Pulling apart the urbanization axis: patterns of physiochemical degradation and biological response across stream ecosystems

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Abstract: Watershed urbanization introduces a variety of physical, chemical, and thermal stressors to receiving streams and leads to well-documented declines in the diversity of fish and macroinvertebrates. Far less knowledge is available about how these urban stressors affect microbial communities and microbially mediated ecosystem properties. We examined 67 chemical, physical, and biological attributes of streams draining 47 watersheds in the metropolitan area surrounding Raleigh, North Carolina. Watersheds ranged from undeveloped to 99.7% developed watershed area. In contrast to prior investigators, we found no consistent changes in habitat structure, channel dimensions, or bed sediment size distributions along the urbanization gradient. Watershed urbanization led to large and consistent changes in receiving stream chemistry (increases in NO_3^-, bioavailable and algal-derived dissolved organic C, and the trace metals Pb, Cd, and Zn) and thermal regimes. These chemical and thermal changes were not associated with any consistent shifts in microbial community structure or taxonomic richness, based on terminal-restriction fragment length polymorphism and pyrosequencing methods, despite the fact that these urban stressors were associated with commonly reported declines in macroinvertebrate taxonomic richness and altered macroinvertebrate community composition. Chemical and thermal changes as a function of % developed watershed area also were unrelated to shifts in microbially mediated biogeochemical processes (C mineralization, denitrification potential, and substrate-induced respiration). A broad urbanization gradient sampled in this region suggests that stream ecosystem responses to watershed urbanization can follow diverse trajectories.
Key words: urbanization, land cover, water quality, water temperature, urban stream syndrome, ecosystem functions, environmental gradients, dissolved organic matter, species composition, benthic macroinvertebrates

Urbanization is associated with a suite of physical, chemical, and thermal stressors on receiving streams (Paul and Meyer 2001, Walsh et al. 2005, Coles et al. 2012). Among the best documented are increases in the frequency and intensity of physical stress as a result of urbanization, with increased peak flows (Booth and Jackson 1997, McMahon et al. 2003, Konrad and Booth 2005, Vietz et al. 2016b), decreased channel stability (Bledsoe and Watson 2001), and an altered sediment regime (Fletcher et al. 2014, Wohl et al. 2015, Vietz et al. 2016a, Russell et al. 2017). Urbanization often increases both the diversity and the degree of chemical stress, with well-documented increases in the concentrations of nutrients and salts (Paul and Meyer 2001, Kaushal et al. 2005, Bernhardt et al. 2008, Steele et al. 2010, Cooper et al. 2014, Wallace and Biastoch 2016), heavy metals (Callender and Rice 2000, Beasley and Kneale 2002, Sharley et al. 2016), and pesticides (Gilliom et al. 2006, Coles et al. 2012, Proia et al. 2013). Urban streams, like the urban heat islands they drain, frequently have modified thermal regimes, with urban stream biota regularly subjected to increased baseflow

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temperatures or pulses of heated stormwater runoff (Herb et al. 2008, Hester and Bauman 2013, Somers et al. 2013, 2016).

In addition to physical, chemical, and thermal stressors, urbanization also can be associated with labile C and nutrient subsidies (Sickman et al. 2007, Kaushal et al. 2011, Newcomer et al. 2012, Hale et al. 2016, Duncan et al. 2017). The combination of urban stressors and subsidies leads to considerable heterogeneity in the physical, chemical, and biological conditions of streams in urban river networks (Wenger et al. 2009, Kaushal and Belt 2012, Mueller Price et al. 2015), a topic of recent research (Booth et al. 2016, Parr et al. 2016, Utz et al. 2016). For example, many studies have highlighted the variability in hydrologic (Hopkins et al. 2015, Bhaskar et al. 2016, Booth et al. 2016, Vietz et al. 2016b), geomorphic (Utz and Hilderbrand 2011, Booth et al. 2016), chemical (Beaulieu et al. 2012), and biological (Feld et al. 2013, Clements et al. 2016, Walsh and Webb 2016) responses to urban land cover (Utz et al. 2016). Together, these studies show that not all urban streams tend to follow the same trajectories (Hale et al. 2016). However, what causes urban streams to follow diverse trajectories or which specific urban stressors have the strongest effects on microbial communities and associated ecosystem processes are not fully understood.

Although the effects of urbanization on macrofauna have been well characterized (Jones and Clark 1987, Lenat and Crawford 1994, Roy et al. 2003, Morgan and Cushman 2005, Brown et al. 2009, King et al. 2011), microbes and microbially mediated biogeochemical functions have received far less attention. Many of the chemical stressors in urban streams that affect sensitive macroinvertebrates and fish may be experienced by microbes as energy subsidies. For example, inputs of labile dissolved organic C (DOC) (Newcomer et al. 2012, Hosen et al. 2014, Potter et al. 2014), nutrients (Grattan and Suberkropp 2001, Gulis and Suberkropp 2003, Greenwood et al. 2007), light (Sudduth et al. 2011), and heat (Acuña et al. 2008) may stimulate microbial activity and increase the rates of microbial processes. We would expect urbanization to alter stream microbial community composition and affect stream ecosystem process rates (Perryman et al. 2008, Wang et al. 2011, 2014), but the direction and magnitude of these responses are poorly known (Wakelin et al. 2008, Perryman et al. 2011).

The suite of urban stressors and subsidies and associated biotic and ecosystem responses has collectively been described as a typical urban stream syndrome (USS; Walsh et al. 2005), but considerable variability occurs in the magnitude and timing of chemical and thermal inputs supplied by urban surfaces to receiving streams (Poole and Berman 2001, Peters 2009, Kaushal and Belt 2012, Ramírez et al. 2014). Broad gradient studies measuring a wide range of variables are helpful for separating out the drivers of heterogeneous responses to urbanization. During the National Water Quality Assessment Program's study on the Effects of Urban Development on Stream Ecosystems (EUSE) (Giddings et al. 2007, Brown et al. 2009, Coles et al. 2012), US Geological Survey (USGS) personnel measured an extensive suite of hydrologic, chemical, and biological variables, but did not include stream ecosystem function and stream microbial community structure. Linking changes in water quality to microbes and microbially mediated processes is critical because these are well-recognized ecosystem functions and services (Wenger et al. 2009). To date, no investigator conducting a broad urbanization gradient study has simultaneously characterized stream microbial and macroinvertebrate communities and microbially mediated ecosystem functions.

We asked: 1) How are physical and chemical properties affected by urbanization in the Raleigh–Durham, North Carolina region? 2) Do macroinvertebrate and microbial responses to urbanization follow similar patterns? 3) Which of the many components of the urban stressor gradient might best predict variation in microbial community composition and function?

METHODS

Site selection

Our study was conducted in the Raleigh–Durham metropolitan area, one of the most rapidly developing regions of the USA. Our goal was to select sites spanning the full urbanization gradient. Details about the site-selection process and land-cover calculations were presented by Somers et al. (2013). We started with sites from a number of sources: 12 previously studied streams (Sudduth et al. 2011, Violin et al. 2011), 16 EUSE sites (Giddings et al. 2007), 44 North Carolina Department of Water Quality (DWQ) sites, and 36 Durham Stormwater (DSW) sites. We characterized the land cover of these watersheds and an additional pool of 270 potential sites in the region chosen from low-order streams upstream of or near roads.

We characterized land cover by using a geographic information system (GIS) to calculate multiple indices describing the extent of development within each watershed (ArcGIS, version 9.3; Environmental Systems Research Institute, Redlands, California). We used 30-m land-cover data (Sexton et al. 2013) to calculate the % developed, forested, and field cover in each watershed as of 2005. Developed land in close proximity to a stream will have a greater effect than more distant development on the stream (King et al. 2005), so we also calculated the percentage of the 100-m stream buffer within the watershed that was developed. To account for transit-related effects, we calculated the density of roads and number of road–stream crossings in each watershed and the distance-weighted traffic volume, which incorporated both traffic intensity and distance from roads to streams along flow paths (NCDOT 2007). Last, we calculated % riparian canopy cover in the 100-m reach upstream of the study

site as the proportion of 10-m grid cells where the stream was visible in 2008 aerial photos (NAIP 2008).

We used % developed watershed area to characterize the urbanization gradient across the study region, and prospective sites were added to the existing research sites such that we fully populated the development gradient across the landscape, rather than focusing on the most and least developed sites (Fig. 1). Working in teams, we conducted initial visits to 118 potential sites on 19 May 2009. On the initial visit, we included sites that were accessible and for which we could delineate a reach ≥100 m upstream of the access point without a road crossing. The final data set for this analysis consisted of 47 sites: 12 sites from our previous research, 8 sites from the EUSE study, 3 DSW sites, 1 DWQ site, and 23 sites from the pool of additional potential sites. Watershed areas ranged from 0.40 to 26.88 km^2 with a median of 3.92 km^2.

