Macroinvertebrate responses to watershed land use and local-scale stream restoration.

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ABSTRACT

CHRISTY ROYER VIOLIN: Macroinvertebrate responses to watershed land-use and local scale stream restoration
(Under the direction of Seth R. Reice)

Human land use practices have resulted in the widespread degradation of waterways draining the surrounding landscape, resulting in poorly functioning streams with lower biological diversity than streams in undisturbed watersheds. Stream restoration has become an increasingly popular method for ameliorating local-scale degradation. Current stream restoration methodology reconfigures channel morphology to reflect a pre-degradation ideal, and relies on habitat provision as the primary means to facilitate biotic community recovery. However, there is little information on the success of this approach. This dissertation focuses on the consequences of urban land use for macroinvertebrate stream community structure and the potential for Natural Channel Design, a common reach scale restoration method, to ameliorate stream degradation due to catchment based land use in various catchment types. In two studies examining macroinvertebrate community response to stream restoration, Natural Channel Design did not lead to improvement in macroinvertebrate community structure, and failed to restore habitat in the urban stream restorations surveyed. A structural equation modeling approach suggests that the factors most associated with community degradation are not currently addressed by reach-scale restoration. This suggests a need to shift

restoration strategies away from a strictly reach-scale approach to a multi-scale approach which incorporates watershed scale processes.

To Jon and Hazel

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Chapter 1

Introduction

Lotic ecosystems are essential to the preservation of the world's freshwater resources. Past and current land use practices have led to widespread damage of these systems and consequently, decreased ecological function (Postel and Richter 2003). River networks drain the terrestrial landscape and perform important ecosystem services, such as the processing of organic matter, nutrients and pollutants. These processes limit the transport of such materials to downstream lakes and estuaries, and thereby increase water quality (Sweeney et al. 2004). In addition, streams and rivers represent distinct and diverse biological communities (Sabo et al. 2005).

Stream impairment arising from land use is multifaceted. Agricultural and urban development leads to stream channel homogenization and pollution, and consequently, reduced ecosystem function and biological diversity. Agricultural and urban runoff leads to eutrophication and pollutant loading (Lenat and Crawford 1994, Carpenter et al. 1998, Bernhardt et al. 2008). High and medium density development, crop tillage, direct livestock access, erosion from unstable stream banks, and altered hydrology have increased sedimentation (Wolman 1967, Costa 1975, Booth and Jackson 1997). Riparian forest loss and fragmentation have increased due to urban and agricultural activities close to stream channels (Benstead et al. 2003, Sweeney et al. 2004). These insults lead to poor water quality, habitat homgenization, altered trophic structure (reviewed in Allan 2004), decreased diversity and ecosystem function, and the loss of ecosystem services (Paul and Meyer 2001, Sweeney et al. 2004, Meyer et al. 2005).

The consequences and extent of stream alteration depend on the type of catchment land use, the proportion of the catchment affected, and the proximity of land use to the stream channel (Wang and Kanehl 2003, Moore and Palmer 2005). Agricultural activities in watersheds and riparian zones cause extensive non-point source pollution (Lenat and Crawford 1994) and erosion, sedimentation, and channelization from crop tillage and stream straightening (Landwehr and Rhoads 2003). Livestock cause heavy nutrient loading and direct, physical degradation of stream banks and benthic habitat (reviewed in Belsky et al. 1999, Strand and Merritt 1999).

The characteristic suite of physical, biogeochemical, and biological stream impairments in urbanized watersheds are termed the "Urban Stream Syndrome" (Walsh et al. 2005b). Geomporhological and hydrological consequences of watershed urbanization include altered base flow and unstable hydrology with frequent short duration high peak floods (Booth and Jackson 1997, Paul and Meyer 2001, Meyer et al. 2005). Modified hydrology leads to channel incision and simplification (Shields et al. 2003, Niezgoda and Johnson 2005, Sudduth and Meyer 2006) and benthic habitat homogenization (FISRWG 1998, Malmqvist and Rundle 2002, Walsh et al. 2005a). Hyperconnectivity with the surrounding landscape through roads, storm drains, and leaky and overflowing sanitary sewers routes watershed contaminants directly into urban channels and leads to elevated nutrient and contaminant concentrations (Bernhardt et al. 2008). Point and non-point inputs and inefficient nutrient removal in hydrologically disconnected riparian zones and streambeds increase channel pollutant concentrations (Groffman and Crawford

2003, Grimm et al. 2005, Meyer et al. 2005).

In the future, human population growth will occur predominantly in urban centers, and therefore an increasing proportion of the world's freshwater ecosystems will become impacted by urban factors (United Nations 2008). Stream macroinvertebrate communities are typically species rich and are strongly affected by land use patterns (Lenat and Crawford 1994, Sponseller et al. 2001, Allan 2004) and aquatic community impairment is associated with urban development across a range of taxa (Wang et al. 1997, Paul and Meyer 2001, Wang et al. 2001, Roy et al. 2003, Cuffney et al. 2005, Roy et al. 2005, Cuffney et al. 2010). Watershed impervious surface is associated with lower invertebrate species richness and higher community tolerance (Morse et al. 2003, Roy et al. 2003, Moore and Palmer 2005, Cuffney et al. 2010). Development in close proximity to or hydrologically connected to stream channels can be particularly detrimental to stream communities (Walsh et al. 2001, Wang and Kanehl 2003, Moore and Palmer 2005). Fragmentation and riparian vegetation removal can limit terrestrial adult dispersal (Wiens 2001, Briers and Gee 2004, Smith and Collier 2005). Urbanization also degrades macroinvertebrate community structure due to stream water chemistry (Roy et al. 2003), sediment particle size (Roy et al. 2003, Violin et al. *in press*), hydrology (Walsh et al. 2001, Walsh et al. 2005a, Cuffney et al. 2010), sedimentation (Minshall 1984, Roy et al. 2003), and metal pollution (Sloane and Norris 2003).

To alleviate channel simplification, habitat and water quality degradation, and loss of aquatic biodiversity and ecosystem function, degraded streams are often targeted for restoration, which seeks to return degraded streams to as close to repimpacted conditions as possible (National Research Council 1992). In North Carolina, a predominant method of stream restoration is Natural Channel Design (Rosgen 1994, 1996). This method reconfigures the pattern, profile and dimensions of a degraded channel to resemble unimpacted regional conditions (Rosgen 2007) by using heavy machinery to re-meander the channel and create a new floodplain. In addition, this approach installs hard structures such as rock vanes to control stream grade, uses vegetation and root wads to stabilize banks, constructs new riffle habitat by adding coarse bed material, and revegetates riparian areas.

To restore degraded aquatic communities, stream restoration relies on the assumption that reconfiguring channel geomorphology to pre-degradation conditions will lead to the recovery of native aquatic organisms, and relies on habitat provision as the primary means to facilitate community recovery (Brooks et al. 2002). This assumption is based on the demonstrated correlation between habitat diversity and species diversity in fish and macroinvertebrate communities (Angermeier and Winston 1998, Brown 2003). There is scant evidence to support the idea that physical habitat restoration (the "field of dreams" hypothesis) is sufficient for community restoration (Palmer et al. 1997, 2010); previous studies of stream restoration success have shown limited or mixed success with regard to geomorphological improvement (Jähnig et al. 2010), and generally show little (Moerke et al. 2004, Palmer 2010) or no improvement in macroinvertebrate community structure (Jähnig et al. 2010, Violin et al. *in press*). However, because of the individual nature of stream restoration projects, lax or varying monitoring requirements, and the variety of restoration strategies, long-term studies of restoration success are lacking (but see Moerke et al. 2004).

In North Carolina, the recent widespread implementation of reach-scale stream restoration is largely funded by the need to mitigate for stream loss elsewhere due to public and private development, as required by the Clean Water Act (Lave et al. 2008, Doyle and Yates 2010). The Clean Water Act requires no net loss of stream function, for which stream length is currently used as a surrogate. In North Carolina, there are no in-stream biological or functional stream restoration success criteria. Rather, practitioners monitor vegetation and geomorphology, and equate geomorphology and adequate live riparian plantings with biological and functional success (Lave et al. 2008, BenDor et al. 2009).

This dissertation focuses on the consequences of catchment urbanization to stream macroinvertebrate communities, and the ability of reach scale restoration to rehabilitate degraded aquatic communities. The overarching goals of this dissertation are to improve our mechanistic understanding of how urban land use degrades macroinvertebrate communities, and to evaluate the extent to which local-scale stream restoration successfully rehabilitates impaired aquatic communities under different land use regimes. A better understanding of both of these issues will facilitate better management of stream ecosystems and may improve restoration design and implementation.

To answer these questions, chapter 2 presents the results of a study of four urban degraded, four urban restored, and four forested streams assayed for a number of biological, functional, and physical attributes. This study addressed the ability of reach scale urban stream restoration to improve physical structure and aquatic invertebrate community structure by comparing urban restored streams to urban degraded and forested reference endpoints. Chapter 3 presents the results of an analysis of long-term stream restoration macroinvertebrate monitoring. This study sought to answer the questions of whether or not local scale stream restoration improved macroinvertebrate community structure in Natural Channel Design restoration projects located in rural, agricultural, and urban catchments throughout North Carolina, whether invertebrate communities improved with time since restoration. To better understand the mechanistic pathways by which urbanization influences invertebrate community structure, chapter 4 utilized a structural equation modeling approach to untangle the myriad pathways through which catchment urbanization can influence community structure, and determine the relative importance of urban vs. non urban pathways. Chapter 5 integrates the findings, implications, and question raised by this dissertation, and attempts to suggest approaches for improving stream protection restoration.

