

# Watershed urbanization affects macroinvertebrate community structure and reduces biomass through similar pathways in Piedmont streams, Georgia, USA

J. L. Sterling<sup>1,2,4</sup>, A. D. Rosemond<sup>1,5</sup>, and S. J. Wenger<sup>1,3,6</sup>

<sup>1</sup>Odum School of Ecology, University of Georgia, Athens, Georgia 30602 USA

<sup>2</sup>Chattahoochee Riverkeeper, 916 Joseph Lowery Boulevard NW, Atlanta, Georgia 30318 USA

<sup>3</sup>River Basin Center, University of Georgia, Athens, Georgia 30602 USA

**Abstract:** Watershed urbanization affects stream macroinvertebrate communities via multiple pathways, including chemical stressors, physical scour, and indirect biological effects such as altered food availability. Different metrics may respond differently to urbanization and may be affected by different pathways. We tested whether macroinvertebrate biomass, community composition, and biotic integrity exhibited consistent responses to watershed urbanization and responded to the same stressors. We quantified macroinvertebrate community composition by biomass and density and estimated aggregate total macroinvertebrate biomass, biomass by functional feeding group, aggregate taxon richness, and biotic indices at 12 sites in urban, suburban, mixed-use, and rural watersheds in the upper Oconee River basin, Georgia, USA. Watershed-scale land use, physicochemical stressors, and biological covariates explained significant variation (~85%) in community structure based on density or biomass across sites; all 3 groups of variables contributed significantly in canonical correspondence analysis hierarchical models. Aggregate macroinvertebrate biomass was predicted by watershed % impervious surface cover (ISC) (–), conductivity and nutrient concentrations (–), and biological covariates (+). Watershed % ISC was as strongly or more strongly related to site-level community structure metrics (biotic indices, taxon richness;  $r^2 = 0.43$ – $0.76$ ) as it was to total macroinvertebrate biomass ( $r^2 = 0.52$ ). Declines in biomass of sensitive and tolerant taxa occurred with increased % ISC, but were steeper for sensitive taxa. Watershed urbanization negatively affected macroinvertebrate biomass and community structure, which were explained by similar drivers. Such alterations in macroinvertebrate communities, including reduced taxon richness and biomass, probably produce substantial changes to ecosystem function in urban streams.

**Key words:** impervious surface cover, functional feeding groups, community structure, multiple stressors, urban streams, chlorophyll *a*, ecosystem function, canonical correspondence analysis

Watershed urbanization alters community structure (Roy et al. 2003, Helms et al. 2009), biomass (Chadwick et al. 2006, Woodcock and Huryn 2007, Johnson et al. 2013), land cover and secondary production of stream macroinvertebrates (Woodcock and Huryn 2007, 2008), but whether different characteristics of macroinvertebrate communities respond in similar ways to watershed disturbance is not known. Urbanization typically involves multiple stressors (physical, chemical, and biological), and their effects on stream structure and function are difficult to disentangle (Wenger et al. 2009). In addition, these stressors may affect different response variables, such as community structure, and collective properties, such as biomass or production,

differently. For example, elevated nutrients in urban streams can increase secondary production, but associated elevated conductivity can reduce taxon richness (Johnson et al. 2013). However, in other studies, stressors, such as contaminants, reduced both secondary production and taxon richness (Woodcock and Huryn 2007). Moreover, in urbanized landscapes, multiple stressors can differentially affect a single response variable. Altered hydrology can have negative effects on macroinvertebrate biomass, whereas elevated nutrients can have positive effects (Riseng et al. 2004). Thus, we need improved understanding of the net effects of stressors associated with urbanization on both macroinvertebrate community structure and patterns of biomass or secondary

E-mail addresses: <sup>4</sup>sterling@chattahoochee.org; <sup>5</sup>rosemond@uga.edu; <sup>6</sup>wenger@uga.edu

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production. Furthermore, we need to assess whether different response variables are similarly affected by stressors in urban streams.

Watershed urbanization affects several physical and chemical stressors, as well as many aspects of food and habitat resources that have been less well studied (but see Riseng et al. 2004, Woodcock and Huryh 2007). Basal food resources are critical for macroinvertebrate biomass and production. For example, several investigators have established quantitative relationships between basal C standing crop and macroinvertebrate production (Wallace et al. 1999, Chadwick and Huryh 2007, Woodcock and Huryh 2007, Walther and Whiles 2011). Retention of detrital C in urban streams may be reduced because of altered hydrology (Miller 2013), nutrient-stimulated accelerated loss rates (Rosemond et al. 2015), or reduced inputs. Algal resources can increase in response to reductions in riparian cover or increased nutrient concentrations or can decrease because of hydrologic disturbance (Walsh et al. 2005). Thus, effects of stressors on urban streams may increase or decrease C resources and thereby affect biomass or production of organisms. In addition, the importance of changes associated with urbanization in food resources relative to changes in physicochemical variables on macroinvertebrate composition and biomass is unknown.

The relative effects of watershed land use, physicochemical stressors, and biological covariates (e.g., food and habitat resources) on macroinvertebrate assemblages indicate ways that stream condition can be predicted and improved. For example, if lack of C retention is a key driver, then control of stormwater flows and local-scale restoration to improve C retention or inputs may be beneficial. If contaminants are the major driver, reduction in the delivery of pollutants to streams should be prioritized. Evaluation of predictive capabilities of variables at different scales requires assessment of the amount of variation in communities that can be explained by watershed-level land use relative to more-proximate drivers (Strayer et al. 2003).

Our goal was to estimate the relative effects of suites of drivers on macroinvertebrate response variables in urban streams. We tested whether the same drivers affected macroinvertebrate community structure (based on density or biomass) and aggregated total macroinvertebrate biomass. We focused on relatively small watersheds and evaluated the effects of drivers operating at different scales (landscape to stream-reach scale) and via different pathways. We identified the scale and type of drivers that best predicted community structure and biomass and quantified their explanatory power. We hypothesized that differential sensitivity of taxa ultimately determines changes in both community structure and biomass and tested this hypothesis by measuring the relative effects of land-cover change on the biomass of sensitive vs tolerant taxa. Our findings can be used to indicate the utility of various predictor and response variables

in studies assessing the effects of urbanization on stream ecosystems.

## METHODS

Study sites were in the upper Oconee River Basin in Athens–Clarke County, in northeastern Georgia, USA (Fig. 1). The research area is situated in the Piedmont physiographic province, which is characterized by red clay soils and granitic–gneiss bedrock. We selected 12 sites in 6 watersheds with a range of land uses across a gradient of impervious surface cover (ISC) (0.1–32.9%) and forest cover (3.9–75.0%) (Table 1). We collected macroinvertebrates at 3 tributaries each in an urban (URB1, URB2, URB3), suburban (SUB1, SUB2, SUB3), and mixed-use watershed (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 3 separate, predominantly undeveloped watersheds (rural; RUR1, RUR2, RUR3). All streams were 2<sup>nd</sup> to 3<sup>rd</sup> order based on US Geological Survey 7.5-minute topographic quadrangles. Urban, suburban, and mixed sites were nested, with sites on 2 tributaries and a downstream site in the same watershed.

