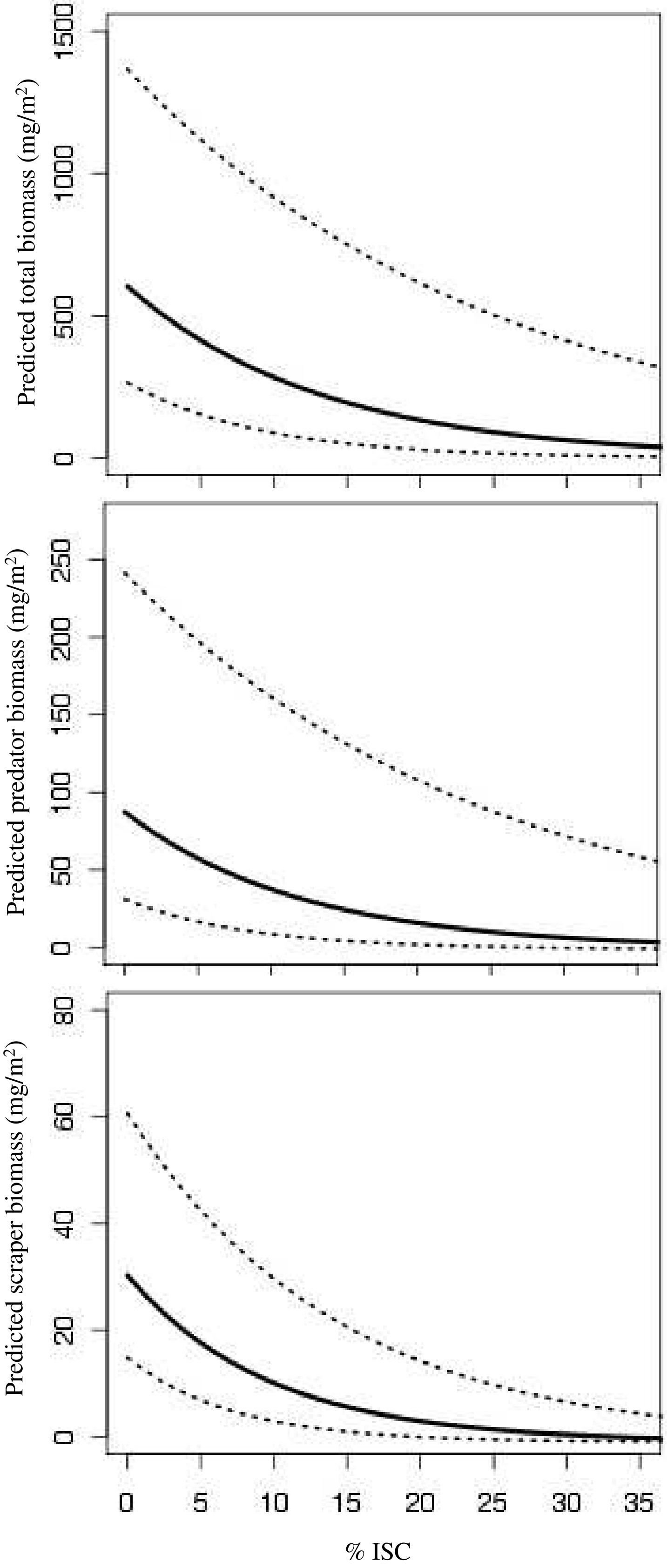


Figure 2.1. Map of the upper Oconee River watershed, Georgia, USA with the 6 watersheds indicated (FOR, forested; MIX, mixed; SUB, suburban; URB, urban).



Predicted total biomass (mg/m²)
1500
1000
500
0
Predicted predator biomass (mg/m²)
250
200
150
100
50
0
Predicted scraper biomass (mg/m²)
80
60
40
20
0
0
5
10
15
20
25
30
35
% ISC

Figure 2.2. Predicted response of (A) total biomass, (B) predator biomass, and (C) scraper biomass to % ISC for best overall models based on Akaike information criteria (AIC). Solid line represents the best model of the relationship between total macroinvertebrate biomass and % ISC. All other variables that were included in the best model are held constant (see Table 2 for parameters and confidence intervals). Dotted lines represent 90% confidence intervals.

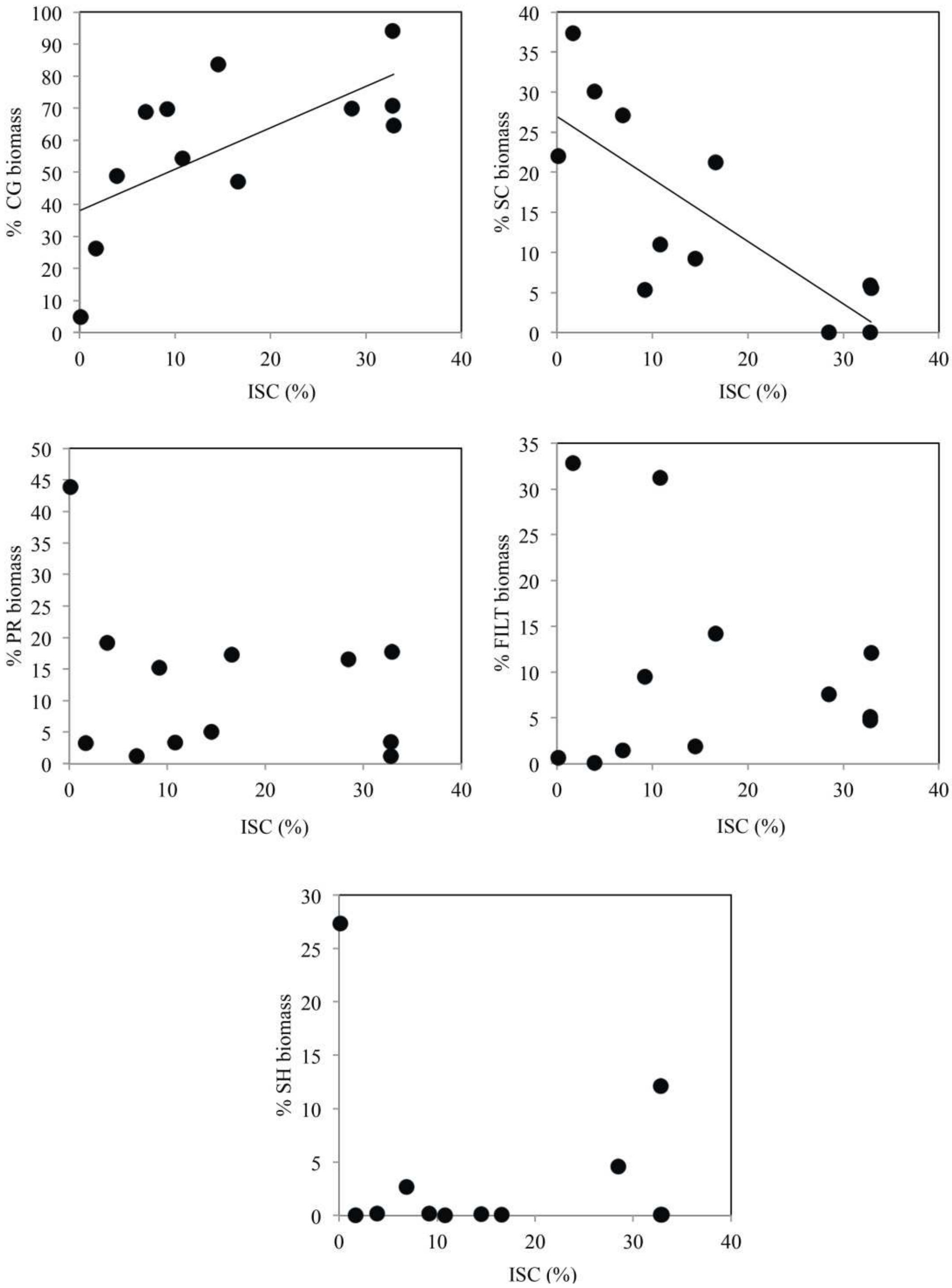


Figure 2.3. Relative contribution of collector-gatherers (CG), scrapers (SC), predators (PR), filterers (FILT) and shredders (SH) to average total biomass at each site vs. % ISC. CG: $r^2 = 0.39$, $p < 0.05$, SC: $r^2 = 0.58$, $p < 0.01$.

Table 2.1. Mean and standard error (in parentheses) for a suite of physical and chemical parameters measured at each sampling site. Abbreviations are: ISC = impervious surface cover, DIN = dissolved inorganic nitrogen, and TSS = total suspended solids.

	ISC	Area	Nutrients (µg/L)				Conductivity	TSS	Turbidity	
Site	(%)	(km^2)	DIN	Total N	PO4	Total P	(µS/cm)	(mg/L)	(NTU)	pH
FOR1	0.1	2,460	598.8 (77.1)	978.9 (117.5)	6.8 (3.9)	37.0 (9.9)	57.9	20.5 (10.9	14.8 (3.4)	6.9 (0.1)
FOR2	1.69	22,258	539.3 (86.3)	867.4 (74.5)	2.3 (1.0)	31.5 (6.0)	40.0	24.1 (14.3)	11.0 (0.9)	6.5 (0.1)
FOR3	3.9	2,512	153.8 (26.3)	239.9 (29.7)	5.4 (1.9)	19.9 (3.8)	39.4	4.6 (0.9)	4.9 (1.0)	7.4 (0.3)
MIX1	10.8	32,423	446.9 (71.2)	748.2 (63.1)	2.7 (1.1)	30.2 (5.7)	55.1	13.8 (2.5)	18.7 (2.5)	7.6 (0.3)
MIX2	9.2	12,763	465.2 (56.8)	805.5 (57.2)	2.1 (1.1)	31.3 (6.9)	52.6	12.8 (2.7)	19.2 (3.4)	7.1 (0.1)
MIX3	6.9	12,128	548.4 (47.8)	816.3 (68.2)	3.2 (1.2)	33.5 (5.2)	51.2	18.2 (3.1)	22.9 (3.1)	6.7 (0.2)
SUB1	16.6	6,922	478.9 (66.5)	628.0 (60.5)	2.6 (1.5)	24.0 (6.2)	68.7	6.1 (1.4)	5.6 (1.5)	7.3 (0.2)
SUB2	14.5	1,146	442.3 (53.8)	603.4 (54.4)	5.1 (2.0)	24.7 (4.9)	66.6	3.7 (0.9)	4.3 (1.3)	7.2 (0.2)
SUB3	28.5	1,077	972.5 (189.0)	1139.8 (177.6)	4.8 (1.3)	19.3 (4.8)	68.5	7.0 (1.8)	4.4 (0.8)	7.0 (0.1)
URB1	32.9	4,902	697.3 (84.7)	926.3 (101.5)	6.0 (2.7)	25.0 (4.4)	99.6	7.8 (5.4)	4.8 (1.5)	7.3 (0.3)
URB2	32.8	1,579	495.4 (123.3)	727.2 (141.4)	5.5 (1.8)	22.5 (5.1)	61.5	4.5 (2.2)	4.2 (1.2)	7.2 (0.3)
URB3	32.8	1,580	714.3 (97.3)	1053.4 (87.0)	3.7 (1.3)	27.7 (6.2)	80.2	3.5 (0.7)	4.8 (1.7)	7.6 (0.2)

Table 2.2. Parameter estimates, confidence intervals and % change for models with the lowest AIC value relating habitat type, % ISC and watershed area to total biomass and FFG biomass. The parameter 'Habitat' refers to changes in biomass in riffles relative to pools (riffle = 1). % Change indicates the expected increase or decrease (-) in macroinvertebrate biomass for each unit change in the predictor variable. An 'N/A' under % Change indicates that confidence intervals for the estimate cross zero and we cannot determine the direction and the magnitude of change in macroinvertebrate biomass.

Parameter	Slope (SE)	10% CI	90% CI	Change
Total biomass				
Intercept	6.40 (0.50)			
Habitat	-0.62 (0.32)	-0.10	-1.15	- 46.3%
% ISC	-0.08 (0.02)	-0.04	-0.11	-7.3%
Collector-gatherer biomass				
Intercept	4.13 (0.37)			
Habitat	-0.63 (0.31)	-0.11	-1.14	-46.50
Filterer-collector biomass				
Intercept	0.12 (0.36)			
Habitat	0.54 (0.36)	1.13	-0.05	N/A
Area	0.11 (0.02)	0.15	0.07	+11.7%
Predator biomass				
Intercept	4.48 (0.62)			
Habitat	-1.20 (0.28)	-0.74	-1.67	-70.0%
%ISC	-0.08 (0.02)	-0.04	-0.12	-7.9%
Area	-0.07 (0.03)	-0.02	-0.12	-6.9%
Scraper biomass				
Intercept	3.44 (0.42)			
% ISC	-0.10 (0.02)	-0.07	-0.14	- 9.9%
Shredder biomass				
Intercept	1.82 (0.53)			
% ISC	-0.05 (0.02)	-0.01	-0.08	- 4.6%
Area	-0.05 (0.03)	-0.01	-0.10	- 5.2%

Table 2.3. Parameter estimates, confidence intervals and % change for models with the lowest AIC value relating habitat type, AFDM and chlorophyll *a* to total biomass and FFG biomass. The parameter 'Habitat' refers to changes in biomass in riffles relative to pools (riffle = 1). Change indicates the expected increase or decrease (-) in macroinvertebrate biomass for each unit change in the predictor variable (chl *a* = 5 mg/m^2, AFDM = 5 g/m^2).

Parameter	Slope (SE)	10% CI	90% CI	Change
Total biomass				
Intercept	5.24 (0.31)			
Habitat	-0.66 (0.31)	-0.15	-1.17	
AFDM	0.35 (0.04)	0.29	0.41	+41.4%
Chl a	0.26 (0.03)	0.21	0.30	+29.1%
Collector-gatherer biomass				
Intercept	4.12 (0.26)			
Habitat	-0.58 (0.28)	-1.04	-0.13	
Chl a	1.32 (0.43)	0.27	0.36	+37.4%
Filterer-collector biomass				
Intercept	1.08 (0.42)			
Habitat	0.52 (0.36)	1.13	-0.05	
Predator biomass				
Intercept	2.51 (0.24)			
Habitat	-1.19 (0.28)	-0.73	-1.65	
AFDM	0.47 (0.04)	0.53	0.42	+61.0%
Scraper biomass				
Intercept	1.78 (0.37)			
AFDM	0.35 (0.05)	0.27	0.44	+42.0%
Shredder biomass				
Intercept	0.62 (0.19)			
AFDM	0.30 (0.02)	0.26	0.34	+35.2%

Table 2.4. Parameter estimates, confidence intervals and % change for models with the lowest AIC value relating (A) stream physical and chemical variables (conductivity, TSS and pH) and (B) water column nutrient concentrations (DIN and SRP) to total macroinvertebrate biomass. The parameter 'Habitat' refers to changes in biomass in riffles relative to pools. % Change indicates the expected increase or decrease (-) in total macroinvertebrate biomass for each unit change in the predictor variable (conductivity = 10 μS/cm, DIN = 50 μg/L).