Chapter 2

Effects of urbanization and urban stream restoration on the physical and biological structure of stream ecosystems.

Abstract

Streams, as low-lying points in the landscape are strongly influenced by the stormwaters, pollutants, and warming that characterize catchment urbanization. River restoration projects are an increasingly popular method for mitigating urban insults. Despite the growing frequency and high expense of urban stream restoration projects, very few projects have been evaluated to determine whether they can successfully enhance habitat structure or support the stream biota characteristic of reference sites. We compared the physical and biological structure of four urban degraded, four urban restored, and four forested streams in the Piedmont region of North Carolina to quantify the ability of reach-scale stream restoration to restore physical and biological structure to urban streams and to examine the assumption that providing habitat is sufficient for biological recovery. To be successful at mitigating urban impacts, the habitat structure and biological communities found in restored streams should be more similar to forested reference sites than their urban degraded counterparts. For every measured reach and patch-scale attribute we found that restored streams were indistinguishable from their degraded urban stream counterparts. Forested streams were shallower, had greater habitat complexity and median sediment size, and contained less tolerant communities with higher sensitive taxa richness than streams in either urban category. Because heavy machinery is used to re-grade and reconfigure restored channels, restored streams had less canopy cover than either forested or urban streams. Channel habitat complexity and watershed impervious surface cover (ISC) were the best predictors of sensitive taxa richness and biotic index at the reach and catchment scale respectively. Macroinvertebrate communities in restored channels were compositionally similar to the communities in urban degraded channels and both were dissimilar to communities in forested streams. The macroinvertebrate communities of both restored and urban degraded streams were correlated with environmental variables characteristic of degraded urban systems. Our study suggests that reach-scale restoration is not successfully mitigating for the factors causing physical and biological degradation.

Key words

benthic macroinvertebrate; biotic recovery; habitat restoration; species composition; stream restoration; urbanization

Introduction

The world's human population is primarily urban, and future population growth will occur predominantly in urban centers (United Nations 2008). Thus, an increasing proportion of our freshwater ecosystems will become impacted by urbanization, and a larger fraction of humanity will rely on waterways degraded by a common set of urban impacts. The physical, biogeochemical, and biological stream impairments that occur specifically in urbanized watersheds have been labeled the "Urban Stream Syndrome" (Walsh et al. 2005b). Physical and hydrological consequences of watershed urbanization are well documented and include altered base flow and unstable hydrology with frequent short duration high peak floods (Booth and Jackson 1997, Paul and Meyer 2001, Meyer et al. 2005, Walsh et al. 2005b). These changes typically lead to channel incision and simplification (Shields et al. 2003, Niezgoda and Johnson 2005, Sudduth and Meyer 2006), and homogenization of benthic habitats (FISRWG 1998, Malmqvist and Rundle 2002, Walsh et al. 2005b).

Coincident with hydrological and geomorphological modification, urban streams have elevated nutrient and contaminant concentrations. Hyperconnectivity with the surrounding landscape through roads, storm drains, and leaky and overflowing sanitary sewers efficiently routes watershed contaminants into urban channels (Bernhardt et al. 2008). Pollutant concentrations increase not only due to increased inputs from point and non-point sources but also as a result of decreased nutrient removal efficiency in hydrologically disconnected riparian zones and streambeds (Groffman and Crawford 2003, Grimm et al. 2005, Meyer et al. 2005).

The inverse relationship between urbanization and native biodiversity and species composition is well established and persists across a range of taxa (Blair 1996, Germaine and Wakeling 2001, Clark et al. 2007, Grimm et al. 2008, McKinney 2008). Macroinvertebrate communities are strongly affected by land use patterns (Lenat and Crawford 1994, Sponseller et al. 2001, Allan 2004). Watershed impervious surface cover is generally associated with a decrease in invertebrate species richness and increasing dominance of highly tolerant taxa (Morse et al. 2003, Roy et al. 2003, Moore and Palmer 2005, Collier et al. 2009, Cuffney et al. 2010). Development that is within riparian areas or that is directly hydrologically connected to stream channels (e.g. road crossings and pipes) can be particularly detrimental to stream communities (Wang and Kanehl 2003, Moore and Palmer 2005, Walsh and Kunapo 2009), and there is thus great interest in riparian reforestation and management for urban stream ecosystem protection (Bernhardt and Palmer 2007). While the impacts of watershed urbanization on stream biota are well documented, it is far from clear what combination of reach and watershed scale management is necessary and sufficient to promote community recovery in urban streams.

In the face of channel incision and bank erosion, water quality degradation, and habitat and biodiversity loss, degraded urban waterways are often targeted for restoration. Stream restoration or rehabilitation encompasses a variety of strategies by which human impacts are mitigated and previous damage is addressed, with the overarching goal of returning the stream to as close to pre-impacted conditions as possible (National Research Council 1992). Urban stream restoration presents unique problems: there is minimal space for rehabilitation, and land acquisition is both expensive and complicated because it generally involves multiple landowners. These challenges typically lead to fewer linear feet being restored and higher per-project costs compared to rural and agricultural stream restoration projects (Bernhardt and Palmer 2007). In fact, for many regions of the US the majority of restoration dollars are invested in a small number of urban stream projects (Hassett et al. 2005, Sudduth et al. 2007). Stream restoration projects are customarily implemented with the specific goals of water quality improvement and provision of aquatic habitat (Bernhardt et al. 2007, Sudduth et al. 2007), yet few projects have been adequately evaluated to determine whether these goals are met (Charbonneau and Resh 1992, Palmer et al. 1997, 2005, Moerke et al. 2004, Moerke and Lamberti 2004, Bernhardt et al. 2005). Given the frequency with which urban stream restoration is employed to mitigate habitat and water quality degradation and the expenses and challenges involved, it is worth understanding whether these efforts are measurably improving habitat and community structure.

The underlying assumption of stream restoration is that altering channel geomorphology to resemble pre-degradation conditions will lead to the recovery of native aquatic organisms. This assumption is based on prior work demonstrating that fish or macroinvertebrate taxonomic richness and spatial heterogeneity are positively correlated (Gorman and Karr 1978, Angermeier and Winston 1998, Vinson and Hawkins 1998, Brown 2003). Although experimental manipulations have demonstrated that high substrate variability does not *per se* lead to higher species richness or faster recovery (Brooks et al. 2002, Spanhoff et al. 2006), stream restoration design employs habitat provision, or increased habitat heterogeneity as the primary mechanism for restoring biotic communities (Brooks et al. 2002). Evidence to support the assumption that successfully restoring physical structure is sufficient for community restoration (the "field of dreams" hypothesis) is lacking (Palmer et al. 1997, 2010, Moerke et al. 2004).

We set out to evaluate the effectiveness of four Natural Channel Design (NCD) projects, a common urban stream restoration approach (*sensu* Rosgen 1994, 1996) in mitigating urban stream degradation. NCD reconfigures the pattern, profile and dimensions of a degraded channel to emulate an unimpacted ideal (Rosgen 2007). This method utilizes heavy machinery to re-grade and reshape a degraded channel and employs hard structures such as log vanes or cross vanes to control grade, installs root wads to stabilize banks, adds coarse bed material to create riffles, and re-vegetates reconfigured or newly created riparian areas.

Effective restoration should recapture the habitat structure and biological communities of forested streams, ideally approaching a stable approximation of "reference" conditions. We tested whether a series of urban restoration projects were achieving or moving towards this goal by examining whether habitat structure and macroinvertebrate community composition in the restored reaches of urban streams were different from similarly situated urban degraded stream reaches and whether the habitat and community structure of these restored reaches more closely matched conditions in nearby forested streams than their unrestored urban counterparts.

Methods

Site selection

Through consultation with staff of the North Carolina Ecosystem Enhancement Program (EEP) and the NC Stream Restoration Institute (SRI) we selected four urban Natural Channel Design restoration projects that practitioners and regulators felt were particularly well-designed and implemented. Our goal in selecting restoration projects was not to select a random sample, but rather to choose a set of projects that represented the best-case scenario for urban restoration based on expert practitioners' opinions. Each restored stream reach was then matched with a similarly situated unrestored urban stream and a forested stream in the Raleigh-Durham area in the Piedmont region of North Carolina. The full comparison thus included 12 study sites: four "forested" (F) sites, within small streams draining forested catchments; four "urban restored" (R) sites, within recently implemented natural channel design restoration projects; and four "urban degraded" (U) sites located in urban parks where future restoration activities are likely (Fig. 1, Appendix A). This suite of sites was selected to determine the potential for ecological restoration to restore the physical and biological structure and ecosystem function of stream ecosystems.

Site descriptions

Four sampling blocks were created from the group of 12, each containing one urban degraded, one urban restored, and one forested stream of similar catchment sizes and underlying geology (Table 1). The study area spans the Northern Outer Piedmont, Slate Belt and Triassic basin ecoregions, and many sites drain multiple ecoregions (Table 1). Soil characteristics affect baseflows and consequently stream size and permanence. Triassic Basin and Slate Belt streams have low summer baseflows due to low clay permeability and low water yield from slate substrate (Griffith et al. 2002). Reduced summer baseflows are not seen in Northern Outer Piedmont streams where streams tend to be larger and less prone to drying. For physical and functional metrics, all streams within a sampling block were sampled within one week with no intervening major storm events. In this way the blocking factor accounts for both differences in watershed size, and staged timing of field analyses.