We delineated watershed boundaries, and calculated watershed area for each of the 12 sites using a geographic information system (ArcGIS, version 10; Environmental Systems Research Institute, Redlands, California) and hand-delineated from topographical quadrangles. We calculated % ISC for each watershed on the basis of an impervious cover layer provided by Athens–Clarke County. The impervious cover layer had a resolution of 15.2 cm and was created by a classification of digital aerial images from 2008. Percent forest cover and % agricultural land use were derived from the 2011 National Land Cover Database (mrlc.gov/nlcd2011.php) by aggregating all classes of forest and agriculture.

### Macroinvertebrate sampling methods

Before sampling, we mapped 50-m reaches at each site and determined the percentage of each habitat type (riffle, pool, and woody debris) every 5 m to characterize habitat differences between sites. We collected benthic macroinvertebrates from 4 riffles and 4 pools at each site on 4–6 April 2008 during baseflow conditions. We sampled riffles with a Surber sampler (0.09 m<sup>2</sup>, 250- $\mu$ m mesh) by scrubbing substrates within the sampler for 3 min. We sampled pools with a core sampler (0.04 m<sup>2</sup>) by removing the top 10 cm of sediment from the core, transferring the sediment to a bucket, and elutriating through a 250- $\mu$ m-mesh sieve in the field.

In the laboratory, we washed samples through stacked 1-mm and 250- $\mu$ m sieves to separate sample material into size class categories of >1 mm and 250  $\mu$ m–1 mm, and fixed

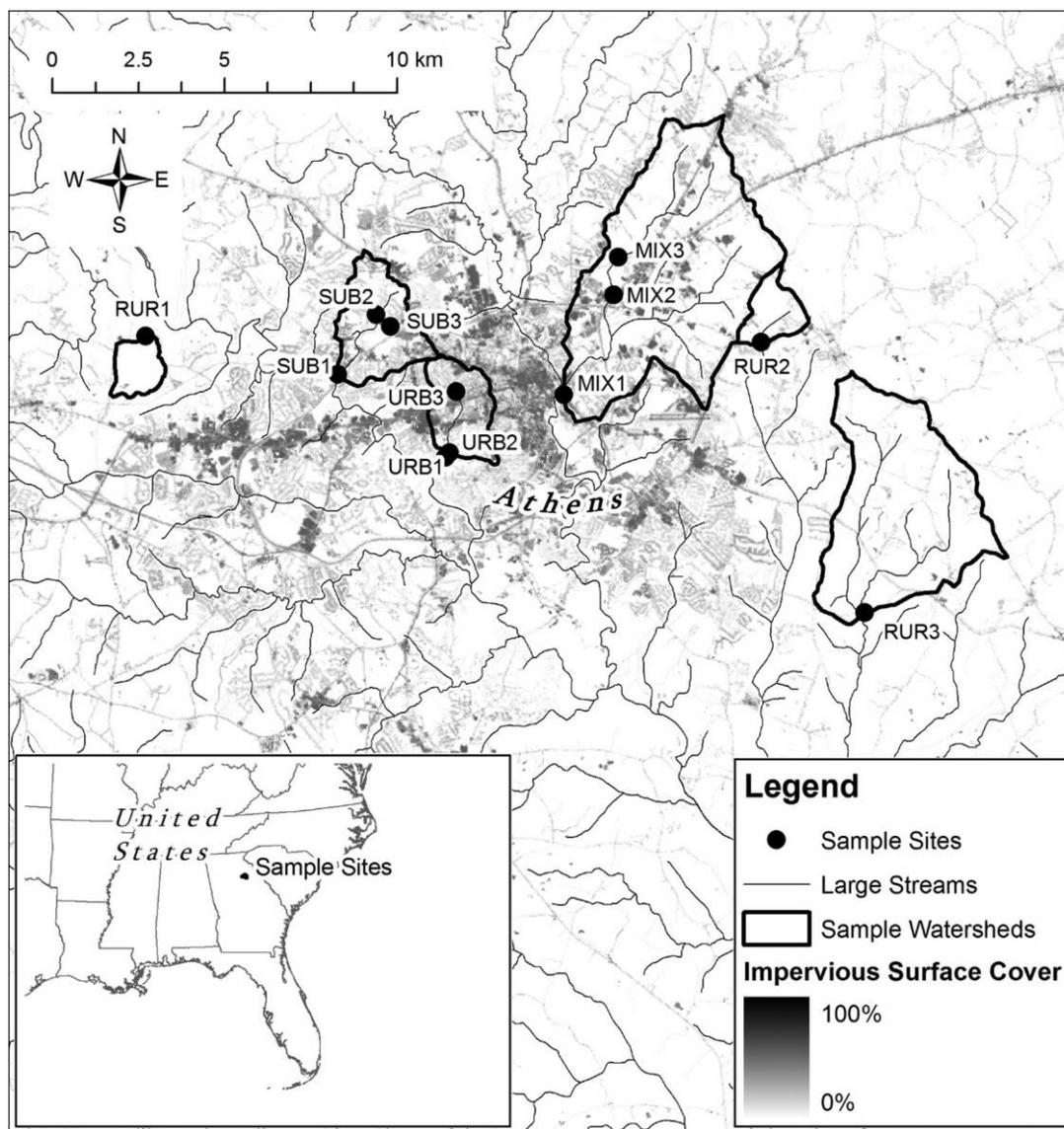


Figure 1. Map of the upper Oconee River watershed, Georgia, USA, with the 6 watersheds indicated (RUR = rural, MIX = mixed, SUB = suburban, URB = urban).

them with 70% ethanol. We separated macroinvertebrates from organic matter and sediment under a dissecting scope at  $10\times$  magnification. If necessary, we subsampled small invertebrates ( $250\ \mu\text{m}$ – $1\ \text{mm}$ ) using a wheel sampler (Waters 1969). We counted, measured (to the nearest 1 mm), and identified most macroinvertebrates to the lowest taxonomic level possible (typically genus) using standard keys (Merritt et al. 2008). We identified non-insects to order and Chironomidae (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 et al. 2008).

We calculated biomass of each individual based on the measured length (to the nearest 1 mm) and published genus-specific length–mass regressions (Benke et al. 1999). If a length–mass regression was not available, we used the regression for the closest related taxon. We then calculated total aggregate macroinvertebrate biomass and biomass by FFG by replicate riffle or pool samples.

We calculated 3 standard measures of community structure—North Carolina Biotic Index (NCBI) (Lenat 1993), Family Biotic Index (FBI) (Hilsenhoff 1988), and total taxon richness. We assigned tolerance values to each taxon based on NCBI (Lenat 1993) and 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

Table 1. Landscape drivers, proximate stressors (physicochemical variables), and biological covariates at rural (RUR), mixed (MIX), suburban (SUB), and urban (URB) streams in 6 watersheds in the Athens, Georgia, USA, area. ISC = impervious surface cover, ag = agriculture, area = watershed area, DIN = dissolved inorganic N, SRP = soluble reactive P, TSS = total suspended solids, Chl *a* = chlorophyll *a*, FBOM = fine benthic organic matter. Numbers in parentheses are standard errors.