Field methods

Physical characterization For each selected site, we delineated a 100-m reach upstream of the access point and installed temperature data loggers (model UA-001-08; Onset Computer Corporation, Bourne, Massachusetts) programmed to collect data at 10-min intervals for a period of 30 d. We conducted physical characterization in the 100-m reach between May and August 2009. We classified habitat by walking the 100-m reach and recording the location and linear distance of each type of flow habitat (riffle, run, or pool). We measured wetted width at each flow habitat transition and every 10 m of reach length. We randomly selected 3 of these transects for cross-sectional measurements by stretching a string level and tape measure across bankfull width and measuring the height from bed to tape at a minimum of 7 points (top and bottom of each bank, thalweg, and both water edges). We calculated channel incision by dividing bankfull width by channel depth at the thalweg. We measured water depth and velocity at 5 points across each transect and canopy cover every 10 m in the thalweg with a spherical densiometer (Forest Densiometers, Bartlesville, Oklahoma) (Lemmon 1957). We also calculated canopy cover over each study reach from 2008 aerial photographs (NAIP 2008) by using a 10- × 10-m grid in ArcGIS and visually counting grid cells where the stream was visible (Somers et al. 2013). We characterized substrate with a Wolman pebble count of particles sampled at 100 randomly selected positions throughout each 100-m reach and used these data to calculate median particle size (D_{50}) and the additional particle-size metrics D_{15} and D_{84} (Wolman 1954). We were unable to classify habitats at 2 intermediate-development sites during late 2009 because of low flows caused by mild drought from reduced late summer rainfall (Palmer Hydrological Drought Index between −0.5 and −1) but returned to make those measurements in May through August 2010. In 2010, we resampled habitat at 13 sites that were sampled in 2009 to provide an interyear comparison for sites spanning the full urbanization gradient.

We collected benthic sediments from 5 transects at randomly selected points within each 100-m reach. For each transect, we collected sediments with 15-cm deep × 5-cm-diameter soil coring tubes. On occasions when a randomly selected transect was bedrock or a boulder and sediment

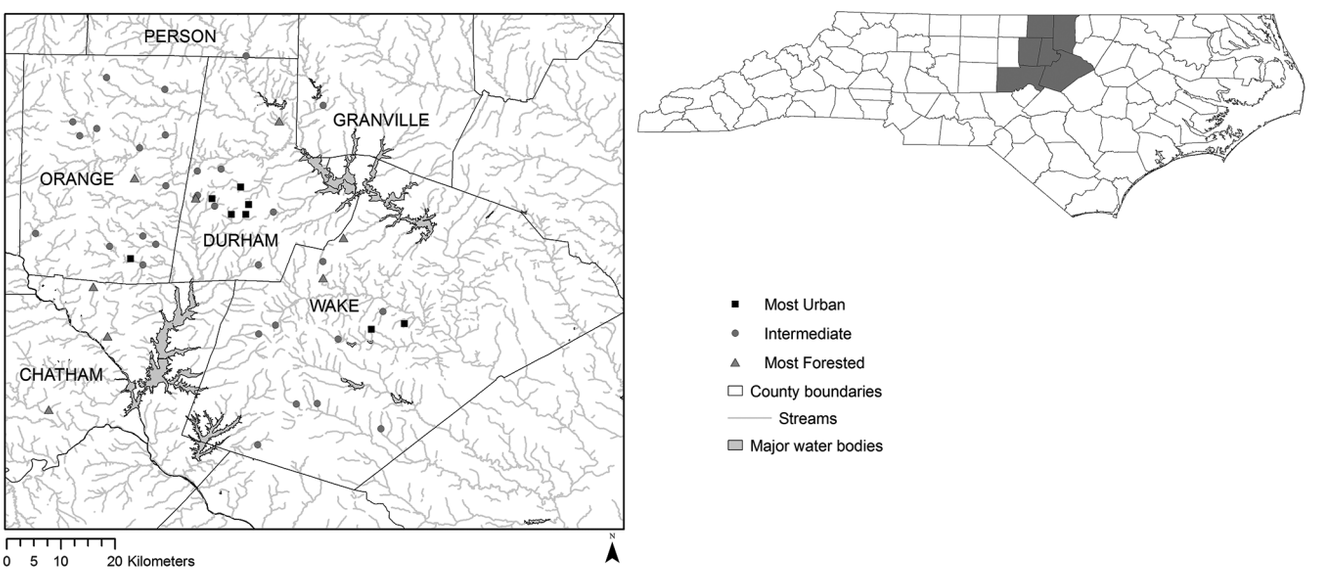

Figure 1. Map of study sites in Piedmont region of North Carolina, USA. Counties shaded gray contain study sites and are shown in the inset.

collection was impossible, we moved to the 1st appropriate point upstream where sediments could be collected. At each transect, we collected sediments until we had amassed a total of 50 cm of sediment depth. All sediments from a reach were composited and homogenized in the field by sieving sediments through a 2-mm sieve. After sieving, the composite sample was thoroughly mixed, and subsamples were collected in sterile specimen cups (for process measurements) and centrifuge tubes (for DNA extraction) and placed on ice until returned to the laboratory. Subsamples for molecular analysis were stored at −80°C, and all other subsamples were stored at 4°C for up to 48 h prior to analysis.

Chemical characterization We collected water-quality samples 3 times over the study: 1) the day of temperature logger deployment in May, 2) the day of temperature logger retrieval in June, and 3) during 1 additional visit in June through August. We collected both filtered and unfiltered water samples from the top of each reach in June through August 2009 to measure dissolved organic matter (DOM) lability. A 250-mL water sample was collected and filtered through a Whatman GF/F filter (nominal pore size = 0.7 μm) to remove particulates and then through a Millipore 0.2-μm membrane filter to remove most microbes. We also collected an unfiltered water sample from each site in a 60-mL acid-washed bottle. We initiated bioavailable dissolved organic C (bDOC) assays in the field by pouring 30 mL of membrane-filtered stream water into 8 amber glass I-CHEM vials that were then inoculated with 0.5 mL of unfiltered stream water from the same site. We immediately acidified 4 vials with 0.2 mL of 2 N hydrochloric acid to serve as preincubation DOC samples.

Biological characterization We sampled macroinvertebrates in May to August 2009 based on the North Carolina Department of Water Quality Qual 4 semiquantitative protocol (NCDWQ 2006). Each composited sample contained 1 kick-net sample (1 m^2 sampled for 2–3 min with 1-mm mesh) from a representative riffle, 1 sweep-net sample (500-μm mesh) from underbank vegetation and root wads, 1 leaf-pack sample from rock or snag habitat, and a visual inspection to collect invertebrates from large rocks or logs that were not easily sampled by the other methods. We sorted samples from debris in the field, preserved them in 95% ethanol, and returned them to the laboratory for identification.

Laboratory methods

Water analyses We analyzed water samples in the laboratory and report mean concentrations across the 3 dates for each site. We analyzed anions (NO_3^-, Cl^-, SO_4^{2-}, and PO_4^{3-}) on a Dionex ICS-2000 ion chromatograph with IonPac AS-18 analytical column (Dionex, Sunnyvale, California). We measured NH_4^+ with the o-phthaldialdehyde method described by Holmes et al. (1999) with a 10-AU field fluorometer (Turner Designs, San Jose, California). We measured total P and total N with a persulfate digestion (Qualls 1989) on unfiltered samples followed by simultaneous PO_4^{3-} analysis by the molybdate method with an PO_4^{3-} manifold and $NO_3^- + NO_2^-$ analysis using a hydrazine reduction method manifold with a QuikChem8500 flow injection analyzer (Lachat Instruments, Loveland, Colorado). We analyzed DOC and total dissolved N (TDN) with a Shimadzu TOC-VCPH with TNM-1 module (Shimadzu Corporation, Kyoto, Japan).

We incubated the bioavailable DOC assays that were inoculated in the field on a shaker table for 30 d in the dark. After incubation, we passed the samples through a 0.2-μm Millipore membrane and immediately acidified them with 0.2 mL of 2 N hydrochloric acid. We analyzed the initial concentration and incubated samples on a Shimadzu TOC-VCPH analyzer for nonpurgeable DOC and compared the initial and final concentrations to estimate DOM bioavailability (McDowell 1985, Qualls and Haines 1992, McDowell et al. 2006).

We used 4 replicate water samples collected on the day of temperature logger retrieval to measure absorbance and fluorescence spectra of the DOM with a Fluorolog-321 spectrofluorometer (Horiba Scientific, Edison, New Jersey). We analyzed fluorescence spectra as excitation–emission matrices (EEMs). We normalized ultraviolet absorbance by DOC concentration to calculate specific ultraviolet absorbance at 254 nm ($SUVA_{254}$) (Weishaar et al. 2003), and we calculated the spectral slope ratio from the absorbance spectra following Helms et al. (2008). All the EEMs were corrected for inner-filter effects and for instrument-specific excitation and emission corrections in Matlab (version 7.7; MathWorks, Natick, Massachusetts; Cory et al. 2010). We calculated the fluorescence index from the corrected EEMs (McKnight et al. 2001), and analyzed corrected EEMs with parallel factor analysis (PARAFAC) to characterize DOM quality (Stedmon et al. 2003). We validated a 5-component PARAFAC model on the data set of 300 EEMs by 4-way split-half analysis (Stedmon and Bro 2008). The DOM components represent 5 different groups of fluorescing molecules: component 1 represents humic DOM, component 2 represents recently microbially processed DOM, component 3 represents the most recalcitrant humic DOM of terrestrial origin, and components 4 and 5 represent N-rich DOM, with component 5 being the most bioavailable fraction (Stedmon and Bro 2003, Cory and McKnight 2005, Hosen et al. 2014, Parr et al. 2015).