Our study included four restored stream reaches – each of which was restored using NCD between 1999 and 2005. The Abbott stream restoration project was implemented in 1999 on a tributary to Walnut Creek, in Raleigh, NC. The goal of this restoration project was,

> *"to restore the stream to the stable dimension, pattern, and profile for a C4 stream type as classified using Rosgen's stream classification methodology (Rosgen 1996)…This type of restoration will reestablish the channel on a previous floodplain, or in this case, the basin of an old pond. Appropriate channel dimensions (width and depth), pattern (sinuosity, beltwidth, riffle-pool spacing), and profile (bed slope) of the new channel will be determined from reference reaches"* (NCDOT 1999).

Rocky Branch is a stream located on the urban North Carolina State University campus in Raleigh, NC and was restored in 2001. The goals of this restoration project included,

> *"Restore a stable self-maintaining morphological pattern in the stream channel; Stabilize stream banks using vegetation; Create and improve habitat for fish and aquatic invertebrates; Improve the quality of stormwater entering the creek through restoring and enhancing riparian buffers and establishing*

stormwater control within the creek's watershed; Provide safe and enjoyable access to the stream and passage through the campus by completing the greenway path adjacent to the creek." (Doll 2003).

Restored in 2004, Sandy Creek flows through the urban Duke University campus in Durham, NC. The Sandy Creek project goals were to,

"Re-contour and restore more than 600 meters of degraded stream to hydrologically reconnect the stream with the adjacent floodplain to improve biogeochemical transformations and stream water quality" (Richardson and Pahl 2005).

Third Fork Creek is a stream flowing through an urban park near downtown Durham, NC, and was restored in 2005. The goals of this project were to,

"Restore stable channel morphology that is capable of moving the flows and sediment provided by its watershed; reduce sediment-related poor water quality impacts resulting from lateral bank erosion and bed degradation; improve aquatic habitat diversity through the reestablishment of riffle-pool bed variability and the use of in-stream structures; restore vegetative riparian buffers utilizing native plant species; and improve natural aesthetics in an urban park setting." (KCI Associates 2003).

All of our urban stream reaches were located in urban parks or protected areas to facilitate access, and are similar to the pre-restoration conditions of our restored study sites. Two of our urban stream sites (reaches of Goose Creek and Ellerbe Creek) were chosen because the NC Ecosystem Enhancement Program listed them as priority stream restoration sites (both were restored after this research effort). Our study reach on Upper Mud Creek is located within the protected Duke Forest, immediately downstream of a 1980's era subdivision. Cemetery Creek is located on city property in Raleigh, NC and drains an older, high- density urban neighborhood.

Forested sites were selected from "reference" sites previously used for stream restoration projects as well as sites within Duke Forest. Lower Mud Creek and the Tributary to Mud Creek are located in Duke Forest, in Durham, NC. Stony Creek is located in Duke Forest near Hillsborough, NC. Pot's Branch is located in Umstead State Park near Raleigh, NC. Because of the land use history of the North Carolina Piedmont, these are not pristine reference sites, but rather post-agriculture reforested streams with primarily forested watersheds; thus there may be legacy effects of prior agricultural land use on geomorphology, vegetation, sediment, and biota (Maloney et al. 2008). There are no primary growth forests of sufficient size to have a permanent stream, rather our "forested" streams are secondary growth and represent the post-agricultural, pre-urban landscape. Due to the lack of undisturbed Piedmont streams, we included one forested stream reach, Lower Mud Creek that had urban development more than 1.5 km upstream of the study reach, and for which the entire watershed within that 1.5 km was ~100 year old mixed deciduous forest. Because Lower Mud Creek is far from an ideal reference stream, we performed all statistical comparisons both with and without this stream.

Land use characterization

We acquired the 1/3" digital elevation model for Durham, Orange, and Wake counties in NC from the USGS Seamless Server and performed analysis using the ArcHydro extension of ArcGIS to calculate flow direction and flow accumulation, and define streams based on a 1000 pixel threshold and delineate watersheds for all sites. Land use and impervious surface cover within study watersheds were analyzed based on 2001 National Land Cover Dataset (NLCD), and the associated Impervious Surface Cover dataset from the USGS Seamless Server (Homer et al. 2004). We classified riparian land use in a 30m buffer around each stream segment using the same technique. NLCD was reclassified into four categories: developed, agriculture, undeveloped, and water and for each watershed we calculated the percent of each land use type and percent impervious surface cover. Percent developed catchment and catchment impervious cover (ISC) were used as predictor variables in subsequent analyses.

Habitat surveys

In each stream we delineated experimental reaches encompassing at least one hour of travel time under June 2006 base flow conditions. We selected the upstream end of each reach by locating an area of constricted flow with the greatest downstream extent of channel uninterrupted by tributary inputs or road crossings. Reach travel time was determined by calculating water travel times using a rhodamine dye release. We used rhodamine tracers because traditional salt tracers proved problematic in several of our urban streams due to high spatial and temporal variation in stream water chloride concentrations. Our study reaches were standardized by water residence time and varied in length from 35 to 200 m. We delineated our study reaches in this manner in order to correctly measure ecosystem function variables (see Sudduth et al. *in press*). Habitat surveys were performed in July and August of 2006. We created habitat maps (see Appendix B for examples) of all experimental reaches by determining the longitudinal boundaries and channel widths of riffle, run, pool, and debris dam habitats within each reach (VT WQD 2009). We used a stadium rod and level to survey longitudinal slope for the entire reach and to generate cross-sectional profiles for five randomly selected points within the reach. Reach canopy cover was measured at each cross section using a spherical densiometer. We conducted pebble count surveys of 100 randomly selected sediment particles spaced evenly throughout the study reach (Wolman 1954) to estimate variation in sediment grain size within each stream reach.

Hydrologic data

We created fine scale flow habitat maps by measuring velocity and depth values at five evenly spaced points across the active channel, with a sixth measurement in the thalweg, at 30 cross-section locations evenly spaced longitudinally in each reach. In October 2006 we deployed Solinst leveloggers in each stream reach to collect continuous measurements of water level. We used HEC-RAS (US Army Corps of Engineers) to estimate discharge water level and surveyed channel dimensions. We used these data to create a flashiness index (Baker et al. 2004) for use as a predictive variable in macroinvertebrate community analyses.

Functional measures

Nutrient and organic matter dynamics were measured concurrently in the same study reaches (for methods and results see Sudduth et al. *in press*). Functional measures were used as potential predictor variables in ordination analyses.

Macroinvertebrate sampling

Macroinvertebrate sampling was conducted at the 12 study sites between May and September 2006 ("summer" sample) and February and March 2007 ("winter" sample) from the same reach as physical and functional measurements were taken. Macroinvertebrate communities were sampled once each season using the North Carolina Department of Water Quality Qual 4 semi-quantitative protocol (NC DWQ 2006). This sampling protocol is designed to assess macroinvertebrate diversity in small streams (drainage area < 7.7 km^2) and is conducted so that sampling effort is consistent among study sites. Each sample consisted of one 2-3 min, 1 m^2, 1 mm mesh kick net sample from a characteristic riffle, one 500 mm mesh triangular sweep net of stream marginal habitats such as root mats and bank vegetation, an approximately 500 g leaf pack sample collected from rock or snag habitats, and visual assessments of habitats not easily sampled with the above methods (e.g., large rocks or logs). Samples were field-sorted and specimens were preserved in 95% ethanol. Non-chironomid taxa were identified at 45x to the lowest possible taxonomic level, typically species (Pennak 1953, Brigham et al. 1982, Merritt et al. 2008). Chironomidae were slide mounted in CMC-10 medium (Master's Chemical Co.), and identified at 400x magnification to genus or species (Epler 2001). Following the NC DWQ protocol, we classified taxa as abundant (>10 individuals), common (3-9 individuals), or rare (1-2 individuals). One of the winter urban degraded samples, Goose Creek, was lost, however field notes conclusively indicate the absence of EPT taxa in this sample. Thus, this sample was included in EPT richness analyses.

Data analyses

Physical data analyses

Habitat complexity was determined by counting the number of transitions between different aquatic habitats (riffle, run, pool, and debris dam classifications) for each experimental reach. The transition counts were normalized for all reaches by converting the counts to number of transitions per 100 m reach length. The average number of transitions and standard error was determined for each stream type (F, R, and U). Velocity and point depth measurement averages for each reach were calculated and used to obtain an average and coefficient of variation for each stream type.

The ratio of active channel width to the active channel depth at the thalweg was determined from the field survey cross-section data for each experimental reach. Also, the maximum (smallest W:D ratio value for each stream) and minimum (largest W:D ratio value for each stream) incision value from the field survey data were calculated. The average and coefficient of variation of percent canopy coverage were determined from spherical densiometer measurements. Physical metrics were compared among stream types using one-way ANOVA with stream type as a single factor (Prism v4, GraphPad Software, La Jolla, CA). Where the overall effect was significant, we performed *post hoc* pairwise comparisons (Student-Newman-Keuls) to test for differences among stream types and calculated the magnitude of effect as ω^2, the variance component of the factor in the ANOVA relative to the total variance (Graham and Edwards 2001).

Macroinvertebrate data analyses

In addition to total species richness, we calculated richness of the orders Ephemeroptera, Plecoptera, and Trichoptera, (EPT) as a measure of pollution-sensitive taxa richness. We also calculated Biotic Index (BI) for each site as a measure of overall macroinvertebrate community pollution tolerance. BI was calculated as a weighted mean of taxa tolerance values relative to their abundance, and higher BI values indicate a more pollution-tolerant assemblage (NC DWQ 2006). Individual taxon tolerance values were taken from the NC DWQ benthos standard operating protocol (Lenat 1993, NC DWQ 2006). Taxa for which BI information was not available represented a small minority of taxa and were excluded from the BI calculations. We compared community metrics among stream types using one-way analysis of variance (ANOVA) with stream type as a factor. Where the overall affect of stream type was significant, post-hoc pairwise comparisons and effect size calculations were performed as for physical metrics.