Site	% ISC	% forest	% ag	Area (km <sup>2</sup> )	DIN (μg/L)	SRP (μg/L)	Conductivity (μS/cm)	TSS (mg/L)	pH	Chl <i>a</i> (mg/m <sup>2</sup> )	FBOM (mg/m <sup>2</sup> )
RUR1	0.1	75.0	2	2.4	598.8 (77.1)	6.8 (3.9)	57.9 (<0.01)	20.5 (10.9)	6.9 (0.1)	20.7 (5.3)	46.5 (24.5)
RUR2	1.7	26.4	33	22.2	539.3 (86.3)	2.3 (1.0)	40.0 (<0.01)	24.1 (14.3)	6.5 (0.1)	39.9 (7.1)	16.9 (5.0)
RUR3	3.9	34.9	38	2.5	153.8 (26.3)	5.4 (1.9)	39.4 (<0.01)	4.6 (0.9)	7.4 (0.3)	36.3 (5.2)	13.7 (5.4)
MIX1	10.8	33.1	11	32.4	446.9 (71.2)	2.7 (1.1)	55.1 (<0.01)	13.8 (2.5)	7.6 (0.3)	24.7 (3.2)	10.0 (1.2)
MIX2	9.2	41.7	9	12.7	465.2 (56.8)	2.1 (1.1)	52.6 (<0.01)	12.8 (2.7)	7.1 (0.1)	29.4 (4.9)	22.8 (3.9)
MIX3	6.9	41.6	10	12.1	548.4 (47.8)	3.2 (1.2)	51.2 (<0.01)	18.2 (3.1)	6.7 (0.2)	46.8 (8.7)	16.2 (7.4)
SUB1	16.6	27.4	0	6.9	478.9 (66.5)	2.6 (1.5)	68.7 (<0.01)	6.1 (1.4)	7.3 (0.2)	31.1 (5.7)	5.9 (1.8)
SUB2	14.5	28.5	0	1.1	442.3 (53.8)	5.1 (2.0)	66.6 (<0.01)	3.7 (0.9)	7.2 (0.2)	30.3 (7.3)	5.0 (1.7)
SUB3	28.5	8.9	0	1.1	972.5 (189.0)	4.8 (1.3)	68.5 (<0.01)	7.0 (1.8)	7.0 (0.1)	26.2 (7.5)	4.8 (1.3)
URB1	32.9	8.7	0	4.9	697.3 (84.7)	6.0 (2.7)	99.6 (<0.01)	7.8 (5.4)	7.3 (0.3)	31.0 (6.6)	25.3 (23.7)
URB2	32.8	19.1	0	1.5	495.4 (123.3)	5.5 (1.8)	61.5 (<0.01)	4.5 (2.2)	7.2 (0.3)	15.0 (3.2)	4.9 (1.8)
URB3	32.8	3.9	0	1.6	714.3 (97.3)	3.7 (1.3)	80.2 (<0.01)	3.5 (0.7)	7.6 (0.2)	55.8 (9.9)	5.0 (1.2)

values for each genus to correct for winter/spring collection (Lenat and Crawford 1994). We also used the NCBI tolerance values to assign taxa to sensitive vs tolerant categories to examine trends in biomass of sensitive vs tolerant taxa across sites (see below).

### Water chemistry

We collected monthly samples for water chemistry at baseflow conditions from June 2009 to May 2010. We field-filtered samples for NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N, and PO<sub>4</sub><sup>3-</sup>-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. We analyzed samples for NH<sub>4</sub><sup>+</sup>-N, NO<sub>3</sub><sup>-</sup>-N and soluble reactive P (SRP) using continuous flow colorimetry (APHA 1998). We used mean values for SRP and dissolved inorganic N (DIN = NH<sub>4</sub><sup>+</sup>-N + NO<sub>3</sub><sup>-</sup>-N) to characterize each stream reach. We quantified total suspended solids (TSS) by filtration (APHA 1998). We measured conductivity and stream temperature continuously in each reach from February 2009 to May 2010 with a data sonde (Manta X2; Eureka, Austin, Texas). A summary of physical and chemical characteristics is given in Table 1.

### Fine benthic organic matter (FBOM) and algal biomass (chlorophyll *a*)

We sampled stream biofilms and analyzed them for chlorophyll *a* (Chl *a*) and organic matter content. We collected biofilms from both hard (erosional areas dominated by gravel, cobble, or bedrock substrates) and soft (depositional areas dominated by fine particle) substrates every

other month from May 2008–April 2010. On each sampling date, we randomly chose 6 transects and collected 5 subsamples at each transect for a total of 6 composite samples. We sampled hard substrates with a modified Loeb sampler (4.9 cm<sup>2</sup> area) (Loeb 1981) or by scrubbing 4.9 cm<sup>2</sup> of cobble in a tray with a toothbrush. Soft substrates were sampled using a polyvinyl chloride (PVC) tube (9.8 cm<sup>3</sup> volume). We collected the top 1 to 2 cm of sediment by slipping a spatula beneath the core opening and then emptied the corer into 500-mL Whirlpack™ bags and filtered the contents in the laboratory onto preweighed 0.7-μm glass-fiber filters. We extracted Chl *a* from one set of filters in the dark in 90% acetone and measured it spectrophotometrically (Wetzel and Likens 2000). We used another set of glass-fiber filters to calculate organic matter content. Filters were dried at 55°C for 48 h, weighed, combusted in a muffle oven at 500°C and reweighed to obtain ash-free dry mass (AFDM). This method quantified all organic matter associated with hard and soft substrates, but we refer to it as FBOM because FBOM dominated the composition of this material. Mean Chl *a* and FBOM used in subsequent analyses were based on 12 bimonthly means (based on both hard and soft substrates) from each site. We did not comprehensively measure all C resources (which would have included coarse fractions, such as leaf litter and wood) in our streams. However, we used FBOM as an indicator of retained organic matter and Chl *a* as an indicator of available algal resources.

### Data analyses

We first examined differences in macroinvertebrate community structure as a function of landscape-level drivers, physicochemical stressors, and biological covariates with

canonical correspondence analysis (CCA). CCA is a constrained ordination technique used to test relationships between a response matrix and a matrix of explanatory variables (ter Braak 1986, Borcard et al. 2011). Our response matrix was composed of 77 macroinvertebrate taxa found among the 12 sites expressed in 2 forms: 1) taxon-specific density (no/m<sup>2</sup>) and 2) taxon-specific biomass (mg/m<sup>2</sup>). Our predictor matrix included landscape (% ISC, watershed area), physicochemical (DIN, SRP, TSS, pH, conductivity) and biotic (FBOM, Chl *a*) variables. We conducted a series of CCAs in which we first added landscape-level drivers, then physicochemical stressors, and then biotic covariates. These 3 groups have a natural hierarchical structure and covary, so our goal was to estimate the amount of variance explained at each level after accounting for that explained at higher levels. Thus, when adding a group of variables, we conditioned on the variables from the higher level(s). We repeated this process for both response matrices (density and biomass). We then ran the reverse process to assess how much variation biotic covariates and physicochemical stressors explained when landscape variables were added last. Examining each variable or group of variables after conditioning on all others (Borcard et al. 2011) is an alternative modeling approach, but the more constrained approach we used reflects our a priori hypotheses for how the system functions (e.g., FBOM and Chl *a* are governed by stressors, and stressors are determined by % ISC, among other factors).