Sediment analyses We initiated all sediment functional assays within 48 h of sample collection with field-moist sediment. We oven-dried subsamples from each site at 60°C and reweighed them to normalize all rates g^{-1} dry mass (DM). Dried subsamples were then combusted in a muffle

furnace at 500°C for 3 h and reweighed to determine % ash-free dry mass (AFDM).

We estimated sediment C mineralization rates based on 5 replicates from each site. We weighed 5 g of field-moist sediment into 40-mL amber glass I-CHEM vials that were then sealed with septa-lined open-top caps (Fierer et al. 2007b). We enclosed vials in a box and incubated them at room temperature. Headspace samples were collected after 24 h with a 1-mL gas-tight syringe, and CO_2 concentration was measured with an infrared gas analyzer (LI-6265; LI-COR, Lincoln, Nebraska) using N_2 as the carrier gas. We calculated C mineralization rates g^{-1} DM in addition to normalizing the rates g^{-1} AFDM.

We measured potential microbial activity with a modified substrate-induced respiration (SIR) assay (West et al. 1986, Fierer et al. 2003, Bradford et al. 2008) using 5 replicates for each site. We weighed 5 g of field-moist sediment into 40-mL amber glass I-CHEM vials and added 10 mL of a yeast extract solution consisting of 12 g autolyzed yeast extract/L of ultrapure deionized water. We sealed vials with septum-lined, open-top caps and incubated them in the dark on a shaker table. We collected headspace samples with a gas-tight syringe 10 min after adding the yeast solution and after 2 and 4 h of incubation and analyzed for CO_2 (as above), with headspace replacement with CO_2-free air after each sample. We calculated SIR rates per g DM in addition to normalizing the rates per g AFDM.

We estimated potential denitrification rates for each site with 5 replicate denitrification enzyme activity (DEA) assays based on the acetylene block technique (Tiedje et al. 1989, Groffman et al. 1999). We weighed 10 g of field-moist sediment into 125-mL Erlenmeyer flasks and added 20 mL of DEA media (containing potassium nitrate, glucose, and chloramphenicol in ultrapure deionized water). We stoppered the flasks and made the slurries anoxic via successive vacuum and N_2 flush cycles. We added acetylene to block conversion of N_2O to N_2 and collected headspace samples after 0, 30, 60, and 90 min. We measured N_2O concentrations on a Shimadzu GC-17A gas chromatograph with a Teledyne Tekmar 7000 headspace autosampler (Teledyne Tekmar, Mason, Ohio). We calculated DEA rates g^{-1} DM in addition to normalizing the rates g^{-1} AFDM.

We used a strong acid digestion of sediment samples to analyze for metals concentrations that could become environmentally available. Prior to digestion, we combined 2 sediment subsamples from each site and passed them through a 1-mm sieve. We dried the ≤1-mm size fraction at 60°C for 48 h and ground it with a mortar and pestle prior to weighing into digestion tubes. We digested 3 replicate samples for each site by EPA method 3050B (USEPA 1996) on a block digester. We ran 3 replicates of certified reference materials STSD-3 (NRC, Canada), and we analyzed 2 method blanks for every 35 samples. Following digestion, we analyzed samples for Zn, Pb, and Cd on an inductively coupled plasma–mass spectrometer (Perkin–Elmer Elan6000; Perkin–Elmer, Waltham, Massachusetts). We normalized metals concentrations to the AFDM values for each site.

Macroinvertebrate identification and analysis We identified all macroinvertebrates except Chironomidae to genus or lowest taxonomic level (Smith 2001, Merritt et al. 2008), and Chironomidae to family. We categorized taxa as abundant (≥50 individuals [ind]), common (10–49 ind), few (3–9 ind), uncommon (2 ind), or single (1 ind). We calculated total richness and richness of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) as a measure of sensitive taxon richness. We calculated the Biotic Index (BI) at the genus level to quantify overall macroinvertebrate community pollution tolerance. We calculated BI as a weighted mean of taxon tolerance values relative to their abundance category value (NCDWQ 2006). A higher BI value indicates a more tolerant community. Tolerance values were taken from the North Carolina Department of Water Quality (NCDWQ) benthos standard operating protocol (Lenat 1993, NCDWQ 2006). The few taxa for which tolerance values were not available were excluded from the BI calculations. We used *ecodist* (Goslee and Urban 2013) in R (version 3.3.1; R Project for Statistical Computing, Vienna, Austria) to ordinate sites in species space based on nonmetric multidimensional scaling (NMDS). Prior to NMDS ordination, we excluded rare taxa (defined as present in <5% of samples) and then created an input matrix of abundance category values, as in the BI calculation. We performed the ordination based on Bray–Curtis dissimilarities of $\sqrt{(x)}$-transformed taxon abundance category values and obtained solutions from 500 runs using random starting coordinates. We rotated the results to principal components, such that NMDS axis 1 had the greatest compositional variation. Variation decreased with successive axes, and all NMDS axes were orthogonal.

We incorporated a 2nd matrix of the functional, thermal, physical, water quality, contaminant, and landscape variables and calculated correlation coefficients of geomorphological and functional variables correlated with macroinvertebrate community structure at different sites. We calculated correlations between each variable and the rotated NMDS ordination axes. To investigate statistical differences in community composition resulting from site category, we performed permutation multivariate analysis of variance (PERMANOVA, Anderson 2001) with the *adonis* function in the *vegan* package (version 2.4-1; Oksanen et al. 2016) in R. We used Bray–Curtis dissimilarity of $\sqrt{(x)}$-transformed taxon abundances and calculated the pseudo *F*-statistic based on 999 permutations.

Microbial community analyses We used terminal-restriction fragment length polymorphism (T-RFLP) analyses to esti-

mate shifts in the composition of microbes carrying the functional denitrification genes (*nirK*, *nirS*, and *nosZ*) (Osborn et al. 2000, Fierer et al. 2007a) and pyrosequencing (e.g., Margulies et al. 2005) to estimate shifts in overall taxonomic richness and microbial community composition. DNA was extracted from triplicate sediment samples with PowerSoil kits (MoBio Laboratories, Carlsbad, California). We ran polymerase chain reactions (PCRs) with Hot Start Taq Master Mix (Sigma Aldrich, St Louis, Missouri). For the functional gene T-RFLPs, we performed PCR with each of 2 primers: *nirK* (functional gene for NO_3^- reductase) and *nosZ* (functional gene for N_2O reductase). We were unable to amplify *nirS* successfully with primer set nirS1F and nirS6R (Braker et al. 1998) and, after repeated attempts, abandoned this analysis.

The *nirK* primer set was nirK1F and nirK5R (Braker et al. 1998). The *nosZ* primer set was nosZ-F and nosZ1622R (Throbäck et al. 2004). We cleaned PCR products with QIAquick PCR purification kits (QIAGEN, Germantown, Maryland), checked them for appropriate sizes by agarose gel electrophoresis, and then digested them with endonuclease HaeIII for *nirK* products and endonuclease MnlI for *nosZ* products to generate T-RFLP profiles. All endonucleases were from New England Biolabs (Ipswich, Massachusetts). We ran subsequent electrophoresis on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, California).

To process T-RFLP data, we used T-REX software (http://trex.biohpc.org/; Culman et al. 2009) to define a baseline fluorescence threshold for filtering true peaks from background noise and to align terminal-restriction fragments (TRFs). The filtering algorithm in T-REX discards peaks that do not meet a user-specified standard deviation (SD) limit (in this case, we used 1 SD) (Abdo et al. 2006). After filtering, we aligned TRFs based on a clustering threshold of 0.5 base pair (bp) (Smith et al. 2005) and eliminated TRFs that appeared in