Parameter	Slope (SE)	10% CI	90% CI	Change
A				
Total biomass				
Intercept	5.25 (0.36)			
Habitat	-0.64 (0.31)	-1.15	-0.13	
Conductivity	-0.39 (0.20)	-0.72	-0.07	-32.5%
B				
Total biomass				
Intercept	5.23 (0.32)			
Habitat	-0.63 (0.31)	-1.14	-0.12	
DIN	-0.22 (0.07)	-0.34	-0.10	-19.9%

Table 2.5. Linear regression models for predicting change in the biomass of sensitive macroinvertebrates and tolerant macroinvertebrates. Macroinvertebrate biomass was log transformed before analysis.

Data	n	Regression model	r^2	p-value
Sensitive biomass	12	(-0.12 * % ISC) + 7.97	0.33	0.029
Tolerant biomass	12	(-0.04 * % ISC) + 8.14	0.25	0.055

CHAPTER 3

THE ROLE OF BIOFILMS IN CARBON AND NUTRIENT STORAGE ACROSS AN URBAN LAND USE GRADIENT[1]

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Abstract

Nutrient retention is an important function in all streams, but it is most important in areas of excess nutrient loading. We assessed the role of biofilms in the storage of nutrients in streams along a watershed impervious surface gradient (% ISC) in the upper Oconee River basin, Georgia, USA. We collected biofilms bimonthly for two years from hard substrates (associated with riffle habitat) and soft substrates (associated with runs and pools). We quantified ash-free dry mass (AFDM), algal biomass (chlorophyll *a*) and biofilm nutrient content (carbon:nitrogen [C:N] and carbon:phosphorus [C:P]), examined patterns on a seasonal basis, and determined relationships to % ISC and other potential drivers (streamwater N and P). AFDM in runs/ pools was roughly an order of magnitude greater than in riffles and was negatively related to % ISC. Algal biomass was greater in riffles than in runs/ pools and was positively related to % ISC during spring months. Biofilm N content was positively related to streamwater N, but biofilm P content was not similarly related to streamwater P. Biofilm nutrient content did not change predictably with % ISC. There was a positive relationship between increased $\delta^{15}N$ in biofilms and streamwater N concentrations, suggesting that anthropogenic sources contributed to greater N loading in these streams. Despite trends for biofilm N content to increase with N availability, when C and N content of biofilms were assessed in terms of reach-scale mass, we found reductions in overall standing crop of C and N as % ISC increased. These results suggest that increased flow disturbance in urban streams may contribute to reduced storage of biofilm-associated nutrients and increased transport of particulate nutrients downstream. Stormwater management strategies to promote the retention of biofilms can likely contribute to increased C and N storage in urban streams.

Introduction

Land use change associated with urbanization often results in higher nutrient sources in urban streams from leaking sewers, failing septic tanks and land application of fertilizers via stormwater inputs (Paul and Meyer 2001, Walsh et al. 2005). The nutrient retention capabilities of different biotic components in small streams are an important factor for watershed managers to consider because upstream retention reduced nutrient transport downstream. Biofilms, a matrix of algae, cyanobacteria, bacteria, fungi, their enzymes and trapped organic materials fixed to the benthic substrate, are one important biotic component of stream ecosystems that contribute to nutrient uptake, retention and transformation in streams (Johnson et al. 2009, Hoellein et al. 2011).

The mass of biofilm material is important in creating greater demand for nutrients and in serving as a nutrient storage pool (Arango et al. 2008). However, different stressors associated with urbanization (e.g., alterations to hydrology and streamwater nutrients) may affect biofilm mass differently. For example, biofilm mass is likely stimulated by increased nutrients, but also may be reduced by altered hydrology in urban streams. Higher percentages of impervious surfaces result in increased runoff of stormwater, which increases the frequency and magnitude of high flow events (Booth and Jackson 1997), resulting in sheer stress and scouring of the channel (Paul and Meyer 2001, Walsh et al. 2005). Such effects may contribute to greater downstream transport of biofilm-derived particulate nutrients (Godwin et al. 2009). Biofilms associated with different habitats may also be differentially affected by watershed urbanization. Hard substrates such as rocks may retain more biofilm than soft substrates such as sand that are more likely to be mobilized during high-flow events.

Additional stressors associated with watershed urbanization can also affect biofilm mass via growth or loss processes. Reduced canopy cover as part of urbanization can increase irradiance, stimulating algal components of biofilms (Catford et al. 2007), which can also be stimulated by streamwater nitrogen and phosphorus concentrations (Dodds et al. 2002). The presence of fewer biofilm consumers in urban systems may also result in higher biofilm biomass (Stepenuck et al. 2002, Riseng et al. 2004). However, hydrologically driven biofilm loss may override stimulatory effects of light, nutrients and fewer consumers. The best models of stream algal biomass include both nutrients and land use (Carr et al. 2005) or nutrients and flood frequency (Biggs 2000).

The nutrient content of biofilms is also important when considering nutrient uptake and retention in streams. Biofilms not only retain nutrients via their mass, but also via capacity for flexibility in nutrient content of their tissues. It has been demonstrated that nutrient content of algal biofilms changes with the type of land use and water column nutrient concentrations (Stelzer and Lamberti 2001, O'Brien and Wehr 2010), suggesting that biofilms may shift their nutrient content relative to supply. It is important to understand the capacity of biofilms to retain nutrients, which may be limited by stoichiometric need.

To understand the mechanisms of nutrient storage and retention in urban streams, we examined biofilm mass and nutrient stoichiometry seasonally in urban, suburban, mixed-use and forested watersheds during two consecutive years of sampling. We quantified ash-free dry mass and algal biomass (chlorophyll *a*) on hard substrates (associated with riffle habitats) and soft substrates (associated with run/pool habitats) along a gradient of percent impervious surface cover (% ISC), a measure of watershed urbanization. We tested whether these measures of biofilm mass differed in relation to % ISC and whether biomass differences were greater in riffle or pool habitats. We tested for effects of streamwater N and P and seasonality in determining patterns in biofilm biomass. We also determined mass of nutrients stored in biofilms and estimated overall standing crop of C and N on a stream reach scale across our gradient in ISC. We used these data to predict how C and N storage changed with % ISC.

We used data on biofilm nutrient content to determine whether nutrient content changed with variation in streamwater nutrient concentrations (dissolved inorganic nitrogen [DIN] and soluble reactive phosphorus [SRP]). To determine whether N in biofilms was derived from anthropogenic sources, we tested whether there was a positive relationship between streamwater N and the $\delta^{15}N$ signature of biofilm. Stable isotopes of N have been used to show qualitative and quantitative relationships between anthropogenic sources of N and the stream benthos (Kaushal et al. 2006, Bannon et al. 2008). Different types of nitrogen sources typically have different $\delta^{15}N$ values, with greater $\delta^{15}N$ values in primary producers retaining nitrogen from sewage and animal waste. Thus, we predicted that biofilms in streams with greater streamwater N would show an increase in $\delta^{15}N$ signature.

Methods

Study Area

Study sites were located in the upper Oconee River Basin in northeast Georgia, USA Sites were chosen in both the North Oconee and Middle Oconee River catchments, which join to form the Oconee River in Athens-Clarke County, GA, USA. We chose twelve sites in six basins with a range of land uses based on the percentage of impervious surface cover. We collected algal biofilms at three tributaries in an urban watershed (URB1, URB2, URB3), three tributaries in a suburban watershed (SUB1, SUB2, SUB3) and three tributaries in a mixed-use watershed (called ‘mixed’; MIX1, MIX2, MIX3). Here, we define a mixed watershed as a watershed that has less impervious cover than the suburban watershed with a combination of light industrial, agricultural and residential land uses and some forest cover. The mixed watershed is slated for an increase in urban and suburban development in the Athens-Clarke County comprehensive land use plan. We also sampled three separate, predominantly forested watersheds (called ‘forest’; FOR1, FOR2, FOR3). All streams were 2nd to 3rd order. The sites represented a gradient of watershed impervious cover (< 5%–35%). Sites were located in the Piedmont physiographic province, characterized by red clay soils and granitic-gneiss bedrock. Urban, suburban and mixed sites were nested (with sites on two tributaries and a downstream site in the same watershed), but watershed area and impervious cover were calculated from the area draining to each sampling site, giving each site independent values.

Impervious surface cover (% ISC) and watershed area were derived from digital aerial images of Athens-Clarke County with a 15.2 cm resolution collected in 2008. Catchment boundaries were delineated using standard GIS procedures using ArcMap (v.10). Percent ISC in each catchment was estimated by overlaying each spatial zone on a land cover map. In this study, we use % ISC to quantify watershed urbanization.

Biofilm sampling methods

Soft and hard substrate biofilms were sampled in three seasons for two years (May 2008–April 2010) at each of the twelve sampling sites. We defined seasons as winter/ spring (January–April; 4 sampling dates, summer (May–July; 3 sampling dates) and fall (September–December; 4 sampling dates). Six transects were randomly chosen on each sampling date, and five subsamples were taken at each transect for a total of six composite samples. Hard substrate samples were taken with a modified Loeb sampler (Loeb 1981) (4.9 cm^2 area), and soft substrate samples were taken with a modified PVC core (9.8 cm^3). In the laboratory, soft substrate samples were elutriated to remove as much sediment as possible. Each hard and soft substrate composite was put into a beaker with a magnetic stir bar to create a slurry. A known volume of slurry was filtered onto two pre-weighed 0.7 µm GFF (Fisher) filters to obtain algal biomass (as chlorophyll *a*) and ash-free dry mass (AFDM). Chlorophyll *a* was extracted in the dark in 90% acetone and determined spectrophotometrically (Wetzel and Likens 2000). Samples for AFDM were dried at 55°C for 48h and weighed, then ashed in a muffle oven at 500°C. Twenty milliliters of the composite slurry was pipetted into a plastic scintillation vial, lyophilized, homogenized, weighed in tin capsules and used to determine %C, %N and isotopic composition (13C/12C and 15N/14N) on a mass spectrometer. Percent phosphorus (%P) of hard substrate biofilms was determined by extraction with Aqua Regia and determined colormetrically (APHA 1998). All biofilm nutrient analyses were conducted in the Odum School of Ecology Analytical Chemistry Laboratory (Athens, GA).

Water Chemistry

Samples were collected for water chemistry monthly at baseflow from June 2009 to May 2010. Samples for NH_4–N, NO_3–N, and PO_4–P were field-filtered through 0.45 µm Whatman nylon-membrane filters into acid-washed polypropylene bottles, returned to the laboratory on ice and frozen until analysis. Samples for total nitrogen (TN) and total phosphorus (TP) were collected from the stream in acid-washed bottles, stored on ice, and frozen until analysis in the University of Georgia Odum School of Ecology Analytical Chemistry Laboratory. Samples for soluble reactive phosphorus (SRP), NH4–N and NO_3–N were measured using continuous flow colorimetry (APHA 1998). We summed NH_4–N and NO_3–N to obtain dissolved inorganic nitrogen (DIN). Nutrient variables were averaged for the three months previous to each sampling date because we expect that stream conditions in the preceding months will impact the biofilm biomass and nutrient content at each sampling date. Total suspended solids (TSS) were measured by filtration (APHA 1998). Conductivity and stream temperature were measured continuously in each reach from February 2009 to May 2010 with a Eureka Multiprobe data sonde (Eureka Manta X2, Austin, TX). A summary of physical and chemical characteristics is listed in Table 3.1.

Scaling

In order to determine the quantity of N stored in algal biofilms on different substrates across a gradient of % ISC, we first quantified the amount of hard and soft substrate at each site. In March 2008, we mapped 50 m reaches at each site and determined the percentage of hard and soft substrates at each 5 m subreach. We analyzed samples of hard and soft substrate biofilms for %C and %N and AFDM for two months, July 2008 and November 2008. We multiplied %C and %N by the amount of AFDM in each sample, averaged values for hard and soft substrates, and then scaled each to the estimates of the amount of hard and soft substrate in each reach.

Data analysis

To test each hypothesis relating response variables (biofilm biomass metrics and biofilm nutrient content) to season, impervious surface cover and streamwater nutrient variables, we used a linear multilevel modeling approach. This approach is beneficial because we sampled sites repeatedly over time, violating the assumption of spatial independence of errors (Gelman and Hill 2007). A multilevel or hierarchical modeling approach accounts for this spatial variability by modeling errors at more than one level of organization. An error term is included at the data level, as well as at the group level (here, we used site). The distributions of all response variables (algal biomass and nutrient content measurements) were checked for normality and heteroscadesity, and were log or log (x+1) transformed if necessary. We standardized all non-factor predictor variables by subtracting the mean and dividing by 2X the standard deviation.

We constructed competing linear models with and without the predictor variable(s) of interest for each hypothesis. We used an information-theoretic approach (Burnham and Anderson 2002) to assess the fit of the competing models (Appendix A). We considered the model with the lowest AIC score to be the best model, but considered all models with AIC weights within 10% of the top model as support of the hypothesis (Appendix A). If the residuals were centered on zero, we determined that the model fit was sufficient. We computed parameter estimates and variances from the best-fitting model. Precision of the parameter estimates was evaluated by examining the 95% confidence limits; if confidence intervals crossed zero, we had less confidence in the direction of the estimate. Each model was fit in using the package lme4 (Bates and Maechler 2009) in R 2.11 (R Development Core Team 2009).

Results

Biofilm biomass

Ash Free Dry Mass (AFDM)—The greatest biofilm mass in these streams was as AFDM in run/ pool habitats (soft substrates) and in sites with lower % ISC (Table 3.1). We found a significant negative relationship between % ISC and AFDM in soft substrates. Model estimates predicted a 3.9% decrease in AFDM on soft substrates for every 1% increase in ISC, suggesting that urbanized streams are generally storing less organic matter on soft substrates (Table 3.1). AFDM on hard substrates was also generally lower as % ISC increased (Figure 3.1A), but confidence intervals on model estimates cross zero (Table 3.3).