We analyzed the relationships between total aggregate macroinvertebrate biomass ( $n = 96$ ) and: 1) landscape-level drivers (% ISC, % forest cover, watershed area), 2) nutrient stressors (DIN, SRP), 3) physicochemical stressors (total suspended solids, pH, conductivity), and 4) biotic covariates (FBOM, Chl *a*) based on a multilevel modeling approach (Gelman and Hill 2007). We separated physicochemical stressors because positive effects of nutrients on macroinvertebrate biomass and production have been observed in other studies (Riseng et al. 2004, Johnson et al. 2013), whereas the effects of other stressors were predicted to be negative (e.g., heavy metals as indicated by conductivity). In multilevel modeling, random effects are used 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 to distinguish it from sample-level variance. We predicted that biomass would differ between pool and riffle habitats (as Roy et al. 2003 found for density), so we included a factor for habitat in the model. If habitat did not improve model fit over the null model, we did not include it in subsequent candidate models. We ranked the resulting models using Akaike's Information Criterion (AIC; Burnham and Anderson 2002). We repeated this process using the log( $x$ )-transformed biomass of each of the FFGs for the models containing landuse variables and biological covariates as predictor variables. To

test whether relative compositions of FFGs differed among watersheds, we averaged biomass estimates from each sample and calculated % 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.

We also tested for shifts in biomass of tolerant vs sensitive macroinvertebrate taxa with increasing % ISC. We summed the biomass of taxa based on each taxon's individual NCBI tolerance value (Lenat 1993). A taxon was categorized as sensitive if it had an NCBI value  $\leq 5.7$  and tolerant if the value was  $> 5.7$ . A score of 5.7 indicates sites with 'good-fair' water quality according to the NCBI index (Lenat 1993). We used linear regression to assess whether biomass of tolerant and sensitive taxa changed across a gradient of % ISC. We used simple linear regression to relate site-level patterns of biotic indices (NCBI and FBI) and taxon richness with % ISC and identified relationships among these variables and macroinvertebrate biomass at each site. We did all calculations in the statistical package R (versions 2.12–3.1; R Project for Statistical Computing, Vienna, Austria).

## RESULTS

We identified 32,900 individual invertebrates across 12 sampling sites. The most common taxa collected at all sites were Chironomidae (Diptera), Oligochaeta, and Copepoda. Other common insects found at most sites were *Ephemera* sp. (Ephemeroptera), *Antocha* sp. (Diptera), and adult and larval Elmidae (Coleoptera). The most common FFG was collector-gatherer, dominated by Chironomidae and Oligochaeta. Shredder was the least common FFG. Benthic macroinvertebrate biomass estimates ranged from 14 mg/m<sup>2</sup> (SUB3) to 1780 mg/m<sup>2</sup> (RUR2) (Table 2), and density ranged from 446 individuals (ind)/m<sup>2</sup> (SUB3; 28.5% ISC) to 9439 ind/m<sup>2</sup> (RUR3; 3.9% ISC). Density and biomass in each sample were weakly but significantly correlated ( $r^2 = 0.06$ ,  $p < 0.05$ ,  $n = 93$ ).

### Effects of predictor variables on community structure

The constraining variables explained a total of 84.8% of the variance in the macroinvertebrate communities when expressed as density and 89.5% when expressed as biomass (CCA). For density, landscape drivers explained 35.5% of the variance alone, whereas physicochemical stressors explained 31% after accounting for landscape drivers, and biotic covariates explained 18.3% after accounting for the other 2 groups of variables (Table 3). Similar patterns were observed for community composition based on biomass, with physicochemical stressors explaining slightly more and biotic covariates explaining slightly less variance than for community composition based on density. Run in reverse, biotic covariates explained ~30% of the variance when entered first, with physicochemical stressors explaining the most

Table 2. Biotic indices, taxon richness, and mean total macroinvertebrate biomass rural (RUR), mixed (MIX), suburban (SUB), and urban (URB) sites in 6 watersheds in the Athens, Georgia, USA, area. NCBI = North Carolina Biotic Index (Lenat 1993; NCBI water-quality class, excellent: <4.18, good: 4.17–5.09, good–fair: 5.10–5.91, fair: 5.92–7.05, poor: >7.05), FBI = Family Biotic Index (Hilsenhoff 1988; FBI water-quality evaluation, excellent: 0.00–3.75, very good: 3.76–4.25, good: 4.26–5.00, fair: 5.01–5.75, fairly poor: 5.76–6.50, poor: 6.51–7.25, very poor: 7.26–10.00), ISC = impervious surface cover.

Site	% ISC	FBI	NCBI	Richness	Total biomass (mg/m <sup>2</sup> )
RUR1	0.1	5.35	5.11	30	1253
RUR2	1.69	5.04	5.58	27	1780
RUR3	3.9	5.86	5.88	25	1117
MIX1	10.8	5.84	5.99	18	425
MIX2	9.2	5.83	5.96	19	224
MIX3	6.9	5.95	5.96	16	443
SUB1	16.6	5.84	5.70	19	278
SUB2	14.5	5.83	5.90	18	343
SUB3	28.5	5.95	5.99	12	14
URB1	32.9	5.71	5.79	18	334
URB2	32.8	5.92	5.94	16	169
URB3	32.8	5.99	5.99	15	581

variance, and landscape variables explaining <10% of residual variation (Table 3). In general, physicochemical stressors explained the most variation in community structure.

### Effects of predictor variables on total and FFG macroinvertebrate biomass

**Effects of landscape-level drivers** We first tested for the effects of land use alone on total macroinvertebrate biomass and found that biomass decreased sharply as watershed % ISC increased (Fig. 2A). Model estimates indicated a ~7% decrease in total biomass for each 1% increase in % ISC (Table 4). Total macroinvertebrate biomass also increased with % forest cover (Table S1), but change in macroinvertebrate response was less for a given change in % forest than in % ISC. For every 1% increase in watershed forest cover, macroinvertebrate biomass increased 4.4%. In addition, macroinvertebrate biomass differed in the 2 habitat types we sampled. Pool habitat made a relatively larger contribution to overall total biomass; biomass was 46.3% lower in riffles than in pools (Table 4).

The biomass of predators, scrapers, and shredders all declined with increasing % ISC (Table 4, Fig. 2B–D), but other FFGs did not. At sites with 0.1–5% ISC, we collected fairly tolerant but large predators in the order Odonata, e.g., *Progomphus* sp. and *Cordulegaster* sp., and Plecoptera, such as *Suwalla* sp. and *Perlesta* sp. The dipterans Ceratopogo-

nidae and Tanyptodinae were a substantial portion of predator biomass at these sites as well. At urban sites with >28% ISC (URB1, URB2, URB3), the only predators collected were Ceratopogonidae and predatory Chironomidae.

For each 1% increase in % ISC, scraper biomass declined by almost ~6% (Fig. 2C, Table 4). Communities at rural sites were dominated by scraper taxa in the order Ephemeroptera, such as *Baetis*, *Centroptilum*, *Ephemerella*, *Drunella*, and *Choroterpes*. Macroinvertebrates in the family Elmidae also were present. We collected both adult and larval Elmidae at all rural sites. Very few scraper taxa were present at the urban sites (URB1, URB2, URB3). Elmidae larvae were present at URB1 and URB3, but no adult Elmidae were present at any urban site. At URB1 and URB2, we collected a small number of *Ephemerella* sp. that contributed a negligible proportion of total biomass. Biomass of collector–gatherers and filterers did not change with increasing % ISC, but filterer biomass increased 11.7% with an increase in watershed area of 1 km<sup>2</sup> (Table 4).