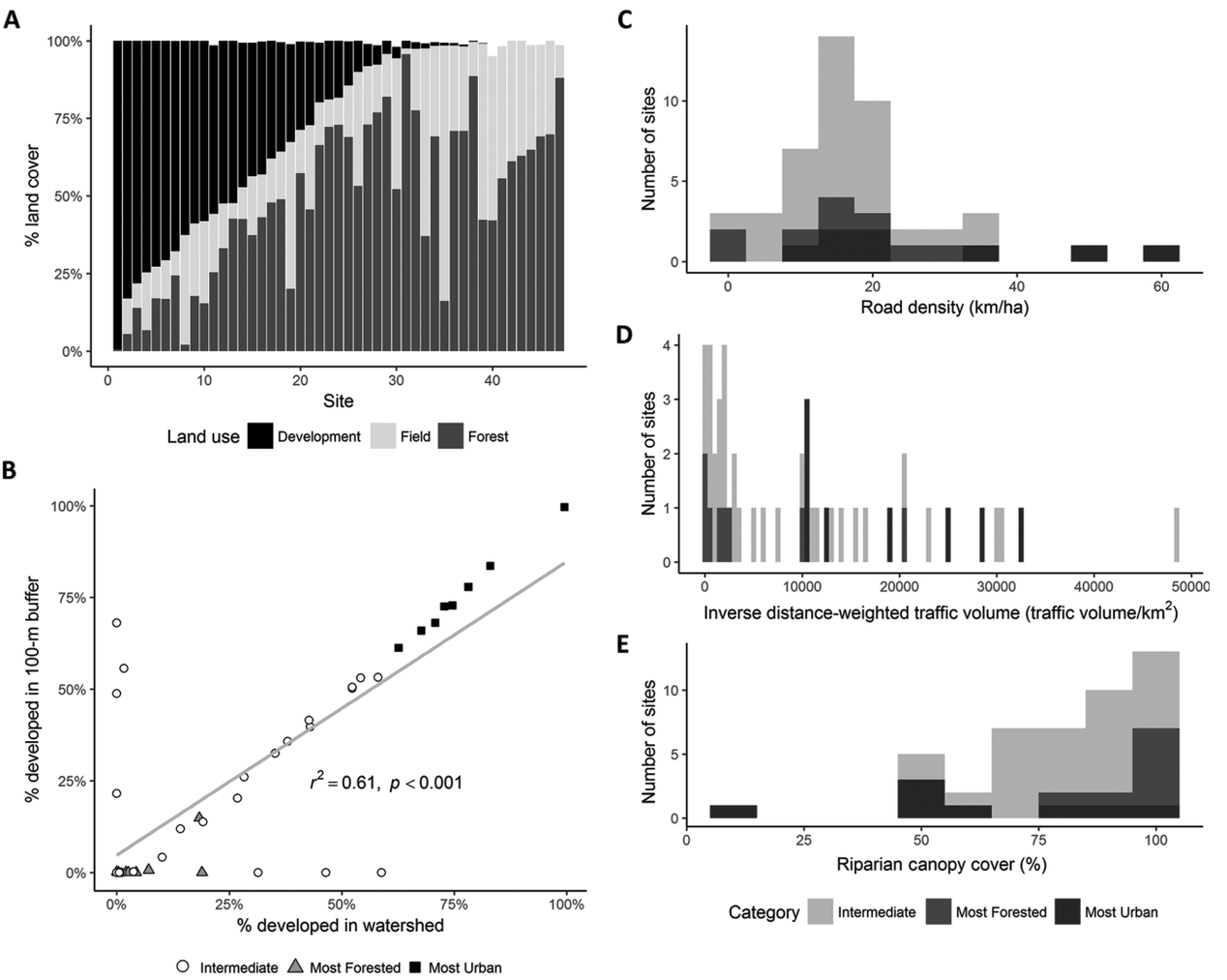

Figure 2. Land-cover distribution across the study watersheds (A), relationship between % developed watershed area and % developed area in 100-m buffer (B), and distributions of road density (C), distance-weighted traffic volume (D) and canopy cover (E) among most urban, most forested, and intermediate sites.

<5% of samples in the data set. Each unique TRF represents an operational taxonomic unit (OTU). For subsequent analyses of compositional differences among sites, we transformed the processed T-RFLP data into a matrix of relativized (by site total relative fluorescence units) abundances.

For the pyrosequencing data set, we used primers 27F-338R to amplify regions V1 and V2 of the 16S rRNA gene from the extracted DNA from 41 sites. Replicate PCRs were pooled and purified using QIAquick PCR Purification Kit (QIAGEN, Valencia, California). PCR products were normalized using SequalPrep™ Normalization Plate Kit (Applied Biosystems, Life Technologies, Grand Island, New York). We added 3 replicates each of purified PCR product with equimolar ratios to a set of barcodes with MID1–10. Samples were sequenced with a Roche 454 Life Sciences Genome Sequencer Flex Titanium Instrument (Branford, Connecticut) at the Genome Sequencing and Analysis Core Resource at Duke University (Durham, North Carolina).

We processed sequences with QIIME1.6 (Caporaso et al. 2010) and removed primers and chimera. Next, we used USEARCH to filter out low-quality sequences, check for chimera, and select OTUs from demultiplexed sequences (Edgar 2010). OTUs were chosen at 97% sequence similarity and identified using the SILVA, version 128 database (Quast et al. 2013).

We used NMDS ordinations in R to visualize patterns of dissimilarity in microbial community composition among study streams. NMDS ordinations of the microbial data set were performed using the *metaMDS* function in the *vegan* package with relativized T-RFLP data and Bray–Curtis distance measures. We incorporated a 2nd matrix of environmental variables in the ordination and calculated Pearson's correlation coefficients between the environmental variables and the ordination axes scores.

Linking land cover and stream data sets

We calculated summary statistics with the *psych* package (Revelle 2016) in R and used Pearson's correlation coefficients between all variables and % developed watershed area to identify the variables that were significantly affected by urbanization. We chose sites to populate the full range of % developed watershed area found throughout the region, but we realized during data analysis that the least-developed sites (8 sites with 0% developed watershed area) had an average of <64% forested land, with an average of 34% field. This situation reflects the reality of the Raleigh–Durham metropolitan area, where sprawling suburbs are slowly taking over a formerly agricultural landscape. This development gradient is not simply an inverse of a % forested gradient, so we used both a gradient and a categorical analysis to examine the effects of development. In the gradient analysis, we regressed each measured variable against % developed watershed area, whereas in the categorical analysis, we compared the 8 most urban to the 8 most forested sites. We were unable to collect macroinvertebrates at 1 of the most forested sites, so for that analysis, $n = 7$ for the most forested group. Missing AFDM data for one most urban site, so $n = 7$ for the most urban category for %AFDM, SIR/g AFDM, CMIN/g AFDM and DEA/g AFDM). For the categorical comparisons, we conducted 1-way analysis of variance (ANOVA) to determine whether significant differences occurred in each measured variable between the most forested and most urban sites. In cases where both correlative and the categorical responses agreed, we concluded that

Table 1. Pearson correlation and analysis of variance (ANOVA) results for physical and thermal differences across the survey watersheds. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

Metric	Full data set ($n = 47$) median (range)	Correlation with % developed	Most forested ($n = 8$) median (range)	ANOVA significance	Most urban ($n = 8$) median (range)
Habitat transitions (riffle–run–pool)/100 m	13.5 (4–40)	NS	17.5 (4–24)	NS	14.5 (5–40)
Channel incision	0.22 (0.04–0.44)	NS	0.23 (0.12–0.33)	NS	0.23 (0.11–0.34)
Median bed particle size (D_{50}) (mm)	13 (1–380)	−0.30*	17.5 (1–130)	NS	5 (1–51)
Degree days	10290 (9245–11580)	0.39*	9843 (9245–10842)	<*	10729 (9713–11565)
Minimum temperature (°C)	19.1 (17.6–21.3)	0.40*	18.6 (17.6–19.8)	<*	19.9 (17.6–20.8)
Maximum temperature (°C)	21.9 (19.3–25.1)	0.34*	20.9 (19.3–23.4)	<*	22.8 (21.2–25.1)
Max temperature change (°C) between subsequent measurements (full record)	0.38 (0.1–4.0)	0.71***	0.3 (0.2–0.3)	<**	1.5 (0.3–4.0)
Max temperature change (°C) during widespread storm	0.2 (0.1–4)	0.63***	0.19 (0.1–0.19)	<*	0.87 (0.1–4.0)
Width:depth ratio	18.8 (4.6–39.5)	−0.35*	21.3 (12.6–32.5)	NS	13.1 (11.1–29.5)

these responses were truly urban effects, whereas effects that were apparent only when end members were compared categorically were more likely to be caused by the loss of forested land cover.

RESULTS

Development effects on physical variables

Many land-cover attributes were strongly associated with urbanization (Fig. 2A–E). In particular, large increases in % developed watershed area in the 100-m buffer (Fig. 2B), road density (Fig. 2C), and distance-weighted traffic volume (Fig. 2D), and were positively correlated with increasing development ($r = 0.78$, 0.58, and 0.51, respectively, $p < 0.001$). Total riparian canopy cover estimated from aerial photographs (Fig. 2E) and % forested watershed area (Fig. 2A) were negatively correlated with development ($r = -0.42$ and -0.81, respectively, $p < 0.005$).