Chlorophyll a—Algal biomass varied strongly by season, was weakly associated with changes in % ISC, and was generally greater on hard than soft substrates. Algal biomass on hard substrates was greatest in spring and showed an interactive positive association with % ISC during that season only. Chl *a* on hard substrates was 256% greater in winter/ spring and 27% greater in summer than in fall across all sites (Table 3.3). Algal biomass on soft substrates was also highest in spring, and exhibited both negative and positive relationships with % ISC depending on season (Table 3.3). As with trends on hard substrates, algal biomass on soft substrates increased by 3.2% for every 1% increase in ISC in winter/ spring months. Streamwater nutrient concentrations did not contribute as explanatory variables of algal biomass, with the exception of SRP concentrations, which were counter-intuitively negatively related to chlorophyll *a* on hard substrates (Table 3.3).

AFDM:chlorophyll a—AFDM:chlorophyll *a* varied by substrate, % ISC and season. AFDM:chl *a* was 312% greater on hard substrates than soft substrates, indicating that hard substrates contain more algae (Figure 3.1E and 3.1F). On hard substrates, AFDM:chl *a* decreased by 2.5% for every 1% increase in ISC, suggesting that a greater proportion of AFDM was made up of autotrophic carbon at those sites (Table 3.3; Figure 3.1E). AFDM:chl *a* was 74% lower on hard substrates in the winter/ spring months than in fall (Table 3.3). There was no apparent relationship between soft substrate AFDM:chl *a* and % ISC (Figure 3.1F; Table 3.3).

Biofilm nutrient stoichiometry

Biofilm C:N was lower in the summer and winter/ spring, consistent with greater relative algal contribution to biofilms at that time. Biofilm nitrogen content was related to streamwater nitrogen. C:N decreased by 14.3% for every 100µg/L increase in DIN (Table 3.4). Percent N in algal biofilms was also positively correlated with water column DIN concentrations. There was a negative relationship between C:P and SRP and a weak positive relationship between %P and SRP, but SRP varied little among sites (Table 3.1).

Biofilm $\delta^{15}N$

Hard substrate biofilm δ^{15}N was related to biofilm C:N, and streamwater N, but not to % ISC in the watershed. To reduce variability in biofilm isotopic signatures, we averaged δ^{15}N at each site and found much stronger relationships. δ^{15}N was negatively related to C:N, so as the amount of N in biofilm tissues increased, the δ^{15}N signature of that biofilm increased as well. Biofilm δ^{15}N was not related to streamwater DIN, but we did find a positive relationship between biofilm δ^{15}N and streamwater TN, suggesting some anthropogenic enrichment of biofilms with streamwater N (Figure 3.3A). There was no relationship between δ^{15}N and % ISC, indicating that increased δ^{15}N in biofilms was not related to the intensity of urban land use. A significant, negative relationship between biofilm C:N and δ^{15}N (Figure 3.3B) indicated that biofilm N enrichment may be coming from anthropogenic sources.

Whole stream nutrient storage

A strength of our approach in determining biofilm nutrient content and quantifying biofilm standing stocks is that by combining these measures, we were able to determine the standing stock of nutrients in streams across a gradient of watershed impervious surface cover. When we scaled carbon (C) and nitrogen (N) storage in the standing crop of biofilms based on the substrate types found at each site, results showed that the storage capacity of C and N are both reduced in a non-linear pattern as % ISC increases (Figure 3.4). Model results predicted that for every 5% increase in ISC, there was a 17.5% reduction in the amount of N stored in the biofilms. Similarly, there was a 21.1% reduction in the amount of C stored in biofilms for every 5% increase in ISC.

Discussion

Nutrient retention in urban stream ecosystems

The capacity of urban streams to store nutrients in biofilms was negatively associated with greater watershed impervious cover. We found the mass of biofilms (as AFDM) to be much greater at sites with lower % ISC in the watershed. The negative relationship of AFDM with % ISC suggested that soft substrates contain stored organic matter (as CPOM, FPOM or algae) which is not retained in urban streams, translating into reductions in both C and N storage. Algae have higher C and N content and a greater ability to take up nutrients from streamwater than other components of biofilms such as terrestrially derived organic matter (Dodds et al. 2004). Although hard substrate biofilms in urban streams are more algal as suggested by the general decrease in AFDM:chl *a* across the % ISC gradient, this increase in algal biomass is not enough to shift to greater biofilm nutrient storage in urban streams.

Biofilms do show a capacity to take up available nutrients, but they are relatively constrained. Generally, we found a negative relationship between DIN concentrations and biofilm C:N and a negative (but not significant) relationship between SRP and biofilm C:P. Two recent studies that have examined C:N ratios across a land use gradient did not find a pattern with streamwater nutrients and biofilm stoichiometry. Both Godwin et al. (2009) and O'Brien and Wehr (2010) found C:N ratios in biofilms to be relatively constrained, and both attributed this to a lack of N limitation in any watershed sampled. In experimental channels, Stelzer and Lamberti (2001) found that biofilm C:N could track streamwater nutrient concentrations particularly at moderate to low streamwater N:P (13:1 and 4:1) and not at higher N:P (50:1). There is evidence that the biofilms collected in streams in our study were not able take up N or P relative to the supply of N or P in streamwater, especially at higher streamwater N:P ratios. For example, the N:P ratio of streamwater is much greater than biofilm N:P. Streamwater N:P ranged from 4:1 to 470:1, while biofilm N:P ranged from 5:1 to 66:1, suggesting that biofilm uptake capacity was at or near saturation and that biofilms had limited capacity to take up N relative to supply.

Algal components of biofilms and watershed land use

Although a much greater mass of biofilm materials occured in depositional habitats (i.e., on soft rather than hard substrates) in our streams, algal components of biofilms were greater on hard substrates. In contrast to patterns of decreased overall biofilm mass (as AFDM) with increased % ISC, algal biomass increased with % ISC. Other studies have reported similar findings of increased benthic algal biomass with increased watershed impervious surface cover (O'Brien and Wehr , Dodds et al. 2002, Taylor et al. 2004). While algal biomass was much greater on hard substrates than soft substrates, soft substrate biomass declined rather than increased with % ISC in the watershed, indicating the lower algal biofilm nutrient uptake potential for depositional habitats. Hydrology of urban streams is usually altered by the efficacy of stormwater delivery during rain events, resulting in widening of the channel and increased scouring of the benthic substrate (Booth and Jackson 1997). Soft substrates such as sand and fine gravel have typically greater bed mobility during high flow events (Roy et al. 2003), so it is possible that algae on soft substrates is dislodged during high flow events and moved downstream, resulting in lower storage.

The algal components of biofilms were poor indicators of nutrient loading, as there were weak relationships between algal biomass and dissolved nutrient concentrations although an increase in algal biomass has been linked to increased streamwater nutrient concentrations in other studies (Biggs 2000, Dodds et al. 2002). In our study, the relationships may be weak due to scouring of the substrate (Biggs 1995) and/or variable inputs of nutrients to streams (Taylor et al. 2004). This is important regarding the management of streams for nutrients, because one strategy to monitor ecosystem effects of nutrient loading is to quantify algal biomass (Stevenson et al. 2006).

What were the best indicators of nutrient loading?

In general, streamwater nitrogen is associated with greater N content of biofilms, and there is evidence that this N is derived from anthropogenic sources. Increased streamwater nutrients were not, as we had predicted, stimulating the biomass of biofilms, however. In particular, the $\delta^{15}N$ signature of biofilms was a good indicator of streamwater nutrient concentrations. The negative relationship between biofilm $\delta^{15}N$ and biofilm C:N suggests that biofilms are retaining some anthropogenic N. Higher $\delta^{15}N$ in primary producers and consumers has been shown to be a result of anthropogenic N inputs, including human sewage (Savage and Elmgren 2004, Steffy and Kilham 2004, Kaushal et al. 2006, Bannon and Roman 2008). Cabana and Rasmussen (1996) reported delta $\delta^{15}N$ values of 3.3‰ in primary producers collected from pristine watersheds and 11‰ in watersheds with high anthropogenic N inputs. We found a weak correlation between $\delta^{15}N$ and average streamwater TN concentrations. These results suggest that N enrichment may be coming from anthropogenic sources and that N is incorporated into biofilms. $\delta^{15}N$ did not increase with urbanization, however. The highest mean $\delta^{15}N$ values were actually found in a forested site (FOR3; 10.82‰) and two mixed-use sites (MIX2 and MIX3; 8.96‰, 10.22‰). High biofilm $\delta^{15}N$ in FOR3 may have resulted either from leaking septic tanks (a few houses were adjacent to the sampling reach) or from light cattle farming upstream of the reach. MIX2 and MIX3 were both impacted by a primary treatment wastewater lagoon upstream of both sites. Our results suggest that N is coming from anthropogenic sources and is incorporated into biofilms, but do not point to the specific source or the quantity of N incorporated into the biofilm.

Implications for nutrient storage and uptake

Decreased retention of organic matter in urban streams has implications for the function of these systems in nutrient storage. Hard substrate biofilms were generally more algal, but there was a significant amount of organic matter that was not necessarily algal stored on the soft substrates, and this storage decreased with urbanization. Likely, this organic matter is contributing to heterotrophic nutrient uptake (Dangelo et al. 1991). In a nutrient diffusing substrata experiment, Johnson et al. (2009) found that community respiration by heterotrophs was less nutrient-limited in urban streams than in forested reference streams, implying that heterotrophic nutrient storage is influenced by both greater streamwater nutrient concentrations and the availability of suitable substrates. We suggest that less organic substrate, suggested by reduced as AFDM on soft substrates, is contributing to lower reach-scale nutrient storage (as C and N; Figure 3.4) in this study. Reduced AFDM also has implications for the rate of uptake. Meyer et al. (2005) found longer NH_4–N uptake lengths in watersheds with greater % ISC, implying reduced N uptake; they attributed this to lower FBOM (and associated heterotrophic nutrient uptake) in the sediments. Overall evidence suggests that biofilm nutrient storage is reduced where it is needed most: in urban and suburban streams where anthropogenic nutrient loading is potentially the greatest. We suggest that innovative stormwater management strategies designed to decrease flows and increase the storage of allocthonous and autochthonous materials in urban streams has the potential to increase nutrient retention in these streams.

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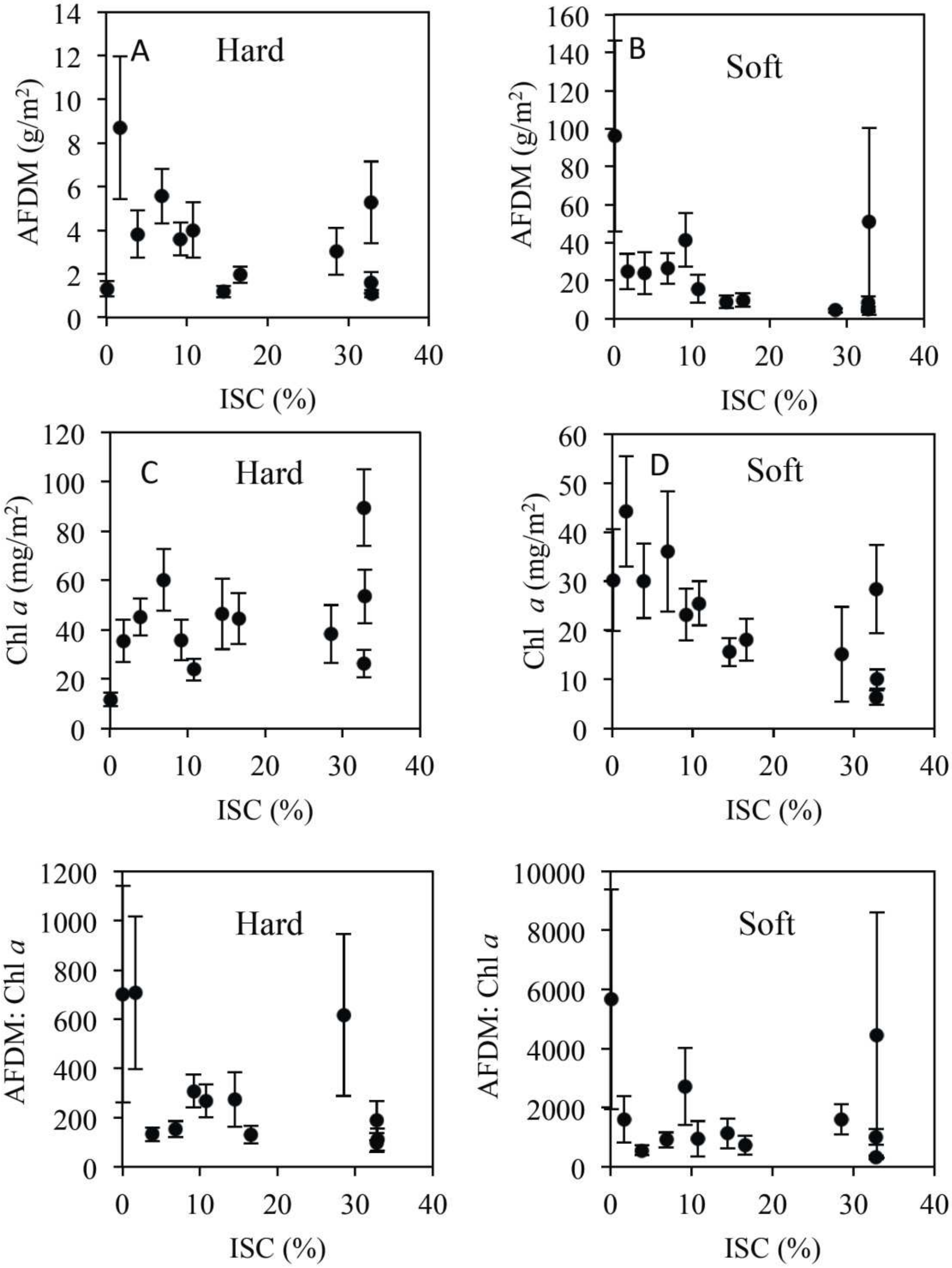


Figure 3.1. Relationships between AFDM (A and B), chlorophyll *a* (C and D), and AFDM:chl *a* (E and F) on hard (rock) and soft (sand) substrates along a gradient of % ISC.