The relative proportions of FFGs changed with % ISC because of differential responses amongst FFGs. The proportion of collector–gatherers increased and the proportion of scrapers declined with % ISC (Fig. 3A, B). The proportions of predator biomass, filterer biomass, and shredder biomass were not significantly related to % ISC (Fig. 3C–E).

Watershed area and habitat (riffles vs pools) were important factors in predicting biomass of predators and collector–gatherers, but not of filterers, scrapers, or shredders. Model estimates predicted 70% lower predator bio-

Table 3. Results of canonical correspondence analysis (CCA) showing variance explained by each group of predictor variables, using taxa-level density (individuals/m<sup>2</sup>) and biomass (mg/m<sup>2</sup>) as response variables across the 12 study sites. In each analysis, groups of variables were added sequentially in the order listed after conditioning on those added previously. Landscape drivers included % impervious surface cover and watershed area; physicochemical stressors included dissolved inorganic N, soluble reactive P, total suspended solids, pH, and conductivity; and biotic covariates included fine benthic organic matter and chlorophyll *a*.

Predictor variable	Density	Biomass
Analysis 1		
Landscape drivers	35.5%	30.7%
Physicochemical stressors	31.0%	50.5%
Biotic covariates	18.3%	8.3%
(Unexplained)	15.2%	10.5%
Analysis 2		
Biotic covariates	31.1%	32.1%
Physicochemical stressors	45.0%	52.0%
Landscape drivers	8.7%	5.4%
(Unexplained)	15.2%	10.5%

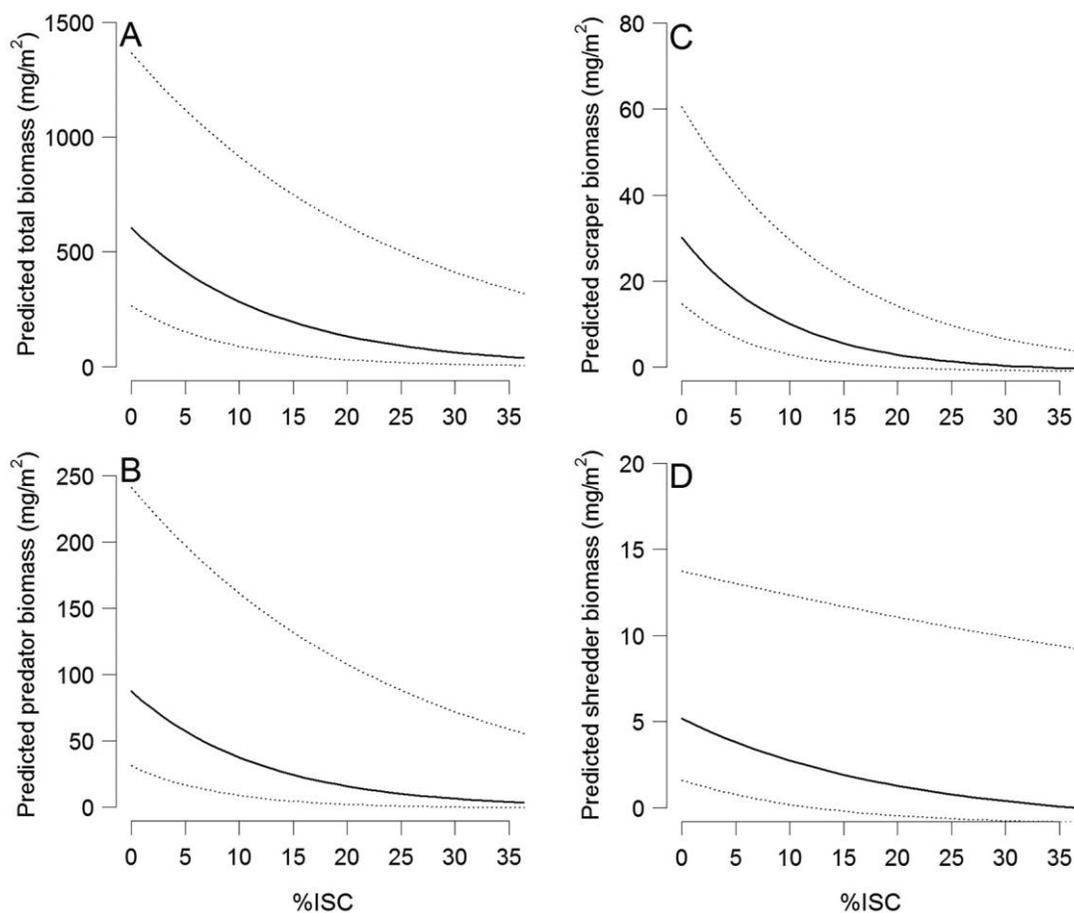


Figure 2. Predicted response of total macroinvertebrate (A), predator (B), scraper (C), and shredder (D) biomass to % impervious surface cover (ISC) for best overall models based on Akaike's Information Criterion. The best model (solid lines) and 90% confidence intervals (gray lines) of the relationship between total macroinvertebrate biomass and % ISC are shown. All other variables that were included in the best model were held constant (see Table 4 for parameters and confidence intervals).

mass and 46.5% lower collector–gatherer biomass in riffles than pools. Increased watershed area was associated with decreased predator and increased filterer biomass. Predator biomass decreased 6.9% and filterer biomass increased 11.7% with an increase in watershed area of 1 km<sup>2</sup> (Table 4).

**Effects of physicochemical and biological variables** DIN and conductivity were negatively related to macroinvertebrate biomass. The best model relating nutrient stressors (DIN and SRP) and habitat to biomass included both habitat type (biomass was greater in pools than riffles) and DIN concentration (Table 5). The best model relating contaminant stressors (habitat, TSS, conductivity, pH) to total macroinvertebrate biomass contained only habitat and conductivity. Total macroinvertebrate biomass declined as conductivity increased (Table 5).

Total macroinvertebrate biomass was positively related to FBOM and Chl *a* (Table 6). Shredder and predator biomass were positively related to FBOM, but filterer biomass was not (Table 6). Scraper biomass and Chl *a* gen-

erally were positively related, but Chl *a* was not included in the best model (Table S2).

#### Responses of biotic integrity and biomass to % ISC

Site-level biotic indices and taxon richness were negatively related to % ISC, which explained more variation for some cases (taxon richness, NCBI) than for site-level biomass (Table 7). Of the biotic metrics tested, total taxon richness was most negatively correlated with % ISC ( $r^2 = 0.76$ ,  $p < 0.001$ ,  $n = 12$ ). For example, for every 5% increase in % ISC, ~11 taxa are lost. Trends for FBI and NCBI were largely driven by RUR1 and RUR2 (Table 2). Macroinvertebrate biomass was significantly related to all biotic indices (Table 7).

#### Responses of tolerant and intolerant macroinvertebrates to % ISC

The biomass of both sensitive and tolerant organisms declined with increasing % ISC (Fig. 4A, B), but the biomass

Table 4. Parameter estimates, confidence intervals (CI), and % change for models with the lowest Akaike Information Criterion (AIC) value relating habitat type, % impervious surface cover (ISC), and watershed area to total macroinvertebrate and functional feeding group biomass. Riffle vs pool is the difference in biomass in riffles relative to in pools (riffle = 1). Change indicates the expected increase (+) or decrease (–) in macroinvertebrate biomass for each unit change in the predictor variable. N/A indicates that CIs for the estimate include 0, and we cannot determine the direction and the magnitude of change in macroinvertebrate biomass. SE = standard error.