We used least-squares linear regression to quantify correlative relationships between macroinvertebrate metrics and environmental physical and functional variables. As sites were grouped into sampling blocks *a priori* according to watershed and geological variables, all analyses should include sampling block as a variable. However, as block was not found to be an important predictor of any of our habitat or macroinvertebrate community metrics (data not shown), it was not included in our analyses in order to maximize our power to detect differences among site types.

We examined seasonal macroinvertebrate species compositional similarity among sites using Non-metric multidimensional scaling (NMS) ordination of sites in species space, using Bray-Curtis similarities of square root transformed abundance values (PC-ORD v.5, McCune and Mefford 2006). Solutions were obtained from 500 runs (250 randomized, 250 with real data) using random starting coordinates. We created joint plots incorporating a second matrix of physical and functional variables. We set a minimum r^2 of 0.30 to identify geomorphological and functional parameters correlated with macroinvertebrate community structure at different sites.

We assessed the importance of time since restoration to macroinvertebrate recovery by evaluating separately collected macroinvertebrate monitoring data from Rocky Branch both within the restoration and at an unmanipulated upstream reference, and from Sal's Branch, a forested reference site in Umstead Park, NC. Monitoring data were collected using the same NC DWQ Qual-4 protocol as for this study. Pre-restoration samples were collected for Rocky Branch in 12/2000, and post-restoration data were collected in 12/2003, 11/2004, 12/2005, and 12/2006. Reference data were collected from Sal's Branch in 3/2002, 3/2003, and 5/2004. We evaluated the importance of time since restoration to total species richness, EPT richness, and community BI for Rocky Branch. We calculated the change in each community metric by subtracting the pre-construction value from the post-restoration value for each monitoring year ($\Delta_{Restoration} = Metric_{post\text{-}restoration\ year\,i} - Metric_{pre\text{-}restoration}$). We accounted for community structure changes due to factors other than restoration by performing the same calculation for the upstream reference (Δ_{Upstream} = Metric$_{\text{upstream year}i}$ – Metric $_{\text{upstream pre-restoration}}$) and then calculated the effect of restoration by taking the difference of the two (Restoration Response = $\Delta_{\text{Restoration}}$ - Δ_{Upstream}). This is similar to the "Raw Effect Score" for taxon abundance calculations from impact assessment studies (Weiss and Reice 2005), but applied to community-level metrics. We evaluated species compositional similarity among these samples using the same NMS ordination protocol as above.

Results

To test our overarching hypothesis that positive restoration outcomes would lead urban restored streams to become more similar to minimally impacted sites, we compared physical and biological attributes among the three stream types. Excluding Lower Mud Creek did not change the conclusions of any of our relationships of physical metrics among stream types and there was no consistent pattern in the effect of removing this site. However, in every case, removing Lower Mud Creek from biological analyses increased the strength of the observed relationship (Fig. 3), and for some analyses, resulted in a stronger overall effect of stream type (Tables 3-4). For all analyses, we show comparisons with and without Lower Mud Creek included as a forested site.

Habitat

Urban streams had significantly deeper channels, smaller substrate sizes and less reach scale habitat variation (transitions between riffles, runs and pools) than their forested counterpart (Table 2, Fig. 2). For each of these metrics, urban restored streams were indistinguishable from their urban degraded counterparts and significantly different from the forested streams. We found a significant difference between urban degraded and urban restored reaches in only a single habitat metric – restored urban streams had significantly lower riparian canopy cover than their unrestored counterparts.

Our hydrologic metrics did not differ between stream types. Stream velocities and flow heterogeneity were highly variable within stream types. There was no difference in either average or maximum degree of incision among stream types (Table 2).

Biological structure

Macroinvertebrate community richness was similar across stream types in summer, while in our winter sampling our three forested sites (excluding LMC) had significantly higher taxa richness than their restored or urban counterparts (Table 3, Fig. 3). In both seasons, species of Chironomidae made up 56.6% (± 4.5) and 44.9% (± 2.6) of the taxa found in urban and restored streams respectively, and only 26.7% (± 5.0) of the taxa in the forested streams (Appendix C). The three forested sites had higher mean EPT richness than urban and restored sites in both summer and winter (Table 3, Fig. 3). Summer and winter biotic integrity scores were lower

(~higher number of sensitive taxa) in these three forested streams than in the urban restored and urban degraded streams (Table 3, Fig. 3).

Among quantified watershed variables, watershed imperviousness was found to be the best single predictor of EPT richness, although the trend was significant only in winter ($r^2 = 0.54$, $P < 0.01$). Biotic index was positively correlated with watershed imperviousness in both summer ($r^2 = 0.50$, $P < 0.01$) and winter ($r^2=0.40$, $P < 0.05$) (Table 4, Fig. 4).

Among the many in-channel structural and functional variables measured, habitat transitions/100 m was the only reliable predictor of EPT richness, with habitat complexity positively correlated with the number of EPT in both summer ($r^2 = 0.54$, $P < 0.01$) and winter ($r^2 = 0.46$, $P < 0.05$). Habitat complexity was strongly negatively correlated with BI scores in both summer ($r^2 = 0.70$, $P < 0.001$) and winter ($r^2 = 0.67$, $P < 0.01$). Removing Lower Mud Creek from the analyses increased the strength of the observed relationships, but had a stronger affect on EPT richness than BI (Table 4, Fig. 4).

NMS Ordination results revealed large differences in community composition between stream types. Two-dimensional NMS solutions were best for both summer and winter. The summer NMS ordination had a final stress of 0.13 and explained 78.2% of compositional similarity, 40.2% along axis 1 and 37.8% along axis 2 (Fig. 5a). The winter NMS had a final stress of 0.078 and explained 88.2% of compositional similarity among sites, 58.6% along axis 1 and 29.7% along axis 2 (Fig. 5b). With the exception of Lower Mud Creek, forested sites clustered closer to one another in winter than in summer (Fig. 5). In spite of their close proximity (<

50 m of forest between the reaches, Fig. 6), Lower Mud Creek and Mud Creek Tributary did not cluster together in either season.

In analyses of long-term data from Rocky Branch, we found no significant effect of time since restoration on total species richness, EPT richness, or BI. A three-dimensional NMS solution was best for explaining compositional similarity (final stress = 0.09, cumulative r^2 = 0.866) among Rocky Branch macroinvertebrate communities collected as part of this study, upstream and restored samples collected for restoration monitoring, and reference data from Sal's branch and the winter forested samples from this study. Regardless of year, restoration monitoring samples from Rocky Branch clustered more closely to restored samples collected as part of this study and impacted upstream reference samples than to forested samples (Fig. 7).

Discussion

We hypothesized that if restoration is effective at improving degraded urban stream ecosystems, both the geomorphology and biota at restored sites would more closely resemble forested sites than would their urban counterparts. While it would be overly optimistic to expect restored stream reaches to become identical to reference sites, successful restoration ought to lead to stream habitat and biological communities that are distinguishable from unrestored urban streams. In this survey, urban restored streams differed significantly from their unrestored urban counterparts in only a single metric – having reduced canopy cover as a direct result of project implementation (Fig. 2). These results suggest that despite expenditures of >$1 million USD per project, these restored streams did not have improved habitat complexity or detectable changes in their macroinvertebrate communities. The deep, sandy, simplified channels in urban catchments suggest that hydrological differences, particularly storm events, are the major habitat structuring force in our study channels. Stormwater is rarely, if ever addressed by NCD, therefore this is likely a significant barrier to urban stream restoration success (Walsh et al. 2005a, Bernhardt and Palmer 2007).

The similarity in summer total species richness among stream types (Table 3, Fig. 3) is likely due to high richness of more tolerant non-EPT taxa in urban and urban restored sites (Appendix C). Higher winter EPT richness probably accounts for the significant effect seen in winter (Table 3, Appendix C). Higher EPT richness at forested sites (Fig. 3) is consistent with the expectation that urbanization typically results in the loss of these sensitive taxonomic groups (Morse et al. 2003, Roy et al. 2003, Cuffney et al. 2010). Urban restored channels did not have higher EPT richness than urban degraded channels (Fig. 3), suggesting that Natural Channel Design is not mitigating the factors responsible for sensitive taxa loss at these locations. The difference in biotic integrity between urban restored and forested channels, and their similarity to urban degraded channels (Fig. 3) indicates that in addition to having lower sensitive taxa richness (i.e. lower EPT richness), these channels contain more tolerant assemblages across all invertebrate groups.

Regression analyses revealed a strong relationship between EPT richness and watershed ISC (Table 4, Fig. 4). Watershed imperviousness is not something easily addressed by reach-scale restoration, thus prioritizing projects with lower ISC or evaluating the spatial arrangement of ISC (Moore and Palmer 2005) during the planning stages may increase the likelihood of successful restoration. However, although ISC cannot be easily altered, its effects may be mitigated by catchment-based stormwater retention efforts (Walsh et al. 2005a).