Contrary to predictions of the urban stream syndrome paradigm, we found no significant differences in the degree of channel incision or the number of flow habitat transitions between the most urban and most forested streams (Table 1). D_{50} was negatively correlated with development ($r = -0.30$, $p < 0.05$), but sediments were not significantly finer in the most urban than in the most forested sites (1-way ANOVA, $p > 0.05$; Table 1). Stream thermal regimes showed a consistent pattern with increasing urbanization. All measures of temperature (total degree days, minimum and maximum daily temperatures, and maximum temperature change between 10-min readings) were significantly greater in streams draining the most urban than in the most forested watersheds (1-way ANOVA, $p < 0.05$; Table 1). The most extreme heat pulse detected across all streams was a 4°C temperature increase in 10 min for a small stream with 78% developed area in the 100-m buffer. The same storm event caused an average increase of 1.5°C over 10 min in

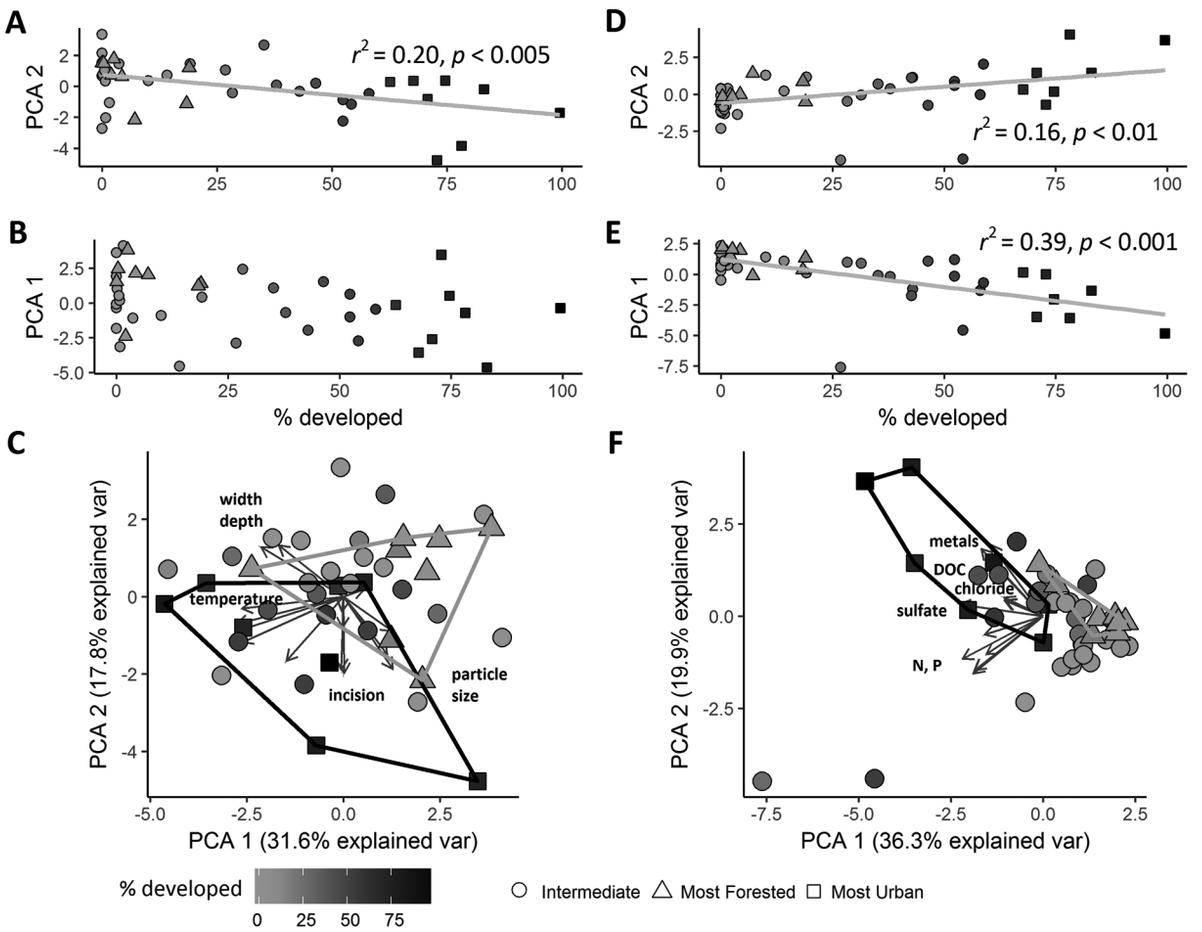

Figure 3. Correlation of principal components analysis (PCA) axis 2 (A, D) and 1 (B, E) loadings with % developed watershed area for physical (A, B) and chemical (D, E) variables and PCA biplots for sites and physical (C) and chemical (F) variables. Sites are identified as most urban, intermediate, and most forested, and color intensity represents the development gradient. Polygons show the most urban and most forested site clusters. Arrows show the strength and direction of correlation of environmental variables with the PCA axes. Var = variability.

Table 2. Pearson correlation and analysis of variance (ANOVA) results for water chemistry across the survey watersheds. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

Metric	Full data set ($n = 47$) median (range)	Correlation with % developed	Most forested ($n = 8$) median (range)	ANOVA significance	Most urban ($n = 8$) median (range)
Chemical substrates:					
NO_3^- (mg N/L)	0.327 (0.037–3.61)	0.29*	0.14 (0.04–0.60)	<*	0.89 (0.17–1.83)
NH_4^+ (mg N/L)	0.028 (<0.001–0.30)	NS	0.03 (0.02–0.04)	NS	0.04 (0.02–0.12)
Total dissolved N (mg N/L)	0.81 (0.16–7.63)	NS	0.4 (0.16–0.97)	<**	1.09 (0.81–2.08)
Total N (mg N/L)	1.12 (0.31–7.56)	NS	0.77 (0.31–1.28)	<*	1.3 (0.79–1.88)
PO_4^{3-} (mg P/L)	0.001 (<0.001–0.990)	NS	<0.05 (<0.05–0.08)	NS	<0.05 (<0.05–0.20)
Total P (mg P/L)	0.45 (0.10–1.56)	NS	0.45 (0.17–0.53)	NS	0.52 (0.28–1.05)
Dissolved organic C (mg C/L)	5.29 (2.35–14.62)	NS	4.09 (2.79–7.18)	NS	6.36 (2.35–9.39)
Chemical stressors:					
Cl^- (mg/L)	13.5 (3.2–105.8)	NS	8.76 (4.87–13.14)	<**	22.4 (16.11–55.69)
SO_4^{2-} (mg/L)	8.4 (1.2–54.7)	0.66***	4.09 (1.16–16.07)	<***	31.64 (5.12–48.18)
Zn (μg/g C)	23.2 (5.3–100.4)	0.55***	10.6 (5.6–36.3)	<*	44.6 (14–100.4)
Pb (μg/g C)	5.2 (1.25–44.5)	0.71***	4.09 (2.67–7.98)	<**	19.21 (5.74–44.52)
Cd (μg/g C)	0.025 (0.009–0.131)	0.72***	0.02 (0.01–0.02)	<**	0.05 (0.03–0.13)

Table 3. Pearson correlation and analysis of variance (ANOVA) results for microbial process rates and dissolved organic matter (DOM) composition and quality. DM = dry mass, AFDM = ash-free dry mass, DOC = dissolved organic C. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

Metric	Full data set ($n = 47$) median (range)	Correlation with % developed	Most forested ($n = 8$) median (range)	ANOVA significance	Most urban ($n = 8$) median (range)
Microbial process rates:					
Substrate-induced respiration (SIR) (mg CO_2 g^{-1} DM h^{-1})	18 (5.4–76)	NS	16 (8.9–42)	NS	24 (11–42)
C mineralization (CMIN) (mg CO_2 g^{-1} DM h^{-1})	0.50 (0.11–3.0)	NS	0.5 (0.29–1.8)	NS	0.84 (0.26–1.9)
Denitrification enzyme activity (DEA) (ng N g^{-1} DM h^{-1})	126 (18.7–941)	NS	107.4 (18.7–297.1)	NS	289 (98.2–562)
% AFDM: % sediment that is organic (by mass)	1.01 (0.1–6.17)	−0.34*	1.03 (0.59–3.6)	NS	0.92 (0.67–1.8)
SIR/g AFDM (mg CO_2 g^{-1} AFDM h^{-1})	15.9 (7.2–52.2)	0.58***	13.8 (7.2–27.6)	<*	25.2 (17.4–49.2)
CMIN/g AFDM (mg CO_2 g^{-1} AFDM h−1)	0.52 (0.13–2.36)	0.34*	0.6 (0–2.4)	NS	0.61 (0.6–2.4)
DEA/g AFDM (ng N g^{-1} AFDM h^{-1})	134 (5.2–710)	0.55***	116 (5–272)	<***	338 (62–710)
DOM composition and quality:					
%C1: humic-like DOM	3.7 (1.3–4.9)	0.6***	3.6 (1.6–4.6)	NS	4.3 (3.7–4.8)
%C2: recently microbially processed DOM	4.4 (1.3–11.8)	0.80***	3.6 (1.3–9.2)	<***	9.3 (7.2–11.9)
%C3: terrestrial humic DOM; most recalcitrant	2.6 (1.1–4.7)	0.42**	2.7 (1.1–4.7)	NS	2.7 (2.5–3.1)
%C4: N-rich amino acid-like DOM	2 (0.65–3.2)	0.72***	1.9 (0.65–2.7)	<**	2.7 (2.3–3.2)
%C5: most bioavailable fraction DOM	1.3 (0.56–3.5)	0.59***	1.3 (0.56–2.4)	<**	2.5 (1.7–3.5)
Fluorescence index (FI); correlated–proportion of microbially derived DOM	1.5 (1.4–1.6)	NS	1.5 (1.4–1.6)	NS	1.5 (1.5–1.6)
$SUVA_{254}$: specific UV absorbance at 254 nm	0.14 (0.03–0.57)	−0.38*	0.13 (0.07–0.17)	NS	0.06 (0.03–0.2)
Bioavailable DOC (mg C/L)	0.43 (0–4.62)	0.36*	0.33 (0–0.82)	NS	1.07 (0–4.62)
Slope ratio: indicative of DOM molecular weight	0.95 (0.67–1.76)	NS	0.89 (0.84–1.58)	NS	0.96 (0.67–1.76)

the most urban sites and an average of 0.2°C in the most forested sites. In a multivariate analysis of all physical variables, we found overlap between conditions in the most urban and most forested sites (Fig. 3A–C) despite significant thermal differences between groups.