Parameter	Slope (SE)	10% CI	90% CI	Change
<b>Total biomass</b>				
Intercept	6.40 (0.50)			
Riffle vs pool	–0.62 (0.32)	–0.10	–1.15	–46.3%
% ISC	–0.08 (0.02)	–0.04	–0.11	–7.3%
<b>Collector–gatherer biomass</b>				
Intercept	4.13 (0.37)			
Riffle vs pool	–0.63 (0.31)	–0.11	–1.14	–46.50
<b>Filterer–collector biomass</b>				
Intercept	0.12 (0.36)			
Riffle vs pool	0.54 (0.36)	1.13	–0.05	N/A
Area	0.11 (0.02)	0.15	0.07	+11.7%
<b>Predator biomass</b>				
Intercept	4.48 (0.62)			
Riffle vs pool	–1.20 (0.28)	–0.74	–1.67	–70.0%
% ISC	–0.08 (0.02)	–0.04	–0.12	–5.9%
Area	–0.07 (0.03)	–0.02	–0.12	–6.9%
<b>Scraper biomass</b>				
Intercept	3.44 (0.42)			
% ISC	–0.10 (0.02)	–0.07	–0.14	–5.9%
<b>Shredder biomass</b>				
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%

of sensitive organisms declined more steeply. Between 5 and 25% ISC, the model predicted a loss of 1444 mg/m<sup>2</sup> of sensitive macroinvertebrate biomass, whereas over the same gradient, the model predicted a loss of 978 mg/m<sup>2</sup> of tolerant macroinvertebrate biomass. A list of taxa, their FFGs and their tolerance categories is provided in Table S3.

## DISCUSSION

### Landscape to proximate drivers

Landscape-scale and more-proximate variables (physicochemical and biological) were similarly important in predicting effects of urbanization on both macroinverte-

brate biomass and community structure. The nature of our community structure models allowed us to assess the relative importance of the different drivers operating at different scales and through different pathways. Landscape-scale drivers explained a considerable amount, but not most, of the variance in assemblages when entered first in CCA. Physicochemical stressors had the greatest effects on community structure. Biotic covariates, if considered first, and physicochemical stressors explained ~75% of the variation in community structure, with little additional variation explained by landscape-level variables. Together, these analyses show that explanations of changes in macroinvertebrate communities can come from multiple levels of analysis ranging from the landscape to interacting biological structural components of streams. In our study, at least 2 hierarchical classes of variables were needed to explain >57% of the variability in macroinvertebrate communities among sites.

Physicochemical, biotic, and landscape variables all affected total macroinvertebrate biomass. We also found simple negative relationships between watershed % ISC and macroinvertebrate biomass, which is associated with an integrated ecosystem-level response, and measures of biotic integrity, which are functions of population-level responses. The fact that increased % ISC was strongly negatively related to both biotic indices and biomass has implications for stream ecosystem response to urbanization. Biological effects on ecosystem processes are a function of the biomass of organisms, populations, and communities, and the identities of the organisms involved (Power et al. 1996). Thus, our finding that urbanization affects both biomass and composition implies a 2-fold effect on stream ecosystem processes. In contrast, Helms et al. (2009) found altered community structure but higher macroinvertebrate biomass in urbanized streams. Systems in which diversity is reduced but biomass is elevated because of the abundance of tolerant taxa (i.e., Oligochaeta and Chironomidae) may still maintain a significant capacity to retain nutrients or organic matter and support higher-level organisms. However, in our study, urbanization resulted in dramatic quantitative declines in biomass (~4× higher in rural than urban sites) and in taxon richness (~2× higher in rural than urban sites). This result suggests significant differences in consumption, processing of materials, and available prey among the sites we studied.

### Mechanisms of impairment

Watershed urbanization was associated with steep declines in macroinvertebrate biomass, and these declines were related to higher watershed % ISC, higher streamwater conductivity, higher streamwater DIN, and lower retention of C resources (FBOM). These results are consistent with presumed mechanisms of degradation of stream health caused by landuse change that occurs via increases in contami-