Urban degradation leads to compositionally distinct macroinvertebrate communities, which is not successfully mitigated by reach-scale restoration. NMS plots revealed that species composition of restored streams were more similar to each other and to urban degraded streams than to forested streams (Fig. 5). The lack of grouping of forested sites in summer illustrates that although forested sites possess multiple sensitive EPT taxa that primarily delineate them from urban sites (Fig. 5a), there are inter-site compositional differences across all taxonomic groups (Appendix C). While this could be due to the fact that sites were sampled over several summer months, it is also likely local scale habitat filters differed among forested sites and influenced community composition (Poff 1997). Additionally, although these sites are best-case scenarios of minimally impacted Piedmont streams, they are still subject to human impacts, the extent of which varies among catchments. The dissimilarity between Lower Mud Creek and Mud Creek Tributary in spite of their proximity further suggests that Mud Creek still experiences urban influences at the lower site. In fact, Lower Mud Creek is more similar to the urban degraded site Upper Mud Creek in both seasonal NMS plots (Fig. 5).

Joint plots suggest that summer species composition is explained firstly by underlying geology (axis 1), and secondly by catchment and reach-scale urban stressors (axis 2). Axis one represents a gradient of high % dilution to high chloride ion concentration (Fig. 5a). Higher percent dilution is characteristic of streams with higher groundwater and hyporheic exchange (Griffith et al. 2002) and this variable correlated mainly with Northern Outer Piedmont sites. High chloride concentration is probably caused by low groundwater and hyporheic exchange and low summer baseflows characteristic of Triassic basin and Slate Belt streams (Griffith et al. 2002). Axis two represents an urban vector that encompasses differences in habitat and water quality, canopy cover, and hydrological differences and consequently separates forested sites from those in urban catchments (Fig. 5a). This axis best represents our original hypothesis that if urban restoration effectively addresses factors responsible for sensitive taxa loss, restored sites would be at least intermediate between forested and urban degraded endpoints. Our analysis finds no evidence of directional change in composition due to restoration.

The closer clustering of forested sites in winter (Fig. 5b) likely reflects the widespread winter prevalence of shredder taxa such as *Tipula*, *Gammarus*, and *Amphinemura*. An urban vector along axis 1 similar to that found in summer was the primary axis separating forested sites from urban and restored sites, and once again we found no evidence that the restored stream benthos were distinct from their unrestored urban counterparts (Fig. 5b). Additionally, stream nitrogen concentrations (TN, NO_3-N) were correlated with urban and restored species composition. Urban catchments deliver more nutrients to streams than undeveloped ones, and nutrient pollution has long been known to influence macroinvertebrate community structure and impair aquatic communities (Bernhardt et al. 2008). There was no clear effect of underlying geology in the winter ordination, in this season chloride concentrations were highly correlated with impervious cover – suggesting that winter chloride concentrations are dominated by road salt use (e.g., Kaushal et al. 2005). Deep streams with high gross primary production (GPP) were delineated from other sites along axis 2 (Fig. 5b). Together the two axes appear to separate the open canopy urban streams with high nitrogen loading and higher temperatures from closed canopy forested streams with the cooler temperatures and high streamwater C:N ratios characteristic of forested heterotrophic streams (Fisher and Likens 1973).

The restored streams we studied more closely resembled urban rather than forested endpoints both structurally and biologically, suggesting that restoration activities have not yet led to the recovery of sensitive macroinvertebrate taxa in these streams. The large number of metrics measured in the context of this study provides an unprecedented opportunity to explore what factors are most important for community recovery.

Lower Mud Creek – an unacceptable reference site proves an effective case study of the field of dreams hypothesis

Due to the extreme difficulty of locating watersheds without significant urban or agricultural activity in the NC Piedmont, we made the decision to include Lower Mud Creek (LMC) as one of our forested streams. Based on its geomorphology and its location within ~100 year old mixed deciduous forest within the protected Duke Forest, the segment we selected on Mud Creek (LMC) appeared to be an acceptable forested stream reach for inclusion in our study. Despite the high habitat heterogeneity, connected floodplain and high canopy cover of LMC, it supports a very depauperate faunal community. Although LMC proved to be a less than ideal forested stream replicate, the mismatch between physical habitat and biological community structure make it an ideal case study for investigating the limitations of reach–scale restoration. Lower Mud Creek was indistinguishable from other forested sites in most measured geomorphic variables, including habitat complexity. Among forested sites however, Lower Mud Creek had the fewest EPT taxa, and the most tolerant macroinvertebrate assemblage (highest BI value).

The positive correlation between habitat complexity and species richness is well documented (Macarthur and Macarthur 1961, Minshall 1984, O'Connor 1991, Downes et al. 1998, Allan 2004) but may not be causal (Palmer et al. 2010). We speculate that our measure of habitat complexity serves as an indicator of hydrologic disturbance as well as a direct measure of habitat suitability. As such, we must caution that the observed strong positive correlation between habitat complexity and sensitive invertebrate taxa (Table 4, Fig. 4) does not necessarily support the assumption that an increase in habitat complexity will improve biological communities. Indeed, our findings suggest that habitat restoration will prove ineffective if urban stormwaters rapidly rehomogenize restored stream segments, as seen in previous urban restorations (Larson et al. 2001, Booth 2005).

Prior work has suggested that landscape or stream network fragmentation or habitat homogenization may represent an important barrier to macroinvertebrate dispersal in urban catchments and thus may inhibit community recovery in restored urban systems (Blakely et al. 2006, Urban et al 2006, reviewed in Smith et al. 2009). The proximity of LMC to Mud Creek Tributary (< 50 m, Fig. 6), the forested site within our dataset with the least impervious cover and the highest diversity of sensitive macroinvertebrate taxa suggests that LMC is not dispersal limited. Indeed, several of the EPT taxa found in the tributary but not in LMC have been sampled in the riparian vegetation surrounding LMC suggesting that dispersal is likely (Violin, unpublished data). The apparent structural integrity and impaired macroinvertebrate community suggests that while habitat complexity is important to faunal diversity, it is, on its own insufficient to support the recovery of biotic communities. This is an important caveat to the utility of the "field of dreams" hypothesis. Mud Creek experiences urban influences along its length, and although the kilometer preceding our LMC study site is entirely forested, this site has a characteristic urban hydrograph due to upstream catchment urbanization (Fig. 6). Hydrologic disturbance is a major driver of macroinvertebrate community structure, as species adapt to local hydrologic conditions (Resh et al. 1988, Townsend et al. 1997, Lake 2000). Previous work has shown that the effective discharge(s) responsible for in-stream habitat structure are not necessarily those responsible for ecological processes such as invertebrate dislodgement (Doyle et al. 2005). Thus, while the urban hydrology of LMC does not cause significant scouring or substrate/habitat homogenization, it may be sufficient to impair aquatic fauna. It is also possible that periods of storm flow introduce urban derived contaminants (e,g. Kolpin et al. 2002, Makepeace et al. 1995, Beasley and Kneale 2002) that may further stress sensitive aquatic taxa. The special case of Mud Creek suggests that even if a restoration project could build intact channels with high floodplain connectivity surrounded by 100 year old trees and in close proximity to source populations – such an effort would be unsuccessful at promoting the recovery of a diverse macroinvertebrate assemblage containing sensitive taxa unless the project also was able to mitigate storm flows or the associated pulses of sediments and contaminants through catchment-based efforts (Walsh et al. 2005a, 2007).

Time since restoration

Restoration itself is a catastrophic disturbance to already impaired stream ecosystems (Tullos et al. 2009), and as a result, we expect time lags between restoration implementation and community recovery. One possible explanation for the lack of significant recovery of habitat or biological communities within our restored streams is that insufficient time was allowed for recovery between the restoration implementation and our sampling effort. Our restored study sites were restored 1 to 7 years prior to our sampling effort, however the small sample size and the lack of pre-restoration data precluded us from evaluating the potential role of time lags in our initial data set. To address this question, we were able to examine long-term data from one of our study sites (Rocky Branch, Raleigh NC). For this dataset we found no significant effect of time since restoration on total species richness, EPT species richness, or biotic index for the 5-year post-restoration monitoring period. Three-dimensional NMS ordination of long term monitoring data for the restored reach of Rocky Branch revealed that macroinvertebrate communities from the restored reach of Rocky Branch remained similar in composition to the unrestored upstream urban reach during the five years of post restoration monitoring and remained consistently different from benthic communities in the closest reference stream Sal's Branch (a tributary to Pot's

Branch) (Fig. 7). Thus, long-term monitoring of Rocky Branch further supports the conclusions of our synoptic sampling effort, with no evidence of directional change in restored stream reaches either away from the pre-restoration composition or towards reference stream conditions. All available evidence suggests that merely waiting longer prior to evaluating a restoration project is unlikely to lead to different conclusions.

Conclusions

Our results demonstrate the limited utility of reach-scale restoration to combat the overwhelming effects of watershed urbanization. Within this study, the only demonstrable effect of restoration activities was to remove riparian trees, a practice that may impede recovery. In our study, restoration failed to improve habitat over impaired urban channels, suggesting that watershed level hydrologic processes are degrading restoration efforts. Expanding urban restoration planning beyond the reach scale to include watershed-scale impacts will lead to better restoration design and more positive restoration outcomes.

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Table 1. Study sites listed by block, stream type, EPA level IV ecoregion, channel, and catchment characteristics.