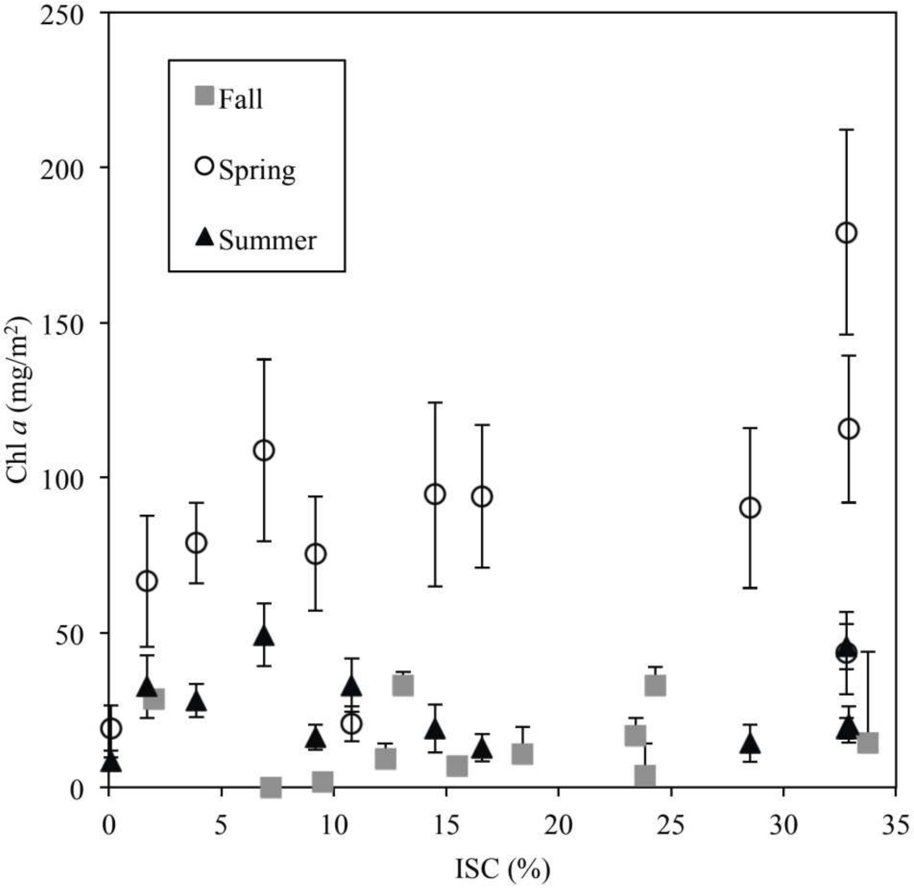
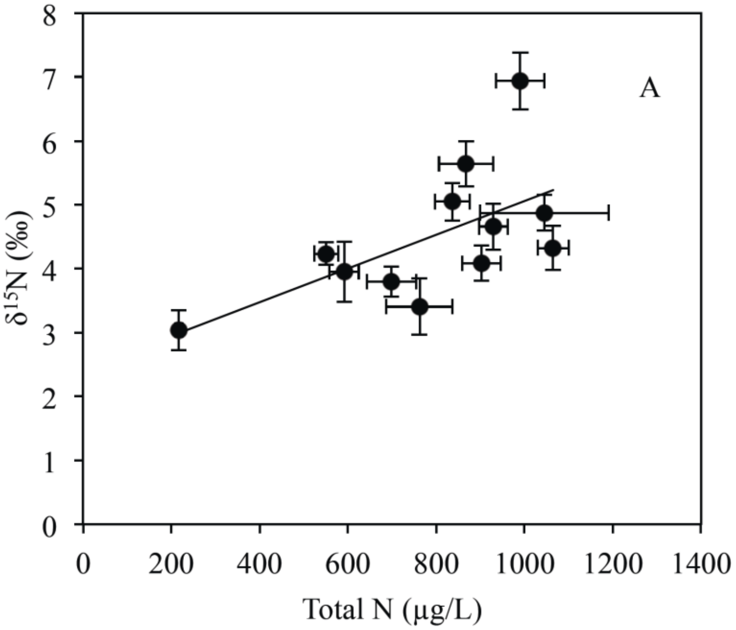


Figure 3.2. Mean algal biofilm biomass (as chl *a*) at each site vs. % ISC in the watershed in Fall (October – December), Summer (April – September), and Winter/ Spring (January – March).



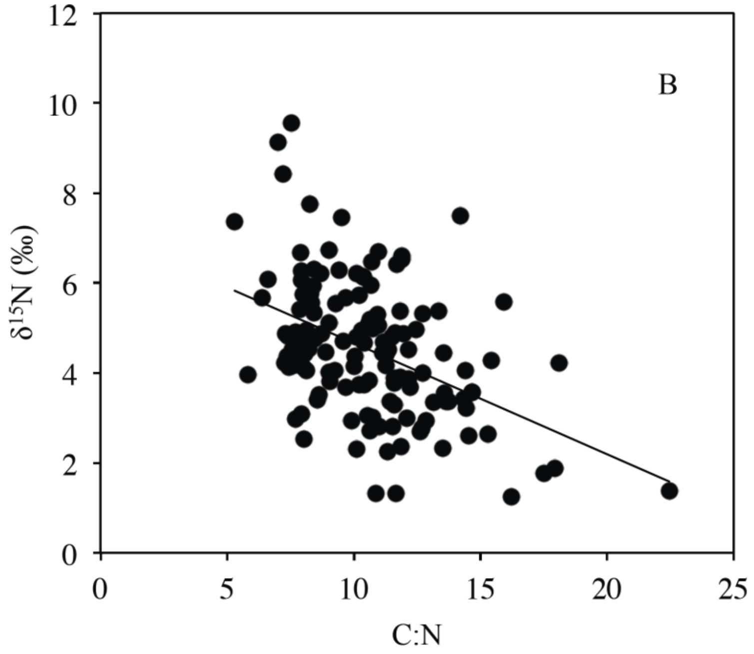


Figure 3.3. Mean biofilm $\delta^{15}N$ on hard substrates vs. mean streamwater TN (A) and biofilm $\delta^{15}N$ on hard substrates vs. biofilm C:N content (B). Regression lines are (A) $\delta^{15}N = 2.64 + 2.41$ TN, ($r^2=0.31$, p=0.03, n=12) and (B) $\delta^{15}N = -0.25 + 7.14$ C:N, ($r^2=0.20$, $p < 0.001$, n=140).

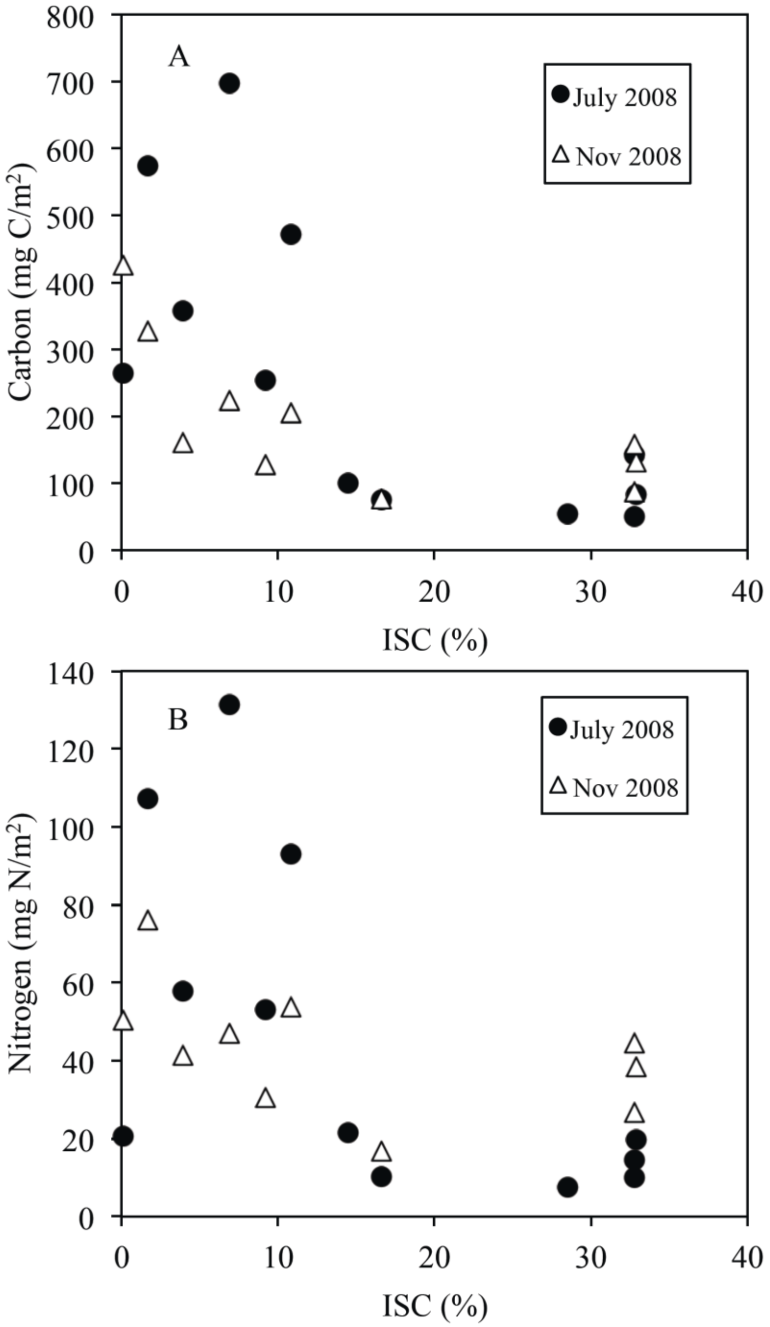


Figure 3.4. Biofilm carbon (A) and nitrogen (B) scaled on the amount of hard (rock) and soft (sand) substrate in each sampling reach. Regressions were fitted to each: log(Carbon (mg C/m^2)) = (– 0.04 * % ISC) + 5.82, (r2=0.49, $p < 0.01$) and log(Nitrogen (mg N/ m^2)) = (– 0.04 * % ISC) + 4.06, (r2=0.30, $p < 0.05$).

Table 3.1. Mean and standard error (in parentheses) for a suite of physical and chemical parameters measured at each sampling site. ISC is impervious surface cover, DIN is dissolved inorganic nitrogen and TSS is total suspended solids.

Site	ISC (%)	Area (km^2)	Nutrients (µg/L)				Conductivity (µS/cm)	TSS (mg/L)	Turbidity (NTU)	pH
			DIN	Total N	PO4	Total P				
FOR1	0.1	2,460	598.8 (77.1)	978.9 (117.5)	6.8 (3.9)	37.0 (9.9)	57.9	20.5 (10.9	14.8 (3.4)	6.9 (0.1)
FOR2	1.69	22,258	539.3 (86.3)	867.4 (74.5)	2.3 (1.0)	31.5 (6.0)	40.0	24.1 (14.3)	11.0 (0.9)	6.5 (0.1)
FOR3	3.9	2,512	153.8 (26.3)	239.9 (29.7)	5.4 (1.9)	19.9 (3.8)	39.4	4.6 (0.9)	4.9 (1.0)	7.4 (0.3)
MIX1	10.8	32,423	446.9 (71.2)	748.2 (63.1)	2.7 (1.1)	30.2 (5.7)	55.1	13.8 (2.5)	18.7 (2.5)	7.6 (0.3)
MIX2	9.2	12,763	465.2 (56.8)	805.5 (57.2)	2.1 (1.1)	31.3 (6.9)	52.6	12.8 (2.7)	19.2 (3.4)	7.1 (0.1)
MIX3	6.9	12,128	548.4 (47.8)	816.3 (68.2)	3.2 (1.2)	33.5 (5.2)	51.2	18.2 (3.1)	22.9 (3.1)	6.7 (0.2)
SUB1	16.6	6,922	478.9 (66.5)	628.0 (60.5)	2.6 (1.5)	24.0 (6.2)	68.7	6.1 (1.4)	5.6 (1.5)	7.3 (0.2)
SUB2	14.5	1,146	442.3 (53.8)	603.4 (54.4)	5.1 (2.0)	24.7 (4.9)	66.6	3.7 (0.9)	4.3 (1.3)	7.2 (0.2)
SUB3	28.5	1,077	972.5 (189.0)	1139.8 (177.6)	4.8 (1.3)	19.3 (4.8)	68.5	7.0 (1.8)	4.4 (0.8)	7.0 (0.1)
URB1	32.9	4,902	697.3 (84.7)	926.3 (101.5)	6.0 (2.7)	25.0 (4.4)	99.6	7.8 (5.4)	4.8 (1.5)	7.3 (0.3)
URB2	32.8	1,579	495.4 (123.3)	727.2 (141.4)	5.5 (1.8)	22.5 (5.1)	61.5	4.5 (2.2)	4.2 (1.2)	7.2 (0.3)
URB3	32.8	1,580	714.3 (97.3)	1053.4 (87.0)	3.7 (1.3)	27.7 (6.2)	80.2	3.5 (0.7)	4.8 (1.7)	7.6 (0.2)

Table 3.2. Means and standard errors (in parentheses) for biofilm mass and nutrient characteristics measured at each sampling site. C:N, C:P and N:P are all molar ratios. Note: C:N and δ^{15}N were measured only in July 2008 and November 2008 on soft substrates, and C:P and N:P were not measured on soft substrates. C:N, C:P and N:P are expressed as molar ratios and δ^{15}N is expressed as ‰. Chlorophyll *a* was measured in mg/m^2 and AFDM was measured in g/m^2.