Development effects on chemical variables

NO_3^--N, bDOC, and several contaminants that are likely to be stressors (Pb, Cd, Zn, and SO_4^{2-}) were each significantly positively correlated with % developed watershed area (Tables 2, 3). Total N, TDN, and Cl^- concentrations were significantly higher in the most urban sites than in the most forested sites (1-way ANOVA, $p < 0.05$). However, these concentrations also were elevated in several of the more agricultural watersheds and, thus, were not significantly correlated with % developed watershed area. NH_4^+, PO_4^{3-}, total P, and DOC showed no significant relationship with % developed watershed area. A principal components analysis (PCA) of water-quality variables showed that the most urban sites were distinctly different from the most forested sites in terms of water-chemistry components. Percent watershed development was the strongest correlate of both PCA axes ($r = -0.62$ and 0.44, $p < 0.005$, respectively; Fig. 3D–F). PCA axis 1 also was significantly negatively correlated with % developed watershed area within the 100-m buffer ($r = -0.57$, $p < 0.0001$), inverse distance-weighted traffic volume ($r = -0.40$, $p < 0.005$), and road density ($r = -0.42$, $p < 0.005$).

Development effects on microbial processes and community composition

Despite large differences in thermal regimes and water chemistry across the development gradient, we were unable to detect any consistent shifts in microbial biomass or the rates of microbially mediated processes of C mineralization or denitrification (Table 3). This lack of a functional response corresponded with the absence of a consistent difference in either OTU richness or microbial community composition between the most forested and most urban sites (Fig. 4A–C). Total bacterial composition from 16S rRNA genes (Fig. 4C) was significantly correlated along NMDS axis 1 with % developed watershed area and % developed area within the 100-m buffer ($r = -0.39$, $p < 0.05$ and $r = -0.45$, $p < 0.01$, respectively) and % watershed forested area ($r = 0.38$, $p < 0.05$). The secondary axis of the NMDS ordination was significantly related to the number of road–stream crossings and the concentration of Zn in the sediments ($r = 0.37$, $p < 0.05$ and $r = 0.53$, $p < 0.001$, respectively).

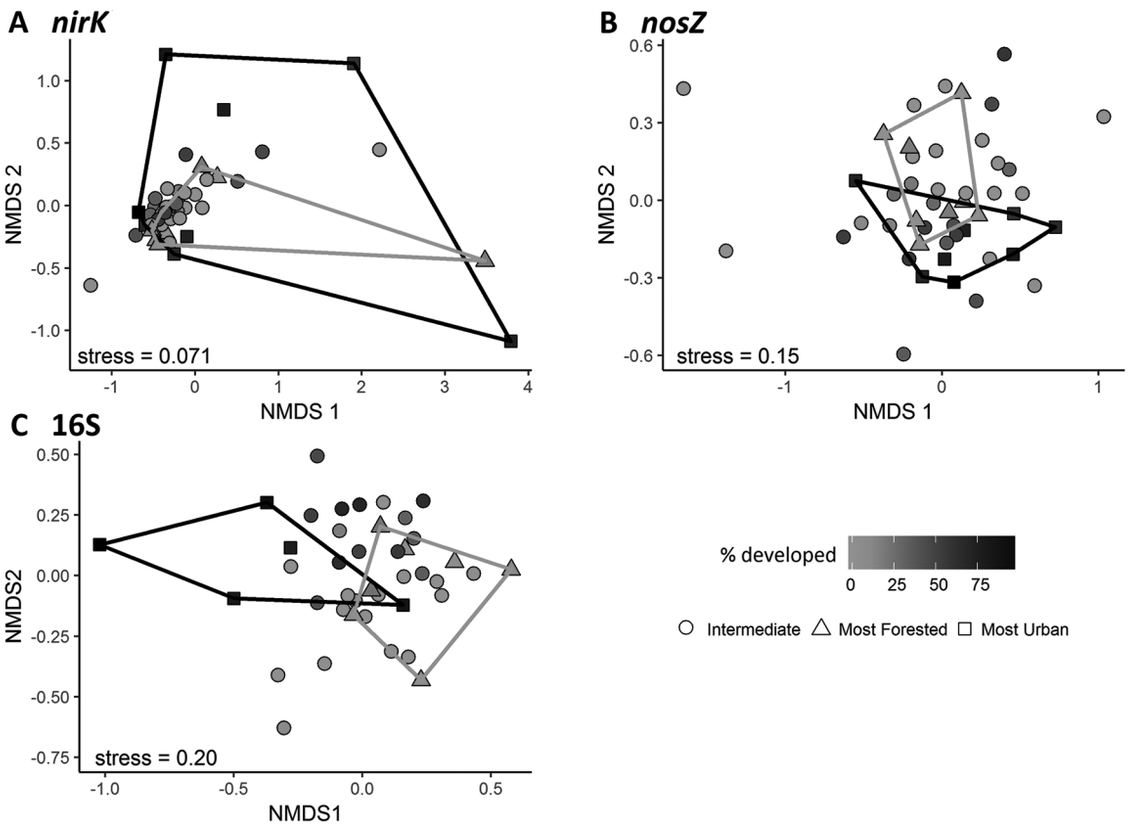

Figure 4. Nonmetric multidimensional scaling (NMDS) ordinations of microbial communities based on *nirK* (A) and *nosZ* (B) functional genes and 16S rRNA genes (C) showing the first 2 axes. See Fig. 3 for explanation of site symbols and colors.

Table 4. Pearson correlations for microbial community metrics with ecosystem functions. NMDS = nonmetric multidimensional scaling, OTU = operational taxonomic unit. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

Variable/axis	C mineralization	Substrate-induced respiration	Denitrification enzyme activity
nirK			
NMDS axis 1	NS	NS	NS
NMDS axis 2	−0.40**	NS	−0.42**
NMDS axis 3	NS	−0.30*	NS
nosZ			
NMDS axis 1	NS	NS	NS
NMDS axis 2	NS	NS	NS
NMDS axis 3	NS	NS	NS
16S			
NMDS axis 1	NS	NS	NS
NMDS axis 2	NS	0.47**	−0.40**
OTU richness	NS	NS	NS
nirK	NS	NS	NS
nosZ	0.30*	NS	NS

The functional groups associated with the *nirK* and *nosZ* genes showed no consistent shifts in composition or richness along the development gradient (Fig. 4A, B). Limited and weak correlations existed between the metrics of microbial community composition and either microbial biomass or microbially mediated processes of C mineralization and denitrification (Table 4).

Development effects on macroinvertebrate communities

Macroinvertebrates responded with the predicted decrease of sensitive taxa along the development gradient. Macroinvertebrate BI was positively correlated with % developed watershed area ($r = 0.63$, $p < 0.001$; Table 5). Total macroinvertebrate genus richness and EPT genus richness were negatively correlated with % developed watershed area ($r = -0.50$ and -0.55, $p < 0.05$; Table 5). Total richness did not differ between most forested and most urban sites (Fig. 5A), whereas EPT richness was significantly greater and BI was significantly lower in most forested than most urban sites (one-way ANOVA, $p < 0.001$; Fig. 5B, C). The NMDS ordination of macroinvertebrates showed no consistent differences between macroinvertebrate communities in the most urban and most forested streams (Fig. 5D, E). PERMANOVA showed a significant effect of landuse category on macroinvertebrate community composition ($F_{2,41} = 1.7$, $p = 0.024$). Correlations of environmental variables with the macroinvertebrate ordination showed that NMDS axis 1 was associated with the urbanization gradient, whereas thermal metrics, % watershed forested area, D_{15}, and D_{84}) were strongly correlated with NMDS axis 3 (Table 6).

Development effects on DOM quality

A PCA of DOM variables indicated a clear separation in ordination space between most urban and most forested sites (Fig. 6A). DOM from most forested sites was enriched in DOM components 1, 3, and 4 and tended to have a high humic content (as measured by high $SUVA_{254}$; Fig. 6A). In contrast, DOM from most urban sites was enriched in component 2 (Fig. 6A). The dominant variation in DOM composition, as measured by PCA axis 1, was significantly related to % developed watershed area ($r = 0.62$, $p < 0.001$; Fig. 6B) and negatively correlated with % watershed forested area ($r = -0.59$, $p < 0.001$). Percentages of DOM components 2 and 4 had the strongest correlations with % developed watershed area ($r = 0.80$ and 0.72, $p < 0.001$, respectively), but all components were significantly positively correlated with % developed watershed area (Table 3). Most urban sites had significantly greater percentages of

Table 5. Biotic variation across the survey watersheds. Correlations are based on Pearson correlations. EPT = Ephemeroptera, Plecoptera, and Trichoptera. OTU = operational taxonomic unit. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, NS = not significant.