Block	Status	Site name	Eco-region	Reach length (m)	Estimated discharge (L/s)	Watershed size (km^2)	% Developed	% ISC
1	forested	Stony Creek	45c	100	0.66	6.9	24.4	3.4
	urban restored	Third Fork Creek	45g	80	4.41	4.4	99.5	32.4
	urban degraded	Ellerbe Creek	45c/g	50	10.41	7.6	88.7	20.8
2	forested	Pot's Branch	45f	140	5.83	4.2	27.4	9.9
	urban restored	Walnut Creek Tributary	45f	200	5.47	1.7	84.5	17.8
	urban degraded	Cemetery Creek	45f	100	11.54	2.2	98	19.1
3	forested	Mud Creek Tributary	45c/g	54	2.08	0.9	4.4	0.5
	urban restored	Rocky Branch	45f	50	1.54	1.5	99.2	34.8
	urban degraded	Goose Creek	45g	35	3.72	1.7	100	39.4
4	forested	Lower Mud Creek	45c/g	102.5	11.58	4.1	58.6	9.5
	urban restored	Sandy Creek	45g	60	12.00	6.7	76.9	16.8
	urban degraded	Upper Mud Creek	45c/g	140	4.86	3.5	66.9	11

Table 2. Mean values (± SE) of habitat complexity, flow heterogeneity, floodplain connectivity, and canopy cover of forested, urban restored, and urban degraded stream types. Results and effect sizes are from one-way ANOVAs with stream type as a factor (Significance codes *0.05, **0.01, ***0.001).

	Forested	Urban restored	Urban degraded	df	F	ω^2
Number of habitat transitions per 100-meter reach length (#)	20.75± 1.89	9.250± 2.14	9.75±1.11	2,9	13.55*	41.1
Average depth from point measurements (m)	0.065± 0.0164	0.175± 0.0131	0.158± 0.012	2,9	17.97**	48.5
Average %CV for depth point measurements	109.3± 12.21	73.73± 7.59	83.03± 9.30	2,9	2.29	
Average velocity from point measurements (m/s)	0.035± 0.008	0.023± 0.007	0.026± 0.012	2,9	0.47	
Average %CV for velocity point measurements	209.2± 46.43	139.4± 5.38	237.0± 46.43	2,9	2.29	
Average degree of incision (W:D)	6.15± 0.37	7.14± 1.39	4.96±0.77	2,8	1.64	
Average maximum degree of incision (smallest W:D)	4.74± 0.31	5.02±1.06	4.40± 0.57	2,8	0.23	
Average longitudinal slope (%)	0.93± 0.49	0.51±0.49	0.29± 0.11	2,8	0.78	
Average canopy cover (%)	87.54± 2.50	53.71± 8.28	81.35± 4.36	2,9	10.37*	34.2
Median substrate size (mm)	35.75±11.35	8.0±6.35	4.75±3.75	2,9	4.75*	17.3

Table 3. Mean values (± SE) of macroinvertebrate community metrics with and without Lower Mud Creek (LMC). Results and effect sizes are from one-way ANOVAs performed with and without LMC, with metric as a factor (Significance codes *0.05, **0.01, ***0.001).

	Forested	Forested w/out LMC	Urban restored	Urban degraded	df	F	df w/out LMC	F w/out LMC	ω² w/out LMC
Summer species richness	20.0± 2.3	22.3± 0.3	15.3±3.9	15.0±-2.6	2,9	0.86	2,8	1.70	
Summer EPT richness	5.8± 2.0	7.3± 1.8	1.8± 0.6	1.5± 0.9	2,9	3.28	2,8	8.79*	32.1
Summer BI	5.8± 0.8	5.4± 1.0	7.7± 0.5	8.0± 0.4	2,9	4.35*	2,8	5.19*	20.2
Winter species richness	29.3± 3.4	32.3± 1.9	15.5± 4.7	14.7± 3.8	2,8	4.19	2,7	5.88*	24.5
Winter EPT richness	10.3± 2.4	12.3± 1.7	1.5± 0.5	1.3± 0.9	2,9	11.46**	2,8	34.47***	67.0
Winter BI	5.6± 0.3	5.4± 0.3	7.0± 0.4	7.3± 0.5	2,8	5.55*	2,7	6.04*	25.1

Table 4. Results from least-squares linear regression analyses of macroinvertebrate community metrics with and without LMC (Significance codes *0.05, **0.01, ***0.001).

Watershed imperviousness	r^2	**df**	**F**	r^2 **(w/o LMC)**	**df (w/o LMC)**	**F (w/o LMC)**
Summer EPT	0.26	1,10	3.44	0.33	1,9	4.43
Summer BI	0.50	1,10	10.18**	0.51	1,9	9.55*
Winter EPT	0.54	1,10	11.85**	0.58	1,9	12.23**
Winter BI	0.40	1,9	6.03*	0.40	1,8	5.24
Habitat transitions						
Summer EPT	0.54	1,10	11.72**	0.86	1,9	56.62***
Summer BI	0.70	1,10	22.91***	0.81	1,9	39.36***
Winter EPT	0.46	1,10	8.61*	0.58	1,9	12.60**
Winter BI	0.67	1,9	18.01**	0.74	1,8	22.41**

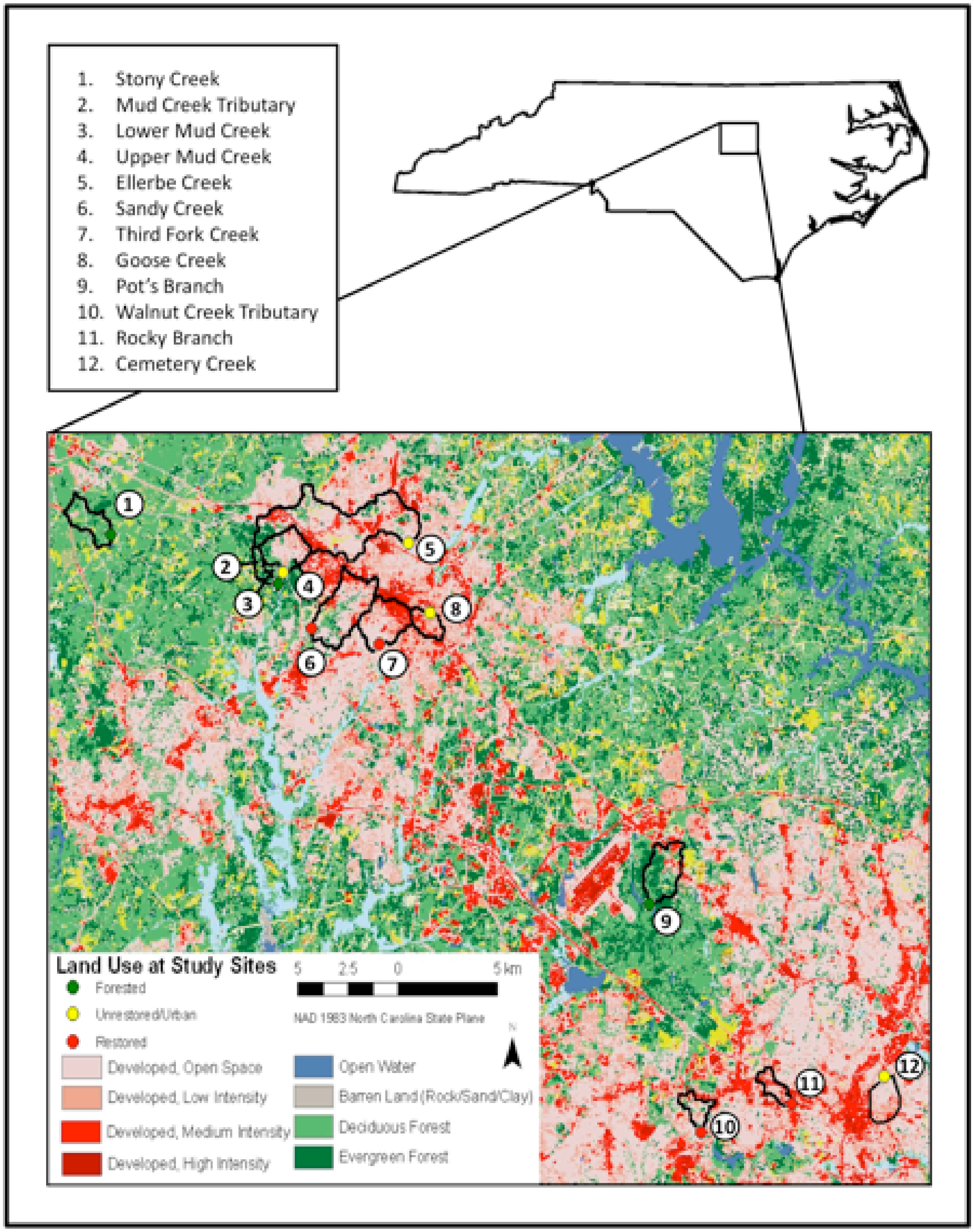


Figure 1. Study site locations and watershed boundaries.