	Forested			**Mixed -Use**			**Suburban**			**Urban**		
	FOR1	FOR2	FOR3	MIX1	MIX2	MIX3	SUB1	SUB2	SUB3	URB1	URB2	URB3
Hard substrate												
Chlorophyll *a*												
Winter/Spring	16.4 (5.9)	55.3 (17.5)	70.8 (12.4)	17.8 (12.4)	66.5 (16.8)	96.1 (24.1)	76.6 (12.5)	74.6 (24.9)	72.8 (22.5)	94.9 (27.9)	35.2 (14.2)	141.1 (38.8)
Summer	9.6 (1.8)	40.5 (13.8)	25.1 (7.2)	44.0 (9.6)	16.9 (4.8)	49.0 (12.2)	14.7 (5.7)	22.6 (10.0)	18.4 (7.8)	25.2 (7.3)	20.4 (4.0)	46.2 (8.5)
Fall	7.2 (2.0)	9.5 (2.7)	23.8 (10.2)	18.4 (8.8)	12.3 (5.0)	13.4 (3.3)	23.4 (5.7)	33.8 (29.2)	1.6 (0.7)	24.3 (6.0)	13.1 (4.3)	47.7 (8.9)
AFDM	1.3 (0.34)	8.7 (3.3)	3.8 (1.1)	4.0 (1.3)	3.6 (.07)	5.5 (1.2)	2.0 (.04)	1.1 (0.3)	5.1 (2.3)	1.1 (0.2)	1.6 (0.5)	5.3 (1.9)
C:N	11.9 (0.8)	8.4 (0.4)	10.9 (0.6)	10.4 (1.1)	11.5 (0.4)	11.4 (0.4)	9.8 (0.7)	9.9 (0.5)	8.0 (0.4)	9.0 (0.5)	11.5 (0.8)	10.8 (0.6)
C:P	195.2 (19.5)	145.5 (20.7)	218.2 (40.4)	115.2 (8.6)	275.6 (30.2)	311.9 (66.0)	159.7 (35.1)	153.7 (17.7)	124.4 (12.7)	171.5 (29.5)	164.7 (19.3)	165.4 (19.3)
N:P	17.7 (12.0)	17.2 (2.3)	19.8 (3.4)	11.5 (1.8)	24.5 (2.8)	26.8 (5.1)	16.2 (4.1)	14.8 (1.2)	15.9 (1.3)	20.1 (4.2)	15.4 (2.6)	15.4 (2.0)
δ^{15}N	3.0 (0.3)	6.9 (0.4)	4.1 (0.3)	3.4 (0.4)	5.1 (0.2)	5.6 (0.4)	4.0 (0.5)	4.2 (0.2)	4.9 (0.3)	4.7 (0.4)	3.8 (0.2)	4.3 (0.3)
Soft Substrate												
Chlorophyll *a*												
Winter/Spring	40.9 (24.1)	34.7 (10.1)	32.4 (13.2)	27.3 (7.9)	41.6 (11.0)	54.4 (29.1)	30.5 (9.6)	27.4 (4.7)	10.6 (2.8)	16.8 (4.6)	12.4 (3.2)	55.5 (20.5)
Summer	38.0 (11.3)	98.1 (33.9)	24.7 (7.6)	32.3 (7.5)	16.9 (6.3)	30.2 (7.2)	14.4 (3.8)	11.4 (3.4)	44.1 (39.5)	10.2 (2.5)	4.7 (1.2)	17.5 (8.8)
Fall	9.4 (4.2)	14.2 (5.9)	27.3 (14.7)	17.4 (1.2)	6.6 (0.4)	18.9 (3.0)	6.4 (1.2)	2.4 (0.7)	0.8 (0.2)	4.3 (1.2)	0.9 (0.4)	6.5 (3.0)
AFDM	96.2 (50.4)	24.8 (9.1)	23.9 (10.8)	15.6 (7.4)	41.5 (14.0)	26.3 (8.1)	9.7 (3.5)	8.8 (3.2)	4.4 (1.1)	51.2 (49.0)	8.2 (3.5)	4.8 (1.4)
C:N	20.1 (1.0)	16.9 (1.0)	16.3 (1.4)	16.0 (1.1)	15.9 (1.0)	17.5 (0.8)	21.6 (2.1)	22.3 (0.4)	26.5 (2.5)	17.5 (1.8)	16.7 (2.8)	15.7 (1.9)
C:P												
N:P												
δ^{15}N	-0.5 (0.29)	4.2 (0.5)	1.8 (0.3)	3.3 (0.3)	4.5 (0.6)	5.6 (0.5)	1.5 (0.3)	1.9 (0.2)	2.9 (0.4)	2.8 (0.2)	2.0 (0.2)	2.3 (0.2)

Table 3.3. Parameter estimates and standard errors (SE) for supported models based on Akaike's Information Criteria (AIC) for biofilm AFDM, chl *a* and AFDM:chl *a.* All models with AIC weights within 10% of the top model for each set of candidate models are represented. AIC scores and model weights are listed in Appendix 3.1. Parameter estimates with confidence intervals that do not cross zero are in **bold**.

	Model	*Predictor*	*Estimate*	*SE*	*Upper CI*	*Lower CI*	*Change*
	AFDM						
	Hard substrate						
A	ISC + season	ISC	-0.010	0.01	0.002	-0.023	
		Spring	0.129	0.09	0.300	-0.041	
		Summer	**0.606**	**0.09**	**0.775**	**0.437**	**+ 83.3%**
	season	Spring	0.131	0.09	0.301	-0.040	
		Summer	**0.604**	**0.09**	**0.773**	**0.435**	**+ 82.9%**
	Soft substrate						
B.	ISC + season	**ISC**	**-0.039**	**0.01**	**-0.025**	**-0.055**	**- 3.9%**
		Spring	0.060	0.13	0.320	-0.199	
		Summer	**1.050**	**0.13**	**1.306**	**0.793**	**+ 185.7%**
Chlorophyll *a*							
	Hard substrate						
C.	season	**Spring**	**1.528**	**0.13**	**1.786**	**1.270**	**+ 256.1%**
		Summer	**0.503**	**0.13**	**0.763**	**0.243**	**+ 27.0%**
	ISC + season	ISC	0.012	0.01	0.037	-0.012	
		Spring	**1.528**	**0.13**	**1.785**	**1.270**	**+ 360.7%**
		Summer	**0.502**	**0.13**	**0.762**	**0.242**	**+ 65.2%**
D.	ISC + season*ISC + season	ISC	-0.033	0.02	0.043	-0.011	
		ISC*Spring	**0.034**	**0.02**	**0.057**	**0.011**	**+ 3.2%**
		ISC*Summer	-0.006	0.02	0.014	-0.026	
		Spring	**1.003**	**0.14**	**1.968**	**1.439**	**+ 449.4%**
		Summer	**1.242**	**0.12**	**0.843**	**0.371**	**+ 83.5%**
E.	DIN + SRP + season	DIN	0.020	0.02	0.064	-0.023	
		SRP	**-0.080**	**0.02**	**-0.032**	**-0.128**	**- 7.7%**
		Spring	**1.551**	**0.19**	**1.924**	**1.179**	**+ 371.8%**
		Summer	**0.923**	**0.26**	**1.433**	**0.414**	**+ 151.8%**

Table 3, *cont*.

	Model	*Predictor*	*Estimate*	*SE*	*Upper CI*	*Lower CI*	*Change*
	Chlorophyll *a*, cont.						
	Soft substrate						
F.	*season + isc*	**Spring**	**1.020**	**0.14**	**1.584**	**1.069**	**+ 276.6%**
		Summer	**1.245**	**0.13**	**1.253**	**0.738**	**+ 170.6%**
		ISC	**-0.027**	**0.01**	**-0.015**	**-0.040**	**- 2.8%**
G.	ISC + season*ISC	**ISC**	**-0.033**	**0.02**	**-0.016**	**-0.051**	**- 1.61%**
	+ season	**ISC*Spring**	**0.034**	**0.02**	**0.057**	**0.011**	**+ 3.2%**
		ISC*Summer	-0.006	0.02	0.014	-0.026	
		Spring	**1.003**	**0.14**	**1.278**	**0.727**	**+ 449.4%**
		Summer	**1.242**	**0.12**	**1.483**	**1.001**	**+ 83.5%**
H.	*season*	**Spring**	**2.100**	**0.17**	**2.007**	**1.257**	**+ 411.5%**
		Summer	**2.407**	**0.21**	**1.684**	**0.713**	**+ 231.5%**
	DIN + SRP + season	DIN	0.007	0.02	0.043	-0.029	
		SRP	0.002	0.00	0.006	-0.003	
		Spring	**2.089**	**0.17**	**2.421**	**1.757**	**+ 707.4%**
		Summer	**2.356**	**0.22**	**2.785**	**1.928**	**+ 955.4%**
	AFDM : Chlorophyll *a*						
	Hard substrate						
I	ISC + season	**ISC**	**-0.029**	**0.01**	**-0.012**	**-0.046**	**- 2.9%**
		Spring	**-1.347**	**0.15**	**-1.054**	**-1.640**	**- 74.0%**
		Summer	**0.311**	**0.15**	**0.603**	**0.020**	**+ 36.5%**
	Soft substrate						
J.	season	**Spring**	**-1.376**	**0.16**	**-1.059**	**-1.692**	**- 74.7%**
		Summer	0.180	0.16	0.493	-0.133	
	ISC + season	ISC	-0.016	0.01	0.007	-0.039	
		Spring	**-1.376**	**0.16**	**-1.059**	**-1.692**	**- 74.7%**
		Summer	0.182	0.16	0.495	-0.131	

Note: Fall is zero. Seasonal effects are interpreted relative to the fall season.

Table 3.4. Parameter estimates and standard errors (SE) for supported models based on Akaike's Information Criteria (AIC) for biofilm %N, %P, C:N and C:P. All models with AIC weights within 10% of the top model for each set of candidate models are represented. AIC scores and model weights are listed in Appendix A. Parameter estimates with confidence intervals that do not cross zero are in **bold**.

	Model	*Predictor*	*Estimate*	*SE*	*Upper CI*	*Lower CI*	*Change*
	C:N						
	Hard substrate						
A.	DIN + season	**DIN**	**-0.155**	**0.04**	**-0.068**	**-0.241**	**- 14.3%**
		Spring	**-1.801**	**0.42**	**-0.534**	**-2.144**	**- 77.7%**
		Summer	**1.609**	**0.53**	**2.649**	**0.568**	**+ 400%**
	% N						
	Hard substrate						
B.	DIN + season	**DIN**	**0.012**	**0.00**	**0.020**	**0.006**	**+ 1.3%**
		Spring	**0.099**	**0.05**	**0.177**	**0.022**	**+ 10.4%**
		Summer	-0.009	0.05	0.087	-0.106	
	C:P						
	Hard substrate						
C.	season	Spring	-0.100	0.078	0.053	-0.253	
		Summer	**-0.332**	**0.097**	**-0.142**	**-0.523**	**- 28.3%**
	SRP + season	**SRP**	**-0.021**	**0.012**	**-0.0002**	**-0.040**	**-2.1%**
		Spring	-0.123	0.080	0.031	-0.276	
		Summer	**-0.402**	**0.102**	**-0.201**	**-0.604**	**- 33.1%**
	% P						
	Hard substrate						
D.	season	**Spring**	**0.020**	**0.008**	**0.035**	**0.004**	**+ 2.0%**
		Summer	**0.047**	**0.010**	**0.067**	**0.028**	**+ 4.8%**
	SRP + season	SRP	0.008	0.008	0.003	-0.001	
		Spring	**0.021**	**0.008**	**0.037**	**0.005**	**+ 2.1%**
		Summer	**0.051**	**0.011**	**0.072**	**0.030**	**+ 5.2%**

Note: Fall is zero. Seasonal effects are interpreted relative to the fall season.

CHAPTER 4

EFFECTS OF URBANIZATION ON WOOD BREAKDOWN RATES IN SOUTHEASTERN PIEDMONT STREAMS

Introduction

In order to manage stream ecosystems impacted by land use change, we need to have knowledge of how both ecosystem structural and functional elements change in response to anthropogenic stressors (Young et al. 2008, Wenger et al. 2009). Assessment of both ecosystem structure and function is important because stressors associated with land use change can alter structure, function, or both (Bunn et al. 1999, Gessner and Chauvet 2002). The breakdown rate of organic matter is one measure of function that has been widely used to assess the impacts of land use change on stream ecosystems. Allocthonous organic matter is an essential source of energy in small streams, supporting higher order consumers (Vannote et al. 1980). In small streams in urban areas, organic matter may remain a major source of energy, but alteration of riparian areas, eutrophication, abrasive flows and loading of metals and toxins may alter the availability of organic matter as a food source to stream consumers.

Measuring leaf breakdown is the most common method used to study organic matter processing in streams across a gradient of land use (Gessner and Chauvet 2002, Woodcock and Huryn 2005, Chadwick et al. 2006, Paul et al. 2006, Imberger et al. 2008). Breakdown rates of leaves across an urban gradient have attributed increases to abrasive flows (Chadwick et al. 2008, Paul et al. 2006) and greater microbial activity stimulated by nutrients (Imberger et al.

2008). Reductions in leaf breakdown rates have been connected with metal pollution (Niyogi et al. 2001, Sridhar et al. 2001) and lower abundances of shredding macroinvertebrates (Huryn et al. 2002).

Wood is another important carbon resource in streams, supplying habitat and substrate for stream biota (Wallace et al. 1999). Wood also provides stability as a food source because it is slow to decompose due to a high concentration of lignin and lower concentration of nitrogen than other sources of organic carbon in streams. Wood breakdown has been used to assess the impacts of increased nutrients (Tank and Dodds 2003, Gulis et al. 2004) and the impacts of agricultural land use (McTammany et al. 2008) on ecosystem functions. No known studies of wood breakdown have been conducted in urban streams (Wenger et al. 2009). In urban streams, woody debris such as rooted vegetation in banks has been shown to be a hotspot for invertebrates and fishes, indicating that wood is likely an important resource in impacted streams (Roy et al. 2003).

Goals of this study were to examine changes in wood breakdown rates across a gradient of land use and to examine patterns in the variability of breakdown rates. Variability has long been suggested as a characteristic of perturbed ecosystems (Odum et al. 1979), so considering variability as a response to disturbance may help us to predict disturbance-driven ecosystem-level changes (Fraterrigo and Rusak 2008). By examining variability in a response, there is the potential to capture differences obscured by looking only at mean responses (Cottingham et al. 2000). Analyzing the variability in responses in addition to mean responses potentially provides another method to quantify disturbance-driven change. Multiple stressors such as those that are associated with watershed urbanization may cause increases and/ or decreases in breakdown rates across gradients of disturbance (Young et al. 2008), resulting in variability in ecosystem responses. Variability may be a good indicator that function is altered even if a specific stressor or suite of stressors cannot be identified.

The objectives of this study were to (1) determine if rates of wood breakdown and associated microbial respiration were different in urban and suburban streams compared to forested streams, (2) identify specific stressors that contributed to rates of wood breakdown, and (3) examine patterns in the variability of wood breakdown rates across a gradient of urban land use. We also examined variability in three other studies that have quantified carbon breakdown rates as a measure of ecosystem function across land use gradients.