Metric	Full data set ($n = 47$) median (range)	Correlation with % developed	Most forested median (range)	Significance	Most urban ($n = 8$) median (range)
Aquatic macroinvertebrates			($n = 7$)		
Biotic index ($n = 43$)	6.05 (4.09–9.08)	0.63***	5.28* (4.39–6.61)	<***	7.58 (6.05–9.08)
EPT richness ($n = 43$)	4.5 (0–15)	−0.55***	6 (2–8)	>*	1.5 (0–4)
Total richness ($n = 43$)	14 (1–24)	−0.50***	15* (4–24)	NS	11 (2–14)
Sediment microbes			($n = 8$)		
nosZ OTU richness	95 (13–239)	NS	89.5 (65–115)	NS	106 (48–162)
nirK OTU richness	67 (2–119)	NS	76.5 (5–88)	NS	43 (2–91)
16S OTU richness ($n = 41$)	664 (355–1067)	NS	711 (523–1067)	NS	503 (420–732)

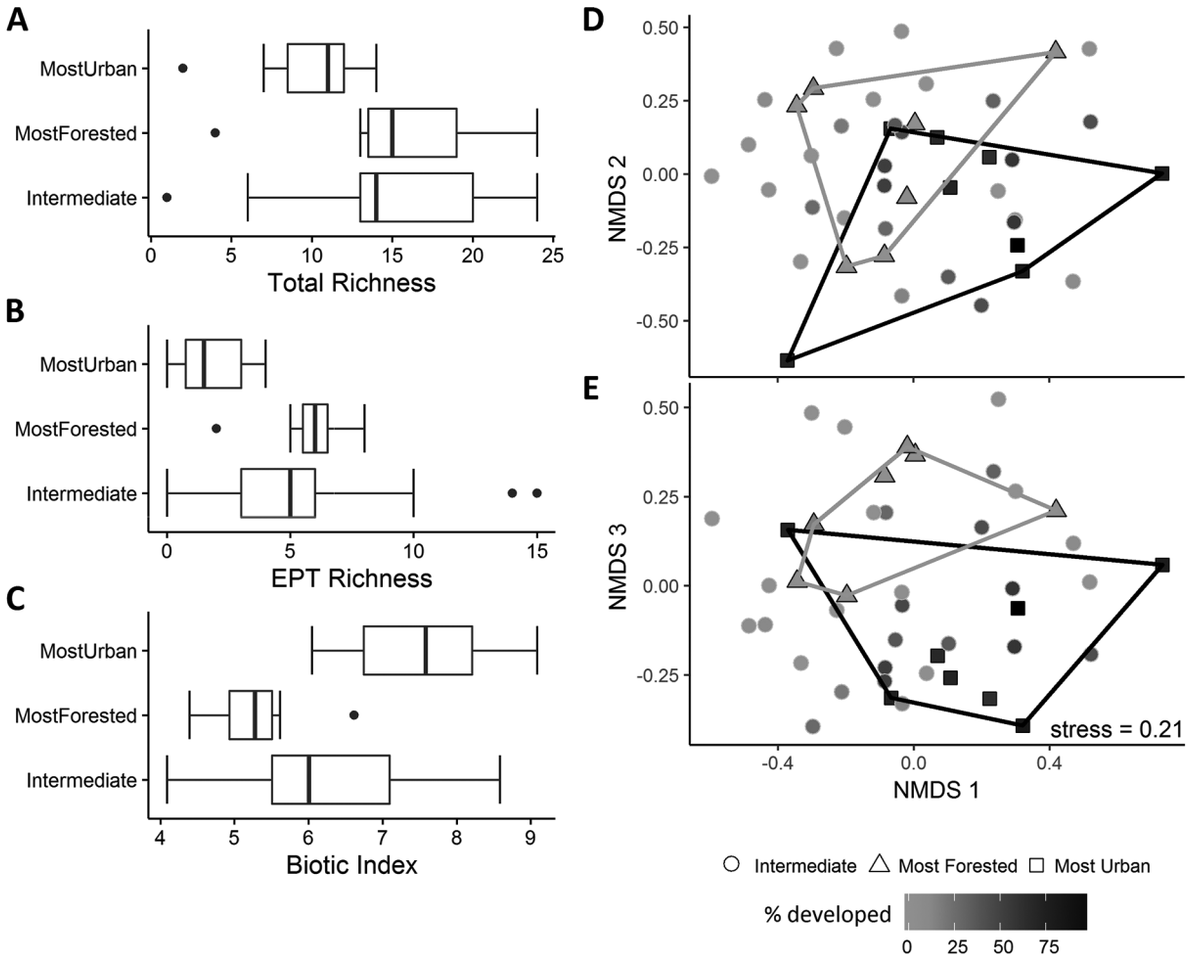

Figure 5. Tukey box-and-whisker plots for macroinvertebrate family richness (A), Ephemeroptera, Plecoptera, Trichoptera (EPT) family richness (B), family biotic index (C), and nonmetric multidimensional scaling (NMDS) axis 1 vs 2 (D) and 1 vs 3 (E) ordinations of macroinvertebrate communities. Lines in boxes are medians, box ends are quartiles, whiskers show the lowest measurement <1.5× the interquartile range, and dots are outliers. See Fig. 3 for explanation of ordination site symbols and colors.

DOM components 2, 4, and 5 than did most forested sites. bDOC was significantly correlated with % developed watershed area ($r = 0.36$, $p < 0.05$), but bDOC concentration did not differ between most urban and most forested streams (Table 3).

In addition to the shift in C quality along the development axis, we also measured an increase in C availability. The % AFDM in the sediment was significantly negatively correlated with % developed watershed area ($r = -0.34$, $p < 0.05$, $n = 46$). SIR and C mineralization (CMIN) rates did not differ between most urban and most forested streams, but both rates were significantly positively correlated with sediment AFDM ($r = 0.74$, $p < 0.0001$, $n = 46$ and $r = 0.63$, $p < 0.0001$, $n = 46$, respectively), suggesting that microbial activity at a site is, to some extent, controlled by the available particulate C supply in which the community developed. When we looked at the possibility of a difference in use of available sediment C supply among the sites, SIR/g AFDM, CMIN/g AFDM and DEA/g AFDM were significantly, positively correlated with % developed watershed area so that our estimates of SIR and DEA/g AFDM were greater in most urban than most forested sites (Table 3).

DISCUSSION

We documented many trends that are consistent with prior research in other urban environments, but we found no consistent changes in channel geomorphology or microbial community composition or function in relation to urbanization. The most urban streams had higher concentrations of salts and labile organic matter and fewer sensitive macroinvertebrate taxa, as has been shown in many previous studies (Paul and Meyer 2001, Roy et al. 2003, Walsh et al. 2005, Coles et al. 2012). However, microbial

Table 6. Pearson correlation coefficients between environmental variables and macroinvertebrate nonmetric multidimensional scaling (NMDS) ordination axes. * = $p < 0.05$, ** = $p < 0.01$, *** $p < 0.001$, NS = not significant.

Metric	NMDS axis 1	NMDS axis 2	NMDS axis 3
Road density	NS	NS	0.36*
Inverse distance-weighted traffic volume	−0.37*	NS	NS
% developed, 1985	−0.36*	NS	0.42**
% developed, 1995	−0.41**	NS	0.45**
% developed, 2005	−0.38**	NS	0.49***
% forest	NS	NS	−0.53***
% development in 100-m buffer	−0.29*	NS	0.32*
Cl^- (mg/L)	NS	0.29*	NS
SO_4^{2-} (mg/L)	NS	0.31*	0.32*
Bioavailable dissolved organic C (mg/L)	−0.39**	NS	NS
Ni (µg/g C)	−0.40**	NS	NS
Cd (µg/g C)	−0.42**	NS	0.32*
Pb (µg/g C)	NS	NS	0.33*
Mean width	0.44**	NS	NS
AFDM	NS	NS	−0.32*
Degree days	NS	NS	0.64***
Mean temperature	NS	NS	0.62***
Mean minimum temperature	NS	NS	0.65***
Mean maximum temperature	NS	NS	0.57***
Short-term temperature change during summer storm	NS	0.31*	NS
Maximum temperature change	−0.32*	0.31*	NS
D_{16}	NS	NS	−0.51***
D_{84}	NS	NS	−0.40**
% canopy cover	NS	NS	−0.38*

communities did not follow the same trends. Denitrifier community composition was highly variable, but this variation was not clearly related to % developed watershed area. Microbial community composition measured using 16S rRNA genes showed weak correlation with urbanization, but with a large amount of variation. Microbial biomass, C mineralization, and denitrification rates were unaffected by urbanization, but increased urbanization led to a decline in sediment organic matter (OM) and increases in labile OM in the water column. Together, these 2 trends appear to be driving higher rates of C mineralization and denitrification per unit OM.