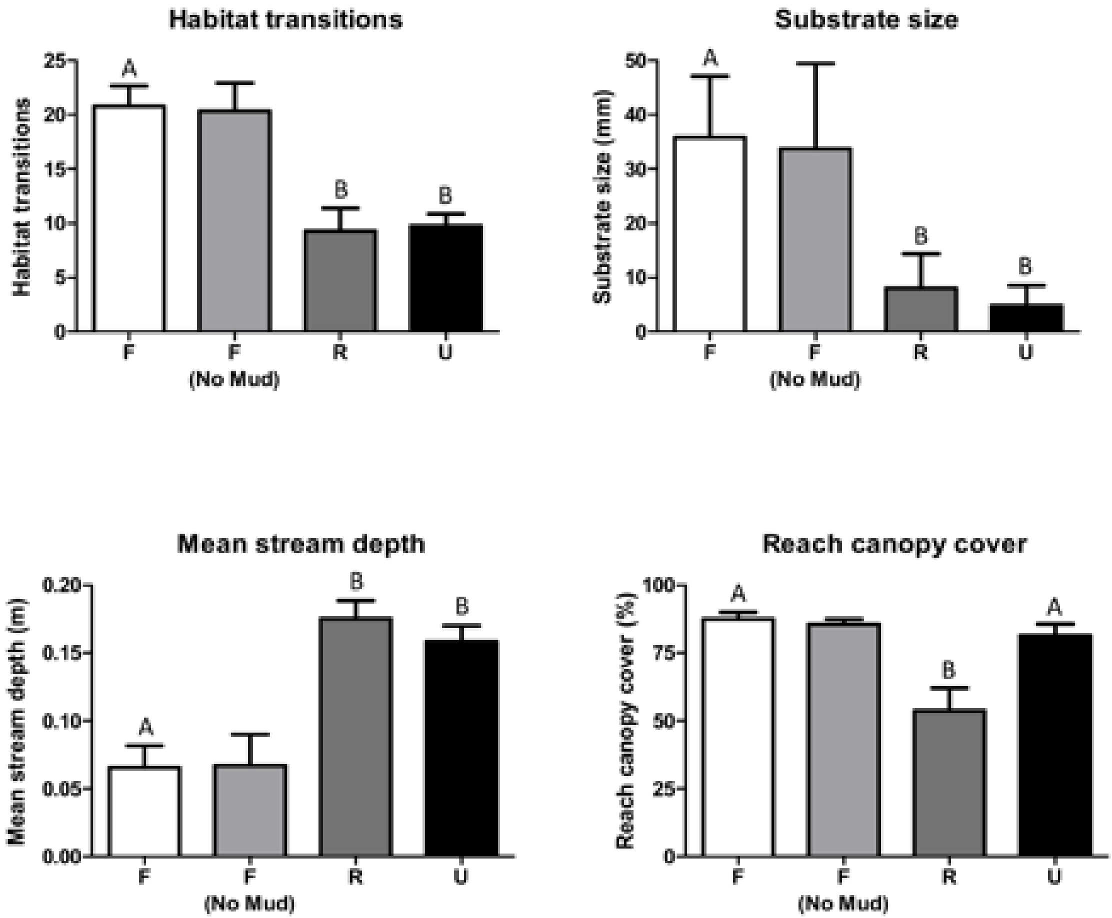


Figure 2. Mean values (± SE) of Habitat transitions (number/100m reach length), Substrate size (mm), Mean stream depth (m), and Reach canopy cover (% covered) for forested sites, forested sites excluding LMC, and urban sites (One-Way ANOVA, $P < 0.05$). Differences among stream types indicated by different letters (Student-Newman-Keuls post hoc multiple comparisons test).

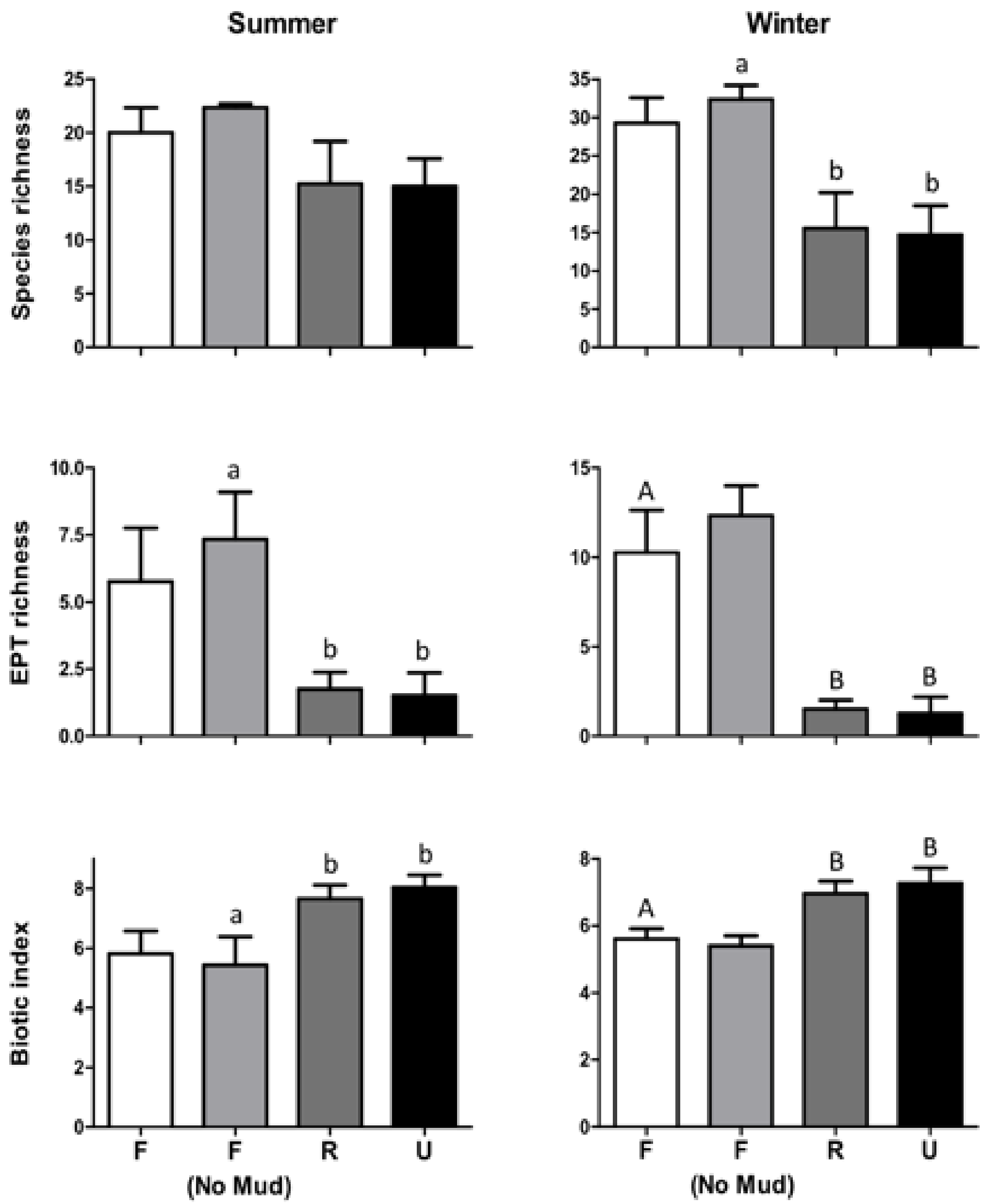


Figure 3. Mean values (± SE) of Summer and Winter species richness, EPT richness, and Biotic index, for forested sites, forested sites excluding LMC, urban restored, and urban degraded sites (One-Way ANOVA, $P < 0.05$). Differences among stream types indicated by different letters (Student-Newman-Keuls post hoc multiple comparisons test). Upper-case letters indicate differences among stream types for the entire dataset, lower-case indicate differences among stream types excluding LMC.