Methods

Study Area

Study sites were located in the upper Oconee River Basin in northeast Georgia, USA. Sites were chosen in both the North and Middle Oconee River catchments, which join to form the Oconee River in Athens-Clarke County, GA, USA We choose ten sites in five basins with a range of land uses based on the amount of impervious cover and designated a representative 50 m reach in each stream. We classified sites as urban (URB1, URB3), suburban (SUB1, SUB2, SUB3) and mixed-use (called ‘mixed’; MIX1, MIX2, MIX3). Here, we are defining a mixed watershed as a watershed that has less impervious cover than the suburban watershed, with combinations of light industrial and residential land uses and some forest. The mixed watershed is also slated for an increase in urban development in the Athens-Clarke County comprehensive land use plan. We also used two separate, predominantly forested watersheds (called ‘forest’; FOR1, FOR2). All streams are 2nd to 3rd order. The catchments are located in the Piedmont physiographic province, characterized by southern red clay soils and granitic-gneiss bedrock.

We derived impervious surface cover (% ISC) and watershed area from digital aerial images of Athens-Clarke County with a 15.2-cm resolution collected in 2008. Catchment boundaries were delineated using standard GIS procedures using ArcMap (v.10). We estimated % ISC in each catchment by overlaying each spatial zone on a land cover map. Land use classifications were based on watershed % ISC (FOR, < 5%; MIX, 6 –14%; SUB, 15 – 29%; URB, >30%).

Physical/chemical variables

We collected samples for water chemistry monthly at baseflow from June 2009 to May 2010 in order to characterize stream conditions. Samples for NH_4–N, NO_3–N, and PO_4–P were field-filtered through 0.45 µm Whatman nylon membrane filters into acid-washed polypropylene bottles, returned to the laboratory on ice and frozen until analysis in the University of Georgia Odum School of Ecology Analytical Chemistry Laboratory. Samples for SRP and NO_3 were measured using a continuous flow colorimeter (APHA 1998). Dissolved inorganic nitrogen was calculated by summing NH_4–N and NO_3–N. We use mean nutrient values for each site to characterize stream nutrient concentrations. Conductivity and stream temperature were measured continuously in each reach from February 2009 to May 2010 with a Eureka Multiprobe data sonde (Eureka Manta X2, Austin, TX). A summary of these variables can be found in Table 4.1.

Wood breakdown

We cut white oak wood veneer into 150 x 25 x 0.5 mm strips and pre-weighed them. We fixed eight veneers horizontally to a 50 cm piece of gutter mesh with plastic cable ties (henceforth called rafts). In November 2008, we placed five rafts randomly in riffles within each 50m reach with gutter nails and tethered to the nearest bank with nylon cord. Two veneers from each raft were collected at days 60, 120, 180, and 220.

To calculate the mass loss of veneers, we cut five 1 cm^2 pieces from each veneer for respiration and the remainder were placed in a metal tin, dried at 60°C, weighed, combusted at 500°C, and reweighed to obtain AFDM. We added the larger veneer piece to the five 1 cm^2 pieces used to measure microbial respiration and calculated breakdown rate (k) for whole veneers per day. Breakdown rate (k) was calculated for each site by regressing the percent AFDM remaining against time.

Microbial respiration

We measured microbial respiration on days 60, 120 and 220 by placing the five 1 cm^2 pieces in a 26 ml glass respiration chamber in the laboratory. The chamber was placed in a water bath at ambient reference stream temperature and covered with foil to prevent any autotrophic respiration. We recorded dissolved oxygen levels at 5-minute intervals over 30 minutes using a YSI 5100 dissolved oxygen meter equipped with a stirrer. We placed the veneer pieces in metal tins, dried them at 60°C, weighed them, combusted them at 500°C and weighed them again to obtain ash-free dry mass (AFDM). The rate of respiration was calculated as the slope of oxygen concentrations versus time and the AFDM of the veneer pieces.

Quantifying abrasion

During the veneer incubation in January 2009, we placed five pre-weighed 50 x 50 x 25 mm CSR Hebelt Thermoblocks attached to gutter mesh (autoclaved lightweight aerated concrete; Xella Aircrete North America Inc., Atlanta, Georgia, USA; henceforth called abrasion blocks) downstream of the wood veneers in each stream (Webb et al. 2006). We removed abrasion blocks from the stream after 60 days, rinsed them, and dried them at 60°C for 72 hours. We re-weighed abrasion blocks and estimated the abrasive force as the percent mass loss of abrasion blocks over 69 days (Webb et al. 2006).

Data analysis

We used an analysis of variance (ANOVA) to assess differences in wood breakdown rates across land use types. We used Tukey's test to determine which land use types differed in their breakdown rates. Due to the low number of sample units (sites; n=10), we used simple linear regressions to determine which independent variables (physical abrasion, streamwater DIN or conductivity) were the most likely drivers of altered breakdown rates in streams and to determine whether microbial respiration rates were correlated to wood breakdown rates. Soluble reactive phosphorus (SRP) did not vary much between sites, so it was not included in regression models. ANOVA models and associated post-hoc tests were conducted in SAS version 9.2 (SAS Institute Inc., Cary, NC, USA), and regressions were all conducted in R (R 2.10.1, R Development Core Team 2010). To measure the amount of variability between sites, we measured the coefficient of variation (CV; standard deviation/mean) for all sites within a land use class.

Results

Wood breakdown rates

Wood veneer breakdown rates ranged from 0.00212 k^{-1} day (URB3) to 0.00618 k^{-1} day (URB 1) and differed significantly among land use classes ($F = 5.99$, $p < 0.0001$). Suburban streams had significantly higher breakdown rates than forested streams (Figure 4.1). Overall, wood breakdown in urban streams was greater than in forested and mix-use streams, but mean breakdown rates were not significantly different due to a large amount of variability among urban sites (Figure 4.1).

Breakdown rates and microbial respiration

Microbial respiration on wood veneer biofilms increased over time. Average respiration across all sites was 0.05 mg O_2/ g AFDM/ hr on Day 60 and 0.22 mg O_2/ g AFDM/ hr on Day 220. Respiration was not significantly correlated with breakdown rates on days 60, 120 or 220, although there was a general positive relationship between respiration and breakdown rates on Day 220 (Table 4.1).

Relationships with physical and chemical drivers

There were no significant relationships between wood breakdown rate (k^{-1} day) and streamwater nutrients (DIN and SRP), physical abrasion, or streamwater conductivity (Table 4.1). In general, slopes of all regressions were positive, suggesting the potential that these stressors could be increasing wood breakdown rates. Wood breakdown rates, respiration rates and physical and chemical variables by site are listed in Table 4.2.

Variability in wood breakdown rates

We found greater variability (CV = 69%, n = 2) among our urban sites than in any other of the land use classes. Both urban sites (URB1 and URB3) represented the highest and the lowest breakdown rates among the 10 sites in this study (Figure 4.2), resulting in high CV for the urban land use class. Forested sites had the lowest CV (25.9%, n = 3).

Discussion

Wood breakdown rates and relationships with physical and chemical drivers

Wood breakdown rates in this study are within the range reported for anthropogenically altered streams (Tank and Webster 1998, Gulis et al. 2004, McTammany et al. 2008). Gulis et al. (2004) found a six-fold increase in wood breakdown on oak wood veneers incubated in streams experimentally enriched with nitrogen and phosphorus. In contrast, McTammany et al. (2008)

did not find any significant differences along an agricultural land use gradient, but in general, breakdown rates were faster in watersheds with low or moderate levels of agriculture than forested or heavily agricultural watersheds. In our study, wood breakdown was generally faster in urban and suburban streams than in forested and mixed-use streams, suggesting that physical, chemical or biological variables are increasing breakdown rates.

Although we found significant differences in breakdown rates among land use classes, we did not find any specific physical or chemical variables that explained differences in wood breakdown rates across sites. Studies have found that both nutrients (Chadwick et al. 2006, Imberger et al. 2008) and physical abrasion (Paul et al. 2006) increase leaf breakdown rates, and metals (Al, Cd, Cu, Fe, Mg, Mn, Ni, Pb, and Zn) decrease breakdown rates (Chadwick et al. 2006). There are several explanations for why we could not elucidate differences in breakdown rates. First, it is possible that there were compensatory effects altering wood breakdown and we did not have any explanatory power to tease out the effects of multiple stressors due to our small sample size (n=10). Next, we may have failed to quantify certain stressors that were important in determining breakdown rates across land use classes. Finally, altered hydrology is also a known stressor (Paul et al. 2006), and it is possible that streamflow was playing a larger role in wood breakdown in our study than we were able to detect. We attempted to quantify that effect via abrasion blocks (Webb et al. 2006, Imberger et al. 2008), but the method might not have captured changes in hydrology that were impacting wood fragmentation and breakdown.

In addition, we did not see any relationship between wood breakdown rates and microbial respiration as we had predicted. This lack of association could be due to burial of the wood veneer rafts in some streams. In several cases, especially in the more disturbed streams, veneers were covered by sediment, even though they were placed in flowing areas to deter sediment accumulation (JLS, pers obs.). In one case, after a storm event, veneers were buried under 10 cm of sediment. This type of disturbance may have increased wood fragmentation and prevented the buildup of a substantial microbial biofilm, decoupling the predicted relationship between microbial respiration and breakdown rate. In fact, McTammany et al. (2008) found a negative correlation between microbial respiration and the amounts of inorganic sediment that accumulated on wood veneers in streams along an agricultural land use gradient.

Variability in wood breakdown rates

Although we only had two urban sites in our design, these sites had the fastest and the slowest wood breakdown rates, resulting in no significant differences in mean breakdown rates between the urban land use class and the other land use classes (Figure 4.1) and high CVs in the urban land use class (Figure 4.2). It has been suggested that increased variability indicates an ecosystem level disturbance that might not be detected in the mean response (Fraterrigo and Rusak 2008). To investigate further if this pattern of increased variability in breakdown rates was characteristic of urban streams, we examined three other studies that measured organic matter breakdown (as leaves) across land use classes that matched our study (Chadwick et al. 2006, Paul et al. 2006, Imberger et al. 2008) (Table 4.3).

Each study documented increased breakdown rates in urban streams, but cited different drivers that altered breakdown. Chadwick et al. (2006) found increased leaf breakdown rates in watersheds with levels of % ISC between 20% and 40% and attributed them to increased streamwater nitrogen and phosphorus concentrations. Levels of % ISC greater then 40% resulted in lower breakdown due to metal pollution. Imberger et al. (2008) documented increased leaf breakdown in urban streams due to more microbial activity on leaves, mediated by increased streamwater nitrogen and phosphorus. Paul et al. (2006) reported increased breakdown in urban streams in Atlanta, Georgia, with abrasive flows as the primary driver.

In the examples cited above, there was greater variability in breakdown rates among urban sites than forested sites (Table 4.3), consistent with the greatest variability in wood breakdown being in urban streams in our study. Although the studies examined here showed a general increase in breakdown rates with urbanization, multiple stressors acting on any one measure of ecosystem function may be causing an increase or decrease in carbon decomposition rates, resulting in increased variability. For example, stressors such as nutrients (Chadwick et al. 2006, Imberger et al. 2008) and physical abrasion (Paul et al. 2006) might increase leaf breakdown rates at some sites, while metals (Chadwick et al. 2006) or lower shredder abundance (Huryn et al. 2002) might decrease breakdown rates at other sites. Ecosystem functional metrics that have been proposed to assess ecosystems (Young et al. 2008) integrate multiple ecosystem components and multiple drivers of change. Since functional metrics are integrative, there is greater potential that they might increase or decrease with urban land use, thus having the net effect of increased variability. We suggest that variability may be a hallmark of disturbance due to the multiple stressors that streams experience with increases in urbanization.

Conclusions and recommendation for future studies

We found generally faster breakdown rates in streams with urban or suburban land use compared to forested streams. Data from other studies suggested that as watershed ISC increased, systems became less retentive of organic matter (Chadwick et al. 2006, Paul et al. 2006, Imberger et al. 2008). In Chapter 2, we found an association between retained organic matter and macroinvertebrate biomass, indicating the critical importance of organic matter in supporting biota in these streams. Trends in mean wood breakdown rates suggested that loss rates of the carbon (wood veneers) that was added was accelerated at urban and suburban sites. Thus, management approaches should focus on the promotion of carbon inputs and slowing loss rates of critical carbon resources in these streams.

Due to the lack of identified drivers of altered breakdown rate in this study, we recommend including a greater number of sites (20+) in future wood breakdown studies in urban streams. Additional sites would allow for the use of a multiple regression approach to elucidate the effect of multiple stressors on breakdown rates. We also advocate for future studies that examine variability ecosystem function in different land uses, especially in studies where functional responses are not altered in their mean. Urban ecosystems are unique in that they are typically experiencing multiple stressors that counteract or interact (Fraterrigo et al. 2005), resulting in variability in ecosystem responses. Evidence presented here suggests that variability in carbon breakdown may be a signal of stressed stream ecosystems.

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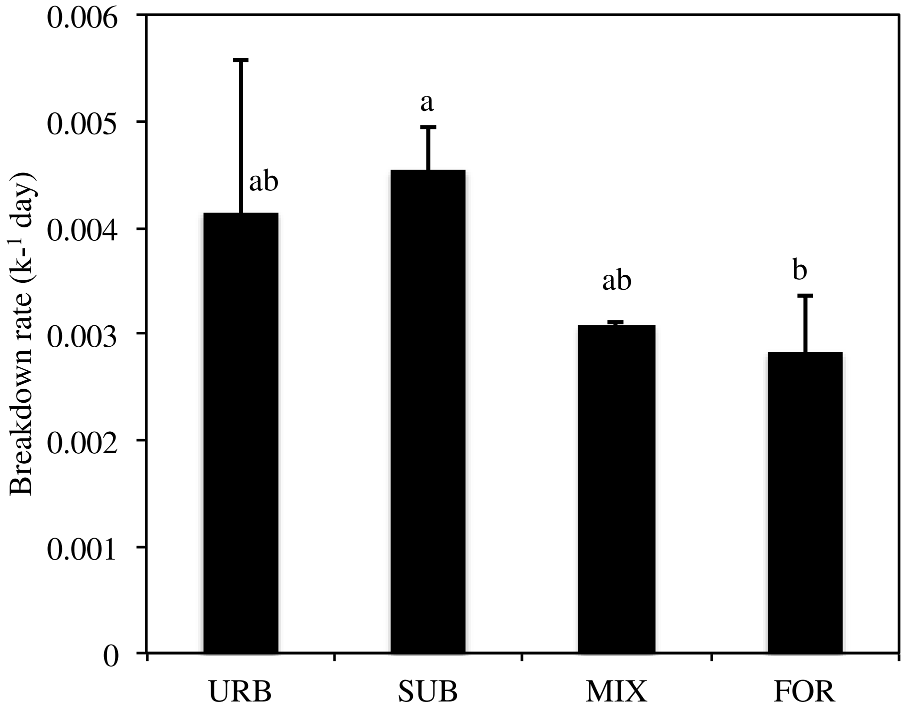

Figure 4.1. Mean white oak wood veneer breakdown rates (k) in each land use class (URB, urban; SUB, suburban; MIX, mixed; FOR, forested). Means for categories with the same letter are not significant ($p < 0.05$).