Microbial biomass per unit sediment OM increased with urbanization, as did rates of C mineralization and denitrification per unit sediment OM. Collectively, these results suggest 3 possibilities: 1) microbes in urban streams may be supported by more labile forms of both dissolved and particulate OM, increasing the efficiency of microbial process rates (Newcomer et al. 2012); 2) increased microbial process rates could be caused by increased temperatures (Acuña et al. 2008, Perkins et al. 2012); or 3) increased microbial efficiency in urban streams could be a result of combined effects of increased nutrients, C, and temperature (Jankowski et al. 2014, Manning et al. 2018). Whatever the mechanism, these results suggest that higher microbial process rates may offset declines in sediment OM observed in our and previous urban stream studies (Beaulieu et al. 2014).

Contrary to our expectations (Perryman et al. 2008, Wang et al. 2011, 2014, Hosen et al. 2017), microbial communities, as measured by OTU richness and community composition, did not shift perceptibly along the development gradient. The 2 denitrifier functional genes we measured with T-RFLP indicated that some community members responded to temperature (*nirK*) or to substrate supply and contamination (*nosZ*) because these variables were correlated with the major axes of variation in composition. Our use of T-RFLP limited our interpretation of the denitrifier communities along the urbanization gradient to the most numerically common organisms at each site, and we may have missed ecologically important, but rare, taxa (Bent and Forney 2008). Therefore, T-RFLP is not useful for assessing species richness or diversity metrics. However, T-RFLP can be used to compare community structure across sites (Osborne et al. 2006, Pilloni et al. 2012), as we did in our ordinations. Finger-printing methods also can be used for correlating environmental variables with community structure (van Dorst et al. 2014), as we did with the denitrifier communities across the urbanization gradient. Wide variation in community composition was seen using both T-RFLP for the denitrifier communities and 454 pyrosequencing for the 16S rRNA genes.

Microbial community composition, measured as 16S rRNA genes, was related to % developed area in the 100-m buffer and in the watershed, % watershed forested area, the number of road–stream crossings, and sediment concentration. Microbial communities were responding to some components of the urban stream syndrome, but our analyses suggest 2 things. First, these stressors are not perfectly correlated with one another across the urban stressor gradient. Second, microbes have far more variable responses to individual urban stressors than do their macroinvertebrate counterparts. Many sensitive macroinvertebrate species are lost from streams regardless of whether degradation is a result of high salinity, lower O_2 availability, or degraded habitat, but microbes may respond differently to each of these changes.

The lack of a consistent microbial response to urbanization might be explained by the numerous mechanisms by which urbanization affects microbial communities. The ef-

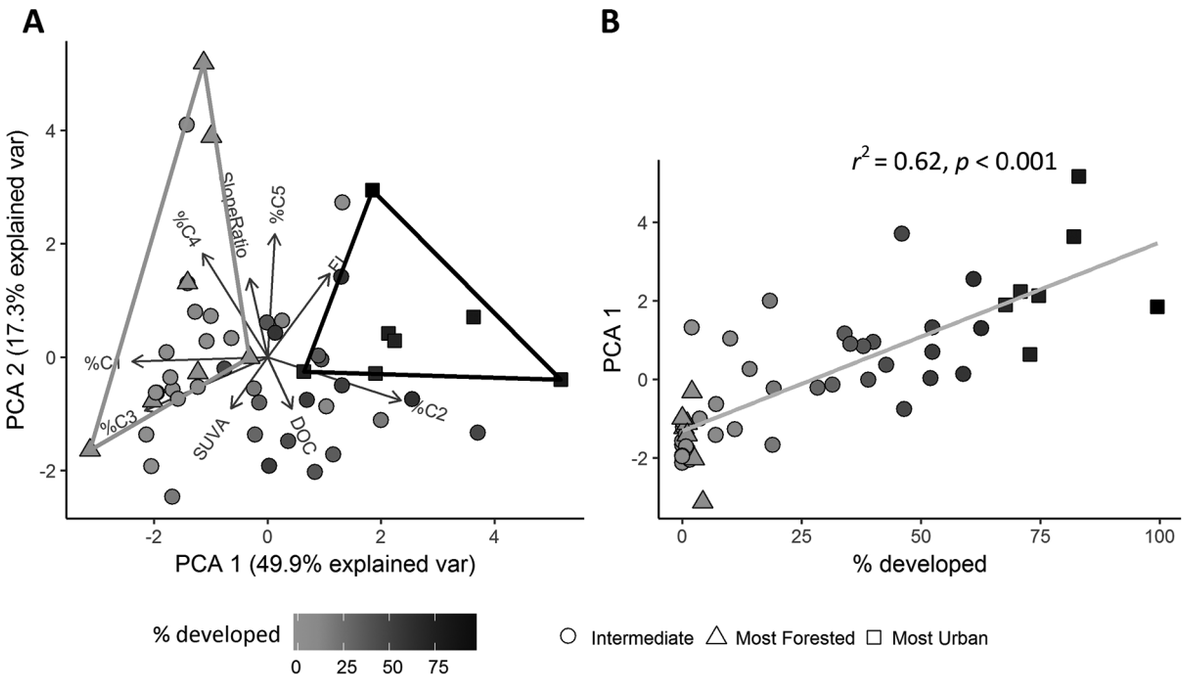

Figure 6. Principal components analysis (PCA) biplot for sites and components of dissolved organic matter (DOM) (A), and the relationship between PCA axis 1 and % developed watershed area (B). The DOM components included in the PCA represent 5 different groups of fluorescing molecules: component C1 represents humic DOM; C2 represents recently microbially processed DOM; C3 represents the most recalcitrant humic DOM of terrestrial origin; and C4 and C5 represent N-rich DOM, with C5 being the most bioavailable fraction. SUVA = specific ultraviolet absorbance at 254 nm.

fects of urban hydrology and water quality on microbial community composition still are not well understood (Hosen et al. 2017). In a synthesis of lotic microbial diversity studies, Zeglin (2015) found that microbial diversity was negatively affected by metals, temperature, substrate type, or hydrology more commonly than by more general effects of land use or nutrient concentrations. Chemical components of effluent inputs (Drury et al. 2013) and other water-chemistry variables (Mykrä et al. 2017) in urban streams can contribute to homogenization of bacterial communities. The interactions of urban influences are likely to simultaneously stress (e.g., increased peak flows, Cl^-, SO_4^{2-}, heavy metals) and subsidize (e.g., increased nutrients, labile OM, temperature) microbial biomass and activity, thereby masking microbial response to urbanization. Functional redundancy (Allison and Martiny 2008) can obscure clear relationships between urbanization and microbially mediated rates of C use or denitrification.

The macroinvertebrate community response to urbanization in this survey was far less consistent and extreme than has been reported in many other studies (Jones and Clark 1987, Roy et al. 2003, Wang and Kanehl 2003, Alberti et al. 2007). We attribute this damped response to 2 important features of this region. First, southern urban streams are not affected as severely by road salting as their northern counterparts (Corsi et al. 2010). Cl^- was higher in most urbanized than at most forested sites, but we did not see a consistent relationship across the urbanization gradient. Another reason for the muted macroinvertebrate community response may be that our sites have a history of agricultural land use and sedimentation, which may have decreased aquatic biodiversity across the entire region regardless of current land use (Burcher and Benfield 2006, Maloney et al. 2008). Investigators also reached this conclusion after a study comparing urban streams across the USA in which urbanization effects on macroinvertebrates were muted in areas developed on formerly agricultural land (Brown et al. 2009, Cuffney et al. 2010).

Historic landuse legacies (Walter and Merritts 2008, Brown et al. 2009, Parr et al. 2016, Utz et al. 2016) also are a probable explanation for why we measured less consistent geomorphic change along this urbanization gradient than others have reported (Booth and Jackson 1997, Wang et al. 2001, Hardison et al. 2009, Shields et al. 2010). Current channel conditions may be a function of physiographic region (Utz et al. 2011, 2016). Urban channels in our study were not consistently incised and did not have consistent shifts in channel habitat. We did observe a decline in grain size in channel beds so that sediments were less stable in the most urban streams.

Conclusion

Our study adds a broad set of data to the emerging views of heterogeneous responses of streams to urban effects (Roy et al. 2016). We found a great deal of variability in the physical status of urban streams, rather than a homogenous and archetypal urban stream channel shape repeated over many sites. We detected an increase in nutrient and contaminant loading along the development gradient, but with wide variation around the mean response. Macroinvertebrates responded predictably to the development gradient with a loss of sensitive taxa and an increase in the dominance of tolerant organisms, but we saw no consistent effect on microbial communities. The microbially mediated processes of C mineralization and denitrification were enhanced in more urban streams, and this stimulation is possibly explained by an increase in labile C loading from urban land uses. The wide range of urban stream responses we observed could be good news for freshwater systems because it means that not all urbanization is created equal and that we might be able to exploit this variation to actively manage for positive outliers of the degrading trend (Carle et al. 2005, Alberti et al. 2007, Parr et al. 2016). If we can identify the aspects of urbanization that are least and most detrimental to the condition of receiving streams, we could use that information to inform future development and retrofitting and restoration efforts.

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