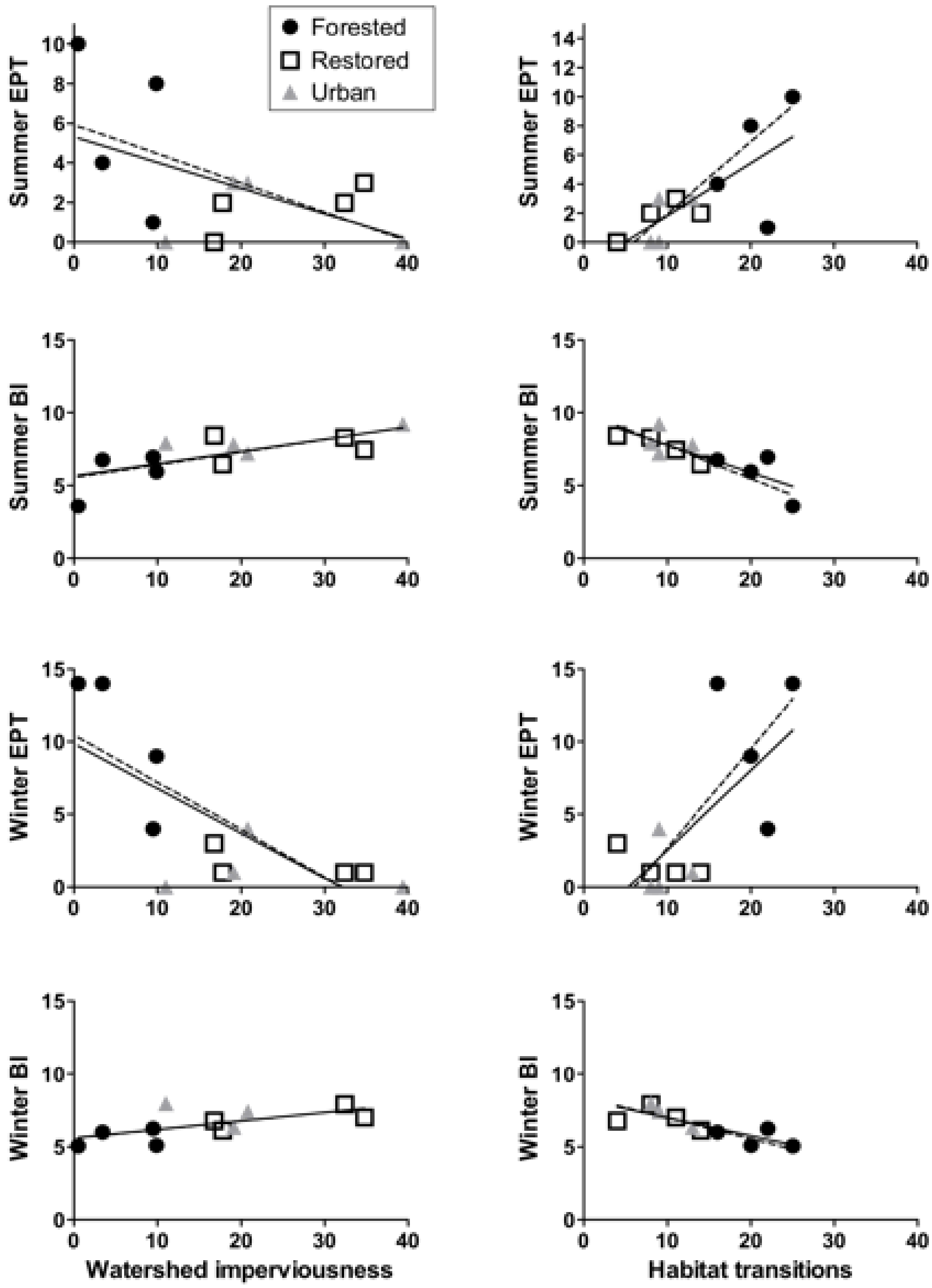
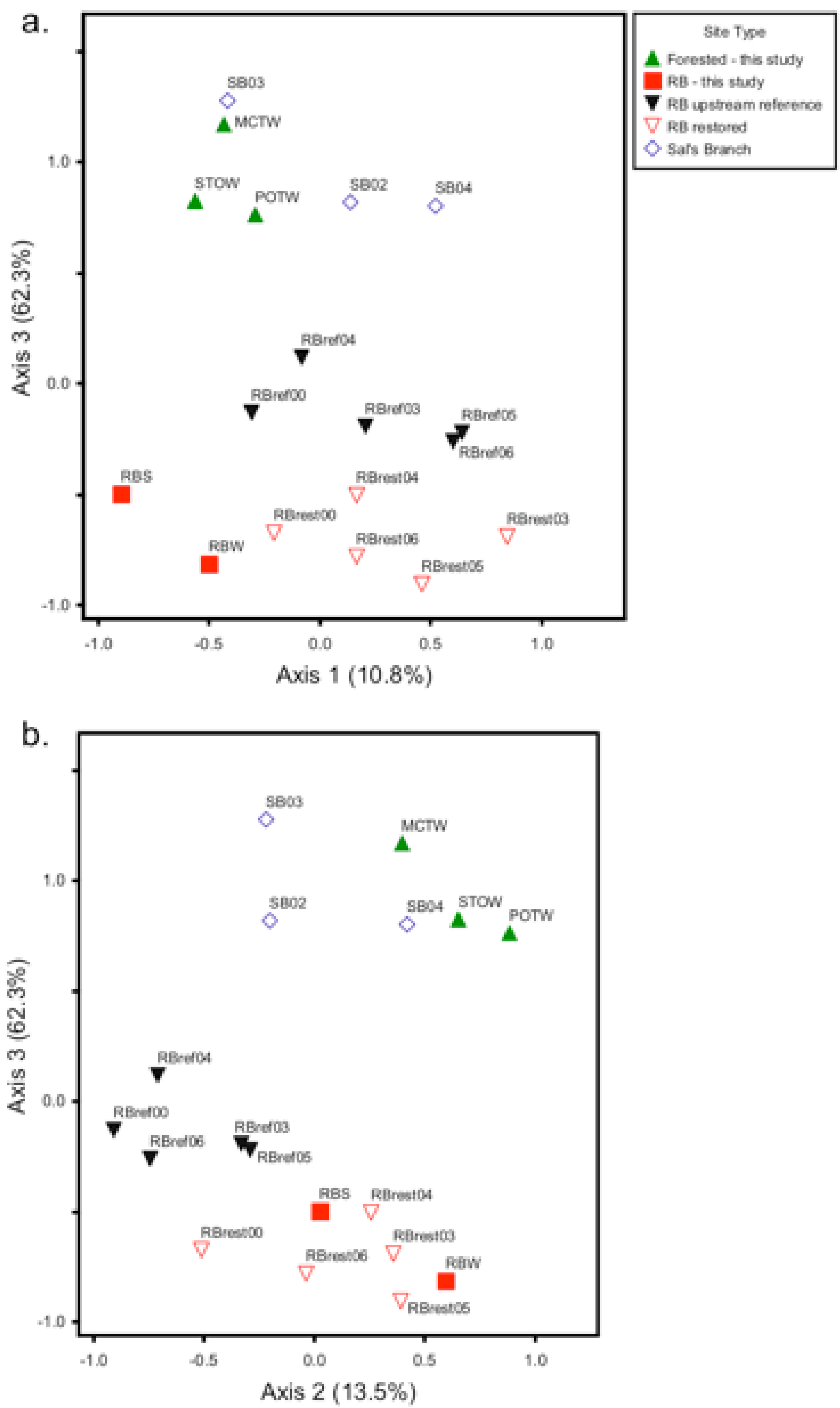


Figure 4. Linear regression of seasonal macroinvertebrate community metrics vs. watershed imperviousness (left panels) and habitat transitions (right panels) with (solid line), and without (dashed line) Lower Mud Creek. R^2 values given in Table 4.

Figure 5. Two-dimensional joint plots of NMS ordination of summer (a) and winter (b) square-root transformation Bray-Curtis similarities with environmental variables. Minimum explanatory r^2 for environmental variables = 0.3. Final stress = 0.13 for summer and 0.078 for winter. Cumulative r^2= 0.782 and 0.882 for summer and winter respectively. Site abbreviations: "AB" = Walnut Creek Tributary; "CEM" = Cemetery Creek; "FH" = Forest Hills; "GSE" = Goose Creek; "LMC" = Lower Mud Creek; "MCT" = Mud Creek Tributary; "NGP" = Ellerbe Creek; "POT" = Pot's Branch; "SAN" = Sandy Creek' "STO" = Stony Creek; " UMC" = Upper Mud Creek. Environmental variable abbreviations: "Hab trans"= habitat transitions; "%CV PD" = percent coefficient of variation of point depth "Ave canopy" = average canopy cover; "Cl^-" = chloride concentration (ppm); "NH4-N" = ammonium-N concentration (ppm); "Ave depth" = average water depth (m); "DON" = dissolved organic nitrogen concentration (ppm); "%ISC" = percent watershed impervious surface cover; "%WS Dev" percent developed watershed; "Point depth" = stream point depth (m); "%Riparian ISC" = percent riparian buffer impervious surface cover; "%CV Canopy" = percent coefficient of variation of canopy cover; "GPP (g/m)" = gross primary production (g/m/d); "NO3-N" = nitrate-N concentration (ppm); "TN" = total nitrogen (ppm); "DD" = degree days, "Ave temp" = average stream temp (^oC); "d50" = median particle size (mm); "DOM C:N" = dissolved organic matter C:N.


a.
Site Type
Forested - this study
RB - this study
RB upstream reference
RB restored
Saf's Branch
SB03
MCTW
STOW
POTW
SB02
SB04
RBref04
RBref00
RBref03
RBref05
RBref06
RBS
RBrest04
RBrest00
RBrest03
RBrest06
RBW
RBrest05
1.0
0.0
-1.0
-1.0
-0.5
0.0
0.5
1.0
Axis 3 (62.3%)
Axis 1 (10.8%)
b.
SB03
MCTW
SB02
SB04
STOW
POTW
RBref04
RBref00
RBref06
RBref03
RBref05
RBS
RBrest04
RBrest00
RBrest03
RBrest06
RBW
RBrest05
1.0
0.0
-1.0
-1.0
-0.5
0.0
0.5
1.0
Axis 3 (62.3%)
Axis 2 (13.5%)

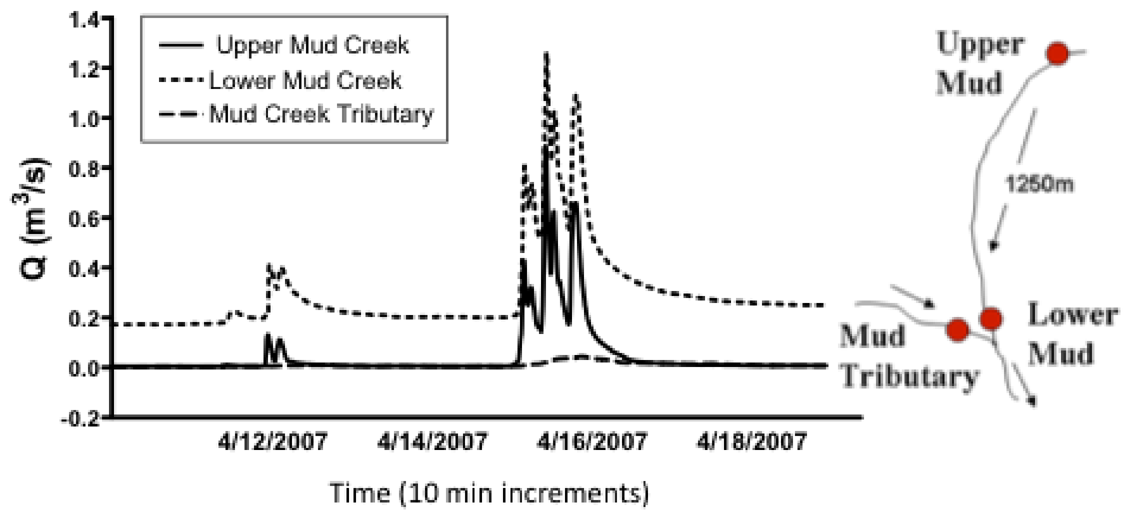