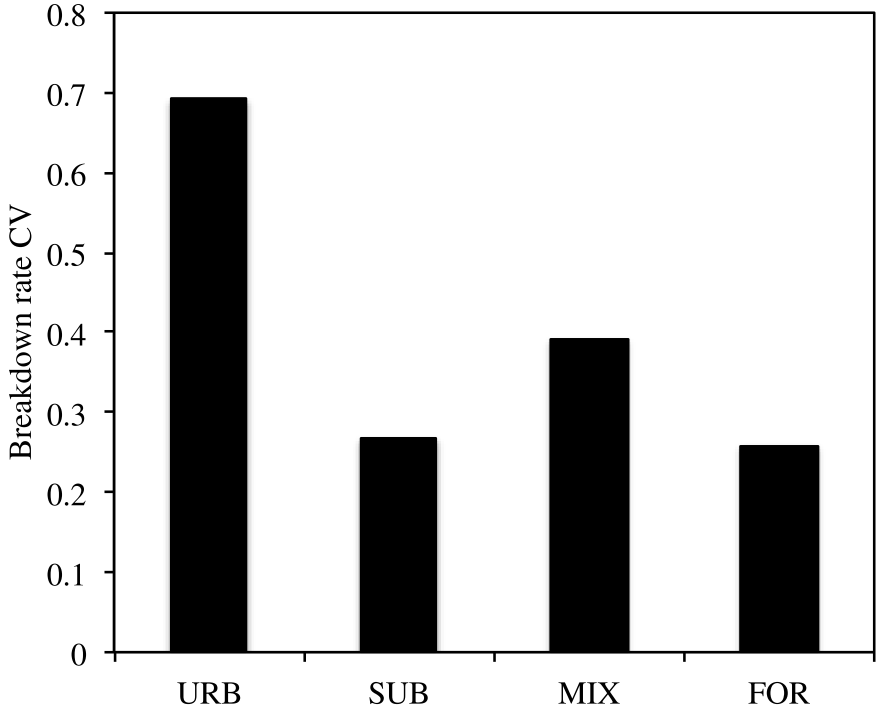

Figure 4.2. Coefficients of variation (CVs) of wood veneer breakdown rates (k^{-1} day) in each land use class (URB, urban; SUB, suburban; MIX, mixed; FOR, forested).

Table 4.1. Mean and standard errors (in parentheses) of wood breakdown rates (k $^{-1}$ day), microbial respiration and selected physical and chemical characteristics at sites within each land use class. Note: ISC = impervious surface cover, DIN = dissolved inorganic nitrogen and SRP = soluble reactive phosphorus.

Land Use	ISC (%)	Breakdown rate (k $^{-1}$ day)	Respiration, Day 220 (mg O_2/g AFDM/hr)	DIN (µg/L)	SRP (µg/L)	Conductivity (µS/cm)	Physical Abrasion (%)
URB 1	32.9	0.0062 (0.0009)		697.3 (84.7)	6.0 (2.7)	99.6	13.3 (2.4)
URB 3	32.8	0.0021 (0.0003)	0.21 (0.02)	714.3 (97.3)	3.7 (1.3)	80.2	10.6 (0.6)
SUB 1	16.6	0.0045 (0.0004)	0.20 (0.06)	478.9 (66.5)	2.6 (1.0)	68.7	10.7 (0.6)
SUB 2	14.5	0.0058 (0.0011)		442.3 (53.8)	5.1 (2.0)	66.6	19.2 (6.4)
SUB 3	28.5	0.0034 (0.0005)	0.54 (0.06)	972.5 (189.0)	4.8 (1.3)	68.5	7.8 (0.4)
MIX 1	10.8	0.0052 (0.0017)	0.12 (0.10)	446.9 (71.2)	2.7 (2.7)	55.1	14.4 (0.4)
MIX 2	9.2	0.0030 (0.0003)	0.21 (0.03)	465.2 (56.8)	2.1 (2.1)	52.6	23.7 (2.3)
MIX 3	6.9	0.0032 (0.0004)	0.03 (0.04)	548.4 (47.8)	3.2 (3.2)	51.2	
FOR 1	0.1	0.0023 (0.0003)	0.32 (0.05)	153.8 (26.3)	5.4 (1.90	39.4	11.9 (0.8)
FOR 2	3.9	0.0034 (0.0002)	0.14 (0.04)	598.8 (77.1)	6.8 (3.9)	57.9	13.8 (0.7)

Table 4.2. Linear relationships between wood breakdown rates (k $^{-1}$ day) and physical and chemical variables at each site. DIN (dissolved inorganic nitrogen), physical abrasion and conductivity were regressed against mean breakdown rate at each site (n=10). Microbial respiration measured on day 220 was regressed against wood breakdown rate on corresponding individual rafts (n=24).

Response variable	n	Regression model	r^2	p-value
DIN (μg/L)	10	(2.97 x 10^{-7} * k) + 3.06 x 10^{-3}	0.12	NS
Physical abrasion (%)	10	5.53 x 10^{-5} * k) + 3.06 x 10^{-3}	0.12	NS
Conductivity (μS/cm)	10	4.17 x 10^{-5} * k) + 1.22 x 10^{-3}	0.15	NS
Microbial respiration (mg O_2 / gAFDM/ hr)	24	2.3 x 10^{-3} * k) + 2.5 x 10^{-3}	0.06	NS

Table 4.3. Coefficients of variation (CVs) for leaf breakdown rates reported in Imberger et al. (2008), Chadwick et al. (2006) and Paul et al. (2006) and wood breakdown rates in this study. CVs were calculated for land use classes based on watershed % ISC (Forested, < 5%; Suburban, 15 – 29%; Urban, >30%).

Urban	Suburban	Forested	Source
0.41	NA	0.13	Imberger et al. (2008)
0.46	0.48	0.12	Paul et al (2006)
0.38	0.21	0	Chadwick et al. (2006)
0.69	0.27	0.25	This study

CHAPTER 5

SUMMARY AND CONCLUSIONS

This thesis project was part of a larger collaboration between the Athens-Clarke County Stormwater Division, the non-profit Upper Oconee Watershed Network and the University of Georgia River Basin Center. The goals of the partnership were not only to quantify ecological condition and quantify aspects of ecosystem structure and function, but also to work towards effective management of watersheds in Athens-Clarke County through the evaluation of watershed stressors. I predicted that each ecosystem component measured in this thesis would respond to anthropogenic stressors in different ways, thus providing a more comprehensive view of how urbanization is impacting streams. I hope the information presented in this thesis will be used to inform future best management practices.

Summary of findings

In Chapter 2, macroinvertebrate biomass was greatly reduced as percent impervious surface cover (% ISC) in the watershed, a measure of urbanization, increased. In addition, the biomass of predator, scraper and shredder functional feeding groups declined with % ISC. Proportionally, the macroinvertebrate community at each site shifted from one representing all functional feeding groups (predators, scrapers, shredders, collector-gatherers and filterers) to one dominated by collector-gatherers and filterers as watershed % ISC increased. Results indicated that lower macroinvertebrate biomass was associated with increased streamwater conductivity and reduced organic matter (measured as ash-free dry mass), an important basal resource. In addition, the loss of predator and scraper taxa and increased dominance of tolerant collector-gatherer taxa with increasing urbanization indicated the emergence of altered community trophic dynamics.

Benthic biofilms are known to be important sites for nutrient retention in streams. Chapter 3 examined how the biomass and nutrient content of hard and soft substrate biofilms changed with increased watershed impervious cover and streamwater nutrients. I found the mass of biofilms (as AFDM) to be much greater at sites with lower % ISC in the watershed. The negative relationship of AFDM with % ISC suggested that soft substrates contained stored organic matter (as CPOM, FPOM or algae). This implied lower organic matter retention in urban streams. Algal biomass was strongly seasonal and generally increased with % ISC. Streamwater nutrients did not, contrary to what I predicted, stimulate algal biomass, but there was a positive relationship between streamwater nitrogen and biofilm $\delta^{15}N$ and negative relationship between streamwater nitrogen and biofilm C:N, suggesting that biofilms were important in the uptake of anthropogenically-derived nutrients. Decreased ash-free dry mass on hard and soft substrate biofilms resulted in reduced carbon and nitrogen storage over the entire reach as % ISC increased. Results from this study suggest that biofilm nutrient storage is reduced where it is needed most—in urban and suburban streams where anthropogenic nutrient loading is potentially the greatest.

In assessing ecosystem health, it is important to measure both ecosystem structure and function (Gessner and Chauvet 2002, Young et al. 2008) because streams may be altered in structure, function or both in response to anthropogenic stressors. Chapter 4 explores alterations to carbon processing (wood), an important ecosystem function, in urban, suburban, mixed-use and forested land use classes. I found that breakdown rates (k) of white oak wood veneers were significantly higher in suburban versus forested watersheds, with intermediate values in urban and mixed-use watersheds. Although I identified differences in wood breakdown rates across land use classes, I could not identify any abiotic variables (streamwater nutrient concentrations, conductivity or abrasive flows) that were driving these differences. I also found more variability in breakdown rates among urban and suburban streams than among forested streams, which may also be an indicator of ecosystem change. While altered breakdown rates and increased variability in breakdown rates indicate altered function in urban and suburban streams, more studies need to be conducted in order to elucidate specific drivers of altered wood breakdown rates. Overall, higher breakdown rates in suburban and urban streams suggest faster carbon processing and increased carbon losses.

Conclusions

Carbon resources provide energetic stability in stream ecosystems (Kominoski and Rosemond 2012) and are important in the production of organisms. Results from this thesis highlight the importance of carbon in urban streams. In Chapter 2, I found decreased macroinvertebrate biomass in streams as streamwater conductivity and nitrogen increased and as organic matter (carbon) decreased. In Chapter 3, I found that biofilms were decreased in their overall carbon and nitrogen storage in urban streams due to reduced organic matter storage. Finally, Chapter 4 documents faster wood breakdown rates in urban streams, indicating lower carbon storage.

Freshwater streams are an important component of the global carbon cycle via storage and respiration (Cole et al. 2007). In fact, terrestrially derived carbon provides most of the energy for the production of organisms and the maintenance of metabolic processes in streams (Kominoski and Rosemond 2012). Watershed urbanization may alter inputs and retention of carbon resources in streams due to altered channel morphology, stream scouring, and decreases in large woody debris (Paul and Meyer 2001, Walsh et al. 2005b, Kominoski and Rosemond 2012), thus impacting biotic assemblages and nutrient retention capacity.

Management recommendations

Altered hydrology (increased storm flows) and watershed inputs (conductivity and nutrients) are two likely causes of altered ecosystem structure and function in streams in this study. In terms of management, it is imperative to address these watershed-scale issues before addressing reach-scale issues in these streams. For example, recent evidence has demonstrated that reach-scale channel restoration projects do not improve macroinvertebrate communities, promote organic matter storage or increase nutrient retention (Bernhardt and Palmer 2011). Reach-scale channel restoration projects increase habitat heterogeneity through the creation of a meandering channel, but they do not address chemical and hydrological stressors that may still impact biota and organic matter retention.

This suggests that management to improve conditions for aquatic life in urban streams should focus on reducing negative watershed inputs and the impacts of altered hydrology. First, management strategies need to reduce streamwater nitrogen and conductivity, which were associated with reduced biomass of macroinvertebrates as examined in Chapter 2. I suggest projects in urban watersheds that promote stormwater infiltration, thus reducing the concentration of nutrients, conductivity-increasing pollutants and sediment. BMPs such as pervious pavement, rain gardens and green roofs (Carter and Jackson 2007) all promote stormwater infiltration, reducing the direct input of pollutants to streams through stormwater pipes (Walsh et al. 2005a)

Organic matter retention is also important in maintaining healthy stream ecosystems. Results from this thesis suggest that the retention of organic matter would provide resources for more sensitive macroinvertebrate consumers (Chapter 2) and increase nutrient retention (Chapter 3). Also, results from Chapter 4 suggest that carbon (wood) breakdown is faster in more urban streams, likely due to abrasive flows (Paul et al. 2006). Again, innovative stormwater management projects such as pervious pavement, rain gardens and green roofs reduce peak flows, thus regaining a more natural hydrograph and reducing abrasive flows and the transport of organic matter downstream. Overall, I suggest that adaptive management that first addresses watershed-scale issues should be considered before reach-scale restoration efforts are implemented.

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APPENDIX 2.1. AIC values from hierarchical models relating (A) total macroinvertebrate biomass and the biomass of each FFG to habitat (riffle vs. pool), % ISC (impervious surface cover) and watershed area (B) total macroinvertebrate biomass to dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP), (C) total biomass to habitat, conductivity, total suspended solids (TSS), and pH, and (D) total macroinvertebrate biomass and the biomass of each FFG to habitat, AFDM in depositional areas, and chlorophyll *a* (chl *a*). The best models for each combination of response and predictor variables are in bold. Parameter estimates and confidence intervals for the best models are listed in Tables 2.2, 2.3 and 2.4.