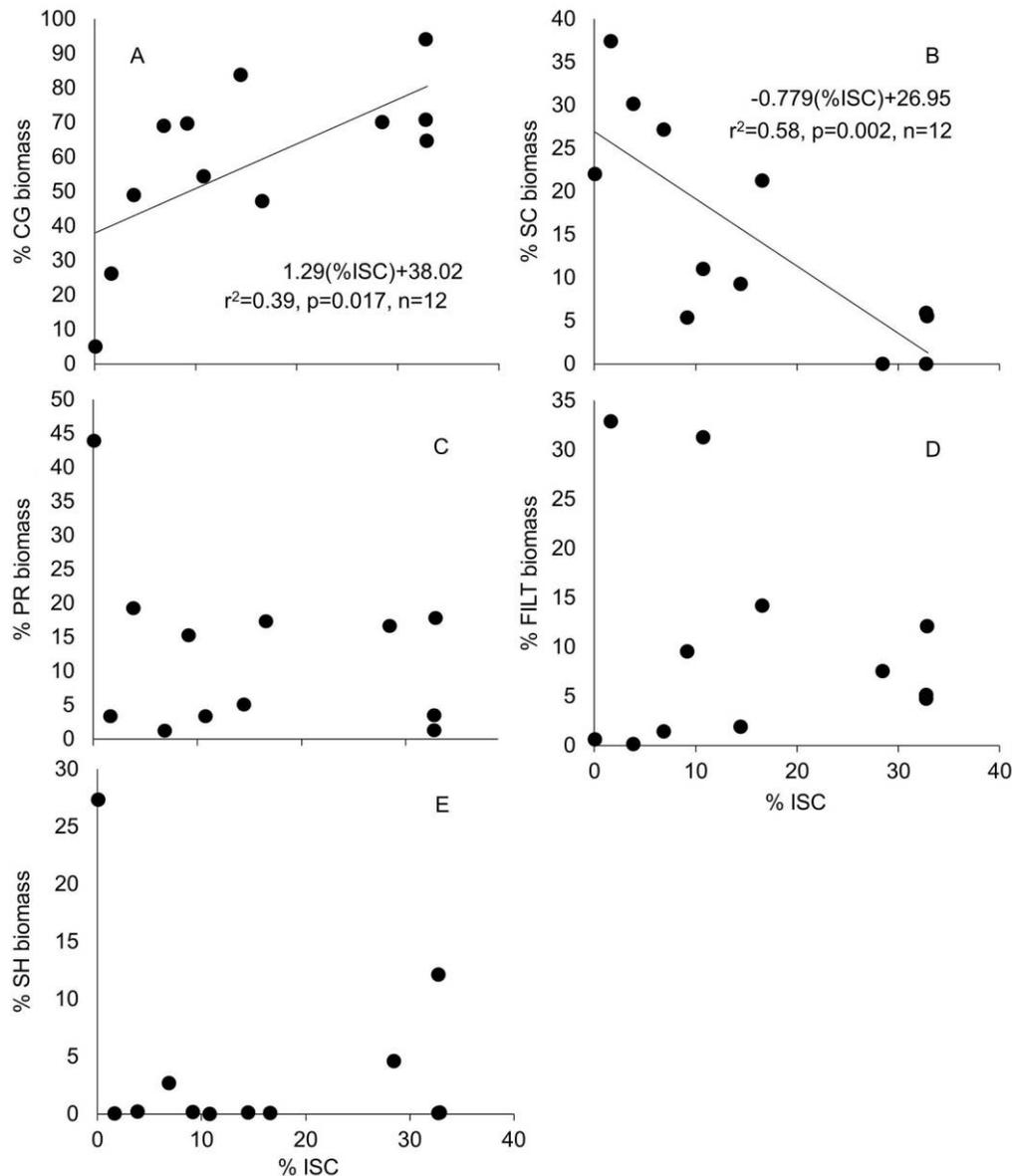


Figure 3. Relative contribution of collector–gatherers (CG) (A), scrapers (SC) (B), predators (PR) (C), filterers (FILT) (D), and shredders (SH) (E) to average total biomass at each site as a function of % impervious surface cover (ISC).

nants associated with urbanization (Woodcock and Huryn 2007, Johnson et al. 2013) and reduced input or retention of organic matter in urban streams (Woodcock and Huryn 2007). In contrast to our a priori hypothesis that macroinvertebrate biomass might be positively related to nutrient concentrations because of indirect effects on algal resources (Riseng et al. 2004) or other factors (Johnson et al. 2013), we found negative effects of DIN on macroinvertebrate biomass. Macroinvertebrate biomass in our study streams ranged from  $13.5 \text{ mg/m}^2$  at a suburban site (SUB2) to  $1780 \text{ mg/m}^2$  at a rural site (RUR2). These ranges are much lower than biomass values reported for some urban streams (e.g.,  $7000\text{--}100,000 \text{ mg/m}^2$  in Georgia, USA; Helms et al. 2009), but are comparable to values from other streams across

urban gradients (e.g.,  $\sim 4\text{--}1400 \text{ mg/m}^2$  in Maine, USA; Woodcock and Huryn 2007). The large reduction in biomass observed across our study sites was a result of negative effects of stressors associated with anthropogenic land use (DIN, conductivity) or other factors that we did not measure directly (e.g., altered hydrology) that are associated with landuse change.

#### Insights from patterns in FFGs

Patterns in FFG response give additional insights into drivers of assemblage structure and predicted effects of urbanization on stream ecosystems. Macroinvertebrate FFG composition varied along the gradient of % ISC with an

Table 5. Parameter estimates, confidence intervals (CI), and % change for models with the lowest Akaike's Information Criterion (AIC) value relating stream physicochemical variables (conductivity, total suspended solids, and pH) and water-column nutrient concentrations (dissolved inorganic N [DIN], soluble reactive P [SRP]) to total macroinvertebrate biomass. Riffle vs pool is the difference in biomass in riffles relative to in pools (riffle = 1). Change indicates the expected increase (+) or decrease (-) in macroinvertebrate biomass for each unit change in the predictor variable (conductivity = 10  $\mu$ S/cm, DIN = 50  $\mu$ g/L). SE = standard error.

Parameter	Slope (SE)	10% CI	90% CI	Change
<b>Physicochemical</b>				
Intercept	5.25 (0.36)			
Riffle vs pool	-0.64 (0.31)	-1.15	-0.13	
Conductivity	-0.39 (0.20)	-0.72	-0.07	-32.5%
<b>Nutrients</b>				
Intercept	5.23 (0.32)			
Riffle vs pool	-0.63 (0.31)	-1.14	-0.12	
DIN	-0.22 (0.07)	-0.34	-0.10	-19.9%

increased dominance of collector-gatherers as % ISC increased. At highly urbanized sites, collector-gatherers, specifically non-Tanytopodinae Chironomidae and Oligochaeta, made up ~60–90% of macroinvertebrate biomass. In rural sites, different FFGs dominated biomass. Predators were dominant at RUR1 (49%), scrapers and filterers at RUR2 (39%), and collector-gathers at RUR3 (48%), but all FFGs were present (Fig. 3A–E). Other investigators who have quantified shifts in the relative abundance (not biomass) of FFGs also have found a significant increase in the proportion of collector-gatherers with urbanization (Stepenuck et al. 2002, Compin and C er ghino 2007). In our study, the increase in dominance of collector-gatherers at sites with higher % ISC was not caused by an absolute increase in collector-gatherer biomass, but rather by a decrease in other functional groups, particularly predators and scrapers. Predator biomass was correlated with total biomass along the urban gradient (JLS, unpublished data). Small tolerant predatory Chironomidae dominated predator biomass in highly urban streams, whereas much larger Odonata and Plecoptera predators dominated predator biomass in less urban streams. The decline in scraper biomass along the urban gradient was dramatic both in absolute and proportional biomass. Biomass of scrapers declined faster than overall biomass and was near 0 at most urban sites (Fig. 3B). These results suggest predicted associated losses in ecosystem function (e.g., reduced processing of organic matter by shredders, removal of algae by scrapers, reduced transfer of energy to macroinvertebrate predators) in urban streams.

We hypothesized that changes in FFG composition could be caused by chemical stressors or by alterations in various food and habitat resources. Evidence supporting the strength of these pathways (negative effects of contaminants, positive effects of algae and retained C) is mixed, with contaminants probably being most important. The decline in more sensitive taxa as watershed % ISC increased and predictive relationships between community structure (richness, FBI, and NCBI) and % ISC suggest that multiple factors associated with urbanization, including increases in delivery of contaminants via stormwater probably were important across our sites. Several investigators have found a decline in sensitive macroinvertebrate

Table 6. Parameter estimates, confidence intervals (CI), and % change for models with the lowest Akaike's Information Criterion (AIC) value relating habitat type, fine benthic organic matter (FBOM), and chlorophyll *a* (Chl *a*) to total macroinvertebrate and functional feeding group biomass. Riffle vs pool is the difference in biomass in riffles relative to in 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<sup>2</sup>, FBOM ash-free dry mass = 5 g/m<sup>2</sup>). N/A indicates that CIs for the estimate include 0, and we cannot determine the direction and the magnitude of change in macroinvertebrate biomass. SE = standard error.

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

Table 7. Simple linear regression relating biotic indices, taxon richness, and site-level biomass to % impervious surface cover (ISC) and mean total biomass (BIOM) at each site. Percent ISC was  $\log(x)$ -transformed before analysis.

Data	<i>n</i>	Regression model	$r^2$	<i>p</i>
% ISC				
Richness	12	$-2.79(\text{ISC}) + 25.33$	0.76	<0.001
NCBI	12	$0.131(\text{ISC}) + 5.54$	0.70	<0.001
FBI	12	$0.119(\text{ISC}) + 5.52$	0.43	0.01
Biomass	12	$-237.08(\text{ISC}) + 1082.27$	0.52	0.004
BIOM				
Richness	12	$0.0087(\text{BIOM}) + 14.38$	0.73	<0.001
NCBI	12	$-0.00031(\text{BIOM}) + 5.99$	0.35	0.025
FBI	12	$-0.00046(\text{BIOM}) + 6.04$	0.67	<0.001

taxa with increased stream pollutants (Stepenuck et al. 2002, Roy et al. 2003, Helms et al. 2009). Macroinvertebrate biomass declines in our study were associated with increasing conductivity and nutrients (as DIN), but whether these variables are actually drivers or simply correlates of other contaminants is unknown. Some of our FFGs were composed of high proportions of taxa categorized as sensitive (scrapers: 91%, predators: 64%), whereas others had proportionally fewer sensitive taxa (filterers: 50%, collector-gatherers: 50%, shredders: 25% [shredder % based on a small number of taxa]). This comparison suggests that sensitivity to pollution could have been a factor driving changes in FFGs (given that the most dramatic decline in FFG biomass was for scrapers, which were largely represented by sensitive taxa) rather than decreased food resources.