Response	*Predictor variables*	*AIC*
A		
Total biomass		
	habitat, % ISC	**359.0**
	habitat, % ISC, area	361.0
	habitat	364.5
	habitat, area	365.3
Collector-gatherer biomass		
	habitat	**356.6**
	habitat, area, % ISC	359.4
	habitat, % ISC	358.0
	habitat, area	357.6
Filterer-collector biomass		
	habitat, area	**371.7**
	habitat, % ISC, area	373.7
	habitat	382.1
	habitat, % ISC	382.5
Predator biomass		
	habitat, % ISC, area	**337.5**
	habitat	342.6
	habitat, % ISC	340.2
	habitat, area	344.1

Appendix 2.1 *cont.*

Response	*Predictor variables*	*AIC*
A, *cont.*		
Scraper biomass		
	% ISC	**359.0**
	% ISC, area	361.0
	area	365.0
	habitat	410.0
Shredder biomass		
	% ISC, area	**324.7**
	% ISC	326.2
	area	327.0
	habitat	327.6
B		
Total biomass		
	habitat + DIN	353.9
	habitat + DIN + SRP	355.9
	habitat	358.6
	habitat + SRP	360.5
C		
Total biomass		
	habitat + **cond**	**357.2**
	habitat + tss	357.9
	habitat + tss + pH + cond	357.9
	hab + tss + cond	358.3
	hab + tss + pH	358.3
	habitat	358.6
	habitat + pH + cond	359.1
D		
Total biomass		
	habitat+AFDM+chl a	**354.7**
	habitat +AFDM	356.1
	habitat	358.6
	habitat + chl a	361.7
Collector-gatherer biomass		
	habitat + **chl a**	**329.1**
	habitat+AFDM + chl a	331
	habitat	334.2
	habitat + AFDM	336.2

APPENDIX 2.1 *cont.*

Response	*Predictor variables*	*AIC*
D, *cont.*		
Filterer-collector biomass		
	habitat	**382.1**
	habitat + AFDM	384
	habitat + chl a	384.1
	habitat + AFDM + chl a	386.1
Predator biomass		
	habitat + AFDM	**328.2**
	habitat + AFDM + chl a	330
	habitat	342.6
	habitat + chl a	344.5
Scraper biomass		
	AFDM	**406.1**
	AFDM + chl a	407.4
	chl a	410.2
Shredder biomass		
	AFDM	**318.7**
	AFDM + chl a	320
	chl a	320.1

APPENDIX 2.2. Estimates of macroinvertebrate density (no./m^2) and biomass (mg/m^2) by taxa for each site sampled. (A) URB1, (B) URB2 and (C) URB3 = Urban; (D) SUB1, (E) SUB2, (F) and SUB3 = Suburban, (G) MIX1, (H) MIX2, and (I) MIX3 = Mixed-use; (J) FOR1, (K) FOR2 and (L) FOR3 = Forested.

A. Brooklyn Creek at Milledge Circle (URB1). 83°24'8" S 33°56'15" E

	Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera			
	Elmidae	25	3.7
Diptera			
	Antocha	803	45.9
	Ceratopogonidae	192	176.2
	Chrysops	25	41.4
	Non-Tanypodinae	15558	329.2
	Simuliidae	156	6.1
	Syrphidae	25	4.5
	Tanypodinae	125	38.1
Ephemeroptera			
	Ephemerella	100	13.8
Plecoptera			
	Plecoptera	33	0.3
Tricoptera			
	Cheumatopsyche	22	21.5
	Hydropsyche	400	1949.5
Other			
	Amphipod	25	0.1
	Collembola	50	0.9
	Cyclopoid	731	0.8
	Hydracarina	78	0.2
	Pulmonata	25	
	Nematoda	531	2.9
	Oligochaeta	9383	35.7
	Tardigrade	100	

B. Brooklyn Creek – McWhortor Branch near Milledge Circle (URB2). 83°24'4" S 33°56'19" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Diptera		
Antocha	189	49.1
Ceratopogonidae	261	12.3
Non-Tanypodinae	18636	339.5
Tanypodinae	33	1.4
Tipula	25	760.7
Ephemeroptera		
Ephemerella	122	55.9
Ephemeropera	25	3.4
Plecoptera		
Chloroperilidae	22	0.8
Tricoptera		
Cheumatopsyche	33	17.6
Other		
Collembola	275	4.9
Corbicula	150	26.3
Cyclopoid	10842	10.9
Hydracarina	1747	4.5
Nematoda	1192	2.5
Oligochaeta	4542	59.6

C. Brooklyn Creek at The Plaza (URB3). 83°24'58" S 33°57'17" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	40	68.2
Diptera		
Antocha	11	0.5
Dixa	100	0.7
Limonia	25	12.7
Non-Tanypodinae	45347	669.9
Syrphidae	25	4.5
Tanypodinae	1700	39.0
Ephemeroptera		
Eurylophella	40	32.1
Tricoptera		
Cheumatopsyche	11	3.1
Hydropsyche	33	43.2
Other		
Collembola	667	11.8
Corbicula	675	1.5
Cyclopoid	3847	3.8
Sphaeriidae	983	
Pulmonata	25	
Hydrocarina	1067	2.8
Nematoda	2144	9.0
Oligochaeta	8282	2979.5
Tardigrade	67	

D. Hunnicutt Creek in Ben Burton Park (SUB1). 83°26'12" S 33°58'31" E.

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	25	89.4
Diptera		
Antocha	944	9.5
Ceratopogonidae	11	0.1
Non-Tanypodinae	26031	508.1
Simuliidae	4089	400.6
Tanypodinae	22	3.5
Ephemeroptera		
Baetis	22	4.1
Ephemerella	256	80.6
Ephemeroptera	22	0.1
Odonata		
Progomphus	25	82.1
Plecoptera		
Chloroperilidae	200	2.7
Plecoptera	358	2.9
Tricoptera		
Hydropsyche	133	769.9
Other		
Amphipoda	11	1.0
Collembola	11	0.2
Cyclopoid	1956	2.1
Hydrocarina	225	0.6
Nematoda	1239	4.6
Oligochaeta	10381	49.1
Tardigrade	61	

E. Hunnicutt Creek at Ashton Drive (HU2). 83°25'30" S 33°58'28" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	300	24.7
Diptera		
Antocha	11	0.2
Ceratopogonids	100	7.7
Non-Tanypodinae	10819	146.7
Simuliidae	236	60.9
Syrphidae	50	13.6
Tanypodinae	33	2.0
Ephemeroptera		
Ephemerella	22	1.6
Odonata		
Progomphus	25	60.9
Plecoptera		
Chloroperilidae	156	1.2
Plecoptera	200	1.6
Suwillia	22	0.1
Tricoptera		
Hydropsych\e	11	0.0
Other		
Collembola	61	1.1
Cyclopoid	1722	1.7
Sphaeriidae	61	
Prosobranchia	25	
Hydracarina	1300	3.4
Nematoda	1358	6.1
Oligochaeta	3994	2408.5
Tardigrade	133	

F. Hunnicutt Creek at Magnolia Blossom Way (SUB3). 83°25'13" S 33°58'17" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Diptera		
Ceratopogonidae	11	4.0
Non-Tanypodinae	1986	23.9
Simuliidae	25	0.6
Tanypodinae	67	1.5
Ephemeroptera		
Hexagenia	11	1.4
Odonata		
Progomphus	25	60.9
Plecoptera		
Plecoptera	11	0.1
Other		
Collembola	89	2.2
Cyclopoid	58	0.1
Hydracarina	47	0.1
Nematode	86	0.4
Oligochaeta	1106	12.9
Tardigrade	44	

G. Trail Creek at Dudley Park (MIX1). 83°22'56" S 33°57'17" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	50	1153.1
Diptera		
Ceratopogonidae	111	3.5
Non-Tanypodinae	43746	422
Simuliidae	11	0.3
Tanypodinae	147	9.6
Ephemeroptera		
Ephemerella	11	2.6
Ephemeroptera	50	25.2
Odonata		
Gomphidae	36	0.3
Trichoptera		
Cheumatopsyche	11	31.1
Chimarra	11	16.6
Other		
Corbicula	303	1484.0
Cyclopoid	4127	4.1
Cladocera	197	0.5
Fingernail Clam	36	
Hydracarina	979	2.6
Isopoda	22	0.1
Nematoda	1572	4.9
Oligochaeta	5931	235.7
Tardigrade	348	

H. Trail Creek at Collins Industrial Boulevard (MIX2). 83°21'2" S 33°59'52" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	211	6.4
Diptera		
Ceratopogonidae	375	52.1
Non-Tanypodinae	27385	402.4
Simuliidae	44	1.1
Syrphidae	25	8.0
Tanypodinae	467	271.1
Ephemeroptera		
Heptagenia	22	24.0
Leptophlebia	58	14.0
Plecoptera		
Beloneuria	11	8.5
Chloroperilidae	22	0.1
Plecoptera	561	4.5
Trichoptera		
Hydropsyche	25	151.6
Other		
Amphipoda	619	8.9
Collembola	86	1.5
Corbicula	75	423.8
Cyclopoid	19037	20.4
Sphaeriidae	461	
Hydracarina	571	1.5
Nematoda	1724	6.6
Oligochaeta	6033	382.5
Tardigrade	75	

I. Trail Creek at Old Hull Road (MIX3). 83°21'58" S 33°59'28" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	11	0.6
Diptera		
Non-Tanypodinae	31043	1.9
Simuliidae	393	27.4
Tanypodinae	161	
Ephemeroptera		
Hexagenia	175	2.5
Macdunnoa	67	10.6
Stenacron	11	48.0
Odonata		
Cordulegaster	11	865.8
Plecoptera		
Plecoptera	911	21.0
Other		
Amphipod	2290	11.3
Ceratopogonidae	400	38.5
Cladocera	2347	5.5
Collembola	1265	27.3
Cyclopoid	6082	6.1
Sphaeriidae	50	1466.0
Hydracarina	736	192.1
Nematoda	2889	813.3
Oligochaeta	3119	7.3
Tardigrade	290	

J. Bear Creek at Cleveland Road (FOR1). 83°30'51" S 33°58'2" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Elmidae (larve)	3058	1777.3
Elmidae (adult)	704	20.6
Diptera		
Antocha	25	16.3
Ceratopogonidae	5194	284.9
Hexatoma	59	9.6
Lype	25	25.7
Non-Tanypodinae	9890	146.6
Simulidae	340	31.1
Tanypodinae	985	92.3
Tipula	58	3879.4
Ephemeroptera		
Baetis	19	19.1
Centroptilum	296	18.9
Ephemerella	380	370.9
Odonata		
Cordulagaster	69	1171.7
Hetaerina	25	251.3
Progomphus	319	427.9
Plecoptera		
Amphinemura	56	21.4
Plecoptera	7467	59.7
Suwillia	292	38.7
Tricoptera		
Cheumatopsyche	11	14.6
Chyranda	100	
Lepodostima	36	37.7
Ptychoptera	100	8.0
Rhyacophilia	11	
Other	25	27.5
Amphipod	1400	6.9
Cladocera	8067	18.8
Clams	100	
Collembola	533	10.6
Cyclopoid	5	5.0
Sphaeriidae	1880	
Prosobranchia	11	
Hydracarina	3796	9.9
Nematoda	7006	9.4
Oligochaeta	23155	17.2
Tardigrade	4800	

K. Big Creek at Bob Godfrey Road (FOR2). 83°16'11" S 33°54'57" E

	Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Diptera			
	Ceratopogonidae	1224	59
	Non-Tanypodinae	27996	877
	Simulidae	203	12
	Tanypodinae	344	61
Ephemeroptera			
	Baetidae	30	0
	Centropotillum	200	28
	Chloroterpes	33	17
	Drunella	67	62
	Ephemerella	1746	9599
	Ephemeroptera	1067	6
	Hexagenia	25	656
	Paraleptophlebia	50	18
Plecoptera			
	Agnetina	11	10
	Amphinemura	52	10
	Neoperla	11	3
	Perlesta	147	1
	Perlinella	119	
	Plecoptera	1107	9
	Seratella	522	475
	Suwilla	504	23
	Calamoceratidae	25	
Other			
	Cladocera	2612	7
	Collembola	30	1
	Corbicula	10800	2008
	Cyclopoid	6095	6
	Prosobranchia	108	
	Hemiptera	30	2
	Hydracarina	2325	6
	Pulmonata	25	
	Nematoda	1757	6
	Oligochaeta	5332	282
	Physidae	11	
	Pulmonata	11	
	Tardigrade	225	

L. Shoal Creek at Winterville Road (FOR3). 83°18'14" S 33°58'10" E

Taxa	*Density (no./m^2)*	*Biomass (mg/m^2)*
Coleoptera		
Ectopria	75	1339.4
Elmidae	181	54.0
Elmidae (adult)	11	1.1
Diptera		
Ceratopogonidae	1681	153.5
Hexatomoa C	25	73.6
Non-Tanypodinae	24947	507.1
Pseudolimnophilla	11	10.8
Simuliidae	11	0.3
Tanypodinae	314	29.8
Tipula	50	20.9
Ephemeroptera		
Centropotillum	56	14.4
Ephemerella	325	650.4
Ephemeroptera	200	1.2
Megaloptera		
Nigronia	25	417.7
Odonata		
Cordulagaster	25	1177.5
Plecoptera		
Amphinemura	11	3.2
Chloroperilidae	11	1.2
Plecoptera	1711	13.7
Suwillia	200	108.2
Other		
Amphipod	133	18.0
Cladocera	2589	6.0
Collembola	603	
Decapoda	11	3225.8
Cyclopoid	18133	18.1
Sphaeriidae	789	
Prosobranchia	75	
Hydracarina	2403	6.2
Nematoda	2419	5.4
Oligochaeta	6122	39.8
Pulmonata	44	

APPENDIX 3.1. Competing models (with and without predictors of interest) for each hypothesis tested for chl *a*, AFDM:chl *a,* AFDM, %N, %P, C:N, C:P and N:P with ΔAIC values and model weights.

Model	*Δ AIC*	*AIC weight*	*Model*	*Δ AIC*	*AIC weight*
AFDM			**Chla:AFDM**		
Hard substrate			*Hard substrate*		
season + ISC	0	56.2	season + ISC	0	92.4
season	0.5	43.8	season	5	7.6
Soft substrate			*Soft substrate*		
season + ISC	0	99.8	season	0	53.7
season	13	0.2	season + ISC	0.3	46.3
Chlorophyll *a*			**C:N**		
Hard substrate			*Hard substrate*		
season	0	62.2	season	9	1.1
season + ISC	1	37.8	season + DIN	0	98.9
season + ISC + season*ISC	0	100.0	**%N**		
season + isc	26	0.0	*Hard substrate*		
			season	8.68	1.3
season + DIN + SRP	0	97.5	season + DIN	0	98.7
season	7.3	2.5			
			C:P		
Soft substrate			*Hard substrate*		
season + ISC	0	98.9	season	1.8	28.9
season	9	1.1	season + SRP	0	71.1
season + ISC + season *ISC	0	98.9	**%P**		
season + ISC	9	1.1	season	0	62.2
			season + SRP	1	37.8
season	0	85.2			
season + DIN + SRP	3.5	14.8			