#### Watershed urbanization and C resources

Total macroinvertebrate biomass was positively related to FBOM quantity, which may be an important measure of C availability or retention in urban streams. Other investigators have found that reduced macroinvertebrate biomass and secondary production in human-altered streams is associated with lower organic matter standing stocks (Walther and Whiles 2011) and reduced coarse particulate organic matter inputs (Gücker et al. 2011). In urban streams, detritus often is an important basal resource, but nutrient loading (Gulis et al. 2004, Imberger et al. 2008) and altered flow regimes (Chadwick et al. 2006, Paul et al. 2006) may increase the rate of organic matter processing, thereby altering the availability of terrestrially derived FBOM as a primary food source for benthic organisms (Kominoski and Rosemond 2012). Multiple factors in urbanizing streams, including loss of stream buffers, may interact to affect inputs, loss rates and retention of both coarse and fine fractions of C resources that are necessary for pro-

duction of organisms (Walther and Whiles 2011). However, hydrological disturbance or lack of inputs also could have affected biomass of both C resources and macroinvertebrates in our study streams. FBOM and % ISC were significantly negatively related across our study sites (Sterling 2012). Thus, FBOM and macroinvertebrate biomass may have been controlled similarly by watershed-scale characteristics (e.g., hydrology, reduced C inputs) without a causal link.

#### Species traits and responses to watershed urbanization

Macroinvertebrate communities in streams with the highest % ISC were characterized by low biomass of tolerant taxa dominated by collector-gatherers. Higher biomass of both sensitive and tolerant taxa in diverse FFGs

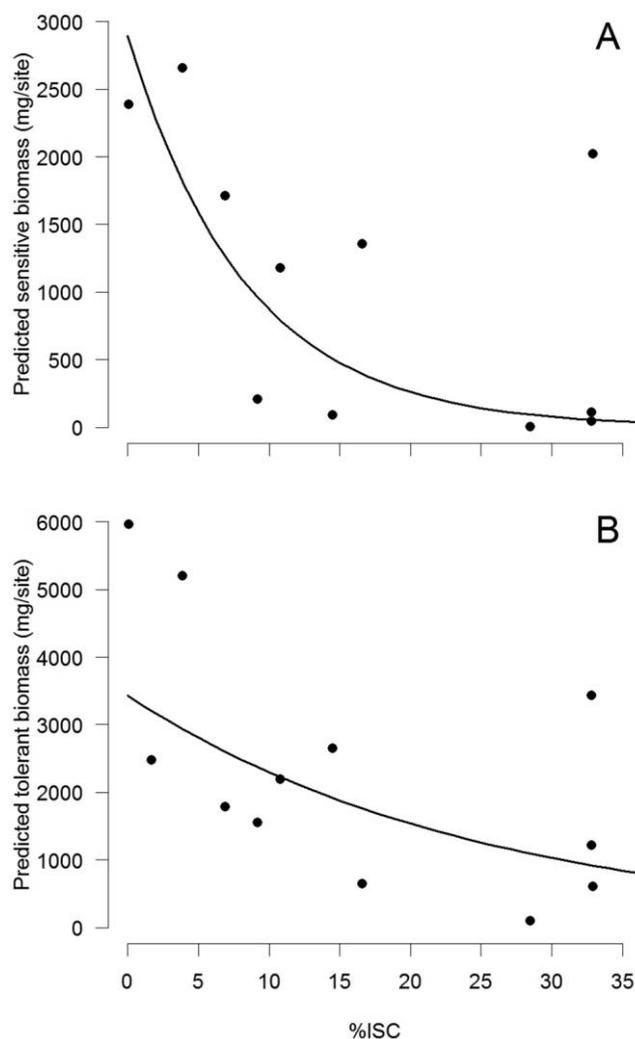


Figure 4. Linear regression models for the predicted biomass of sensitive (A) and tolerant (B) macroinvertebrates as a function of % impervious surface cover (ISC). Macroinvertebrate biomass was  $\log(x)$ -transformed before analysis.

characterized sites with lower watershed % ISC. We calculated a crude proxy of body size (biomass:density ratio) for all taxa, tolerant taxa, and sensitive taxa. This body-size proxy variable decreased with increasing % ISC for sensitive but not for tolerant or total taxa (but these taxa groupings showed similar trends; Sterling 2012). This result suggests that body size (i.e., biomass:density ratio) and feeding mode were important determinants of the macroinvertebrate community response to urbanization, consistent with much finer-scale analyses of macroinvertebrate traits and their response to landscape features (Lamouroux et al. 2004).

### Coupled community structure and biomass responses

We found that macroinvertebrate community structure and biomass were affected by the same urban-associated drivers. This result makes intuitive sense because both arise from the same phenomenon, which is the loss of or reduced colonization by individual organisms. However, these variables may not show similar responses under all circumstances. For example, individuals could be lost evenly across all taxa, leading to a loss of biomass but not a change in community structure, or sensitive taxa could disappear but be balanced by a compensatory increase in biomass of tolerant taxa. The similarity in response we observed was a result of differential sensitivity of taxa to stressors associated with urbanization. Large and sensitive taxa in certain feeding guilds were more susceptible to loss with watershed urbanization than were smaller and tolerant taxa. Whether altered community structure and biomass are similarly coupled in other systems is an important question that should be addressed in future studies. Either loss of biomass or a shift in community structure is expected to alter ecosystem function. The fact that the two are responding in synchrony in this case suggests that the effect on ecosystem functional responses could be even stronger than has been indicated previously by studies that were focused on community structure alone.

### Implications for stream ecosystem function and management recommendations

We showed that urban streams have lower macroinvertebrate biomass, simplified trophic structure, and reduced richness, indicating that macroinvertebrate contribution to energy flow and processing of materials was reduced across the landscape gradient that we studied. The loss of predator and scraper taxa and increased dominance of tolerant collector-gatherer taxa with increasing % ISC indicates altered community trophic dynamics. These changes were driven by increases in physicochemical stressors and reductions in C resources. This material may have been more abundant at less disturbed sites because of a more stable hydrologic regime or increased C inputs. To promote and restore biomass and functional diversity of macroinver-

tebrates in urban streams, we recommend restoration that promotes the input and storage of organic matter and reduces the delivery of contaminants. Creative stormwater management solutions that reduce stormflow inputs and increase stormwater filtration, especially during smaller, more frequent storm events (Walsh et al. 2005) provide improvements to both of these pathways simultaneously. Higher biotic integrity and associated macroinvertebrate biomass-driven ecosystem functions in streams can potentially be achieved where such solutions can be implemented.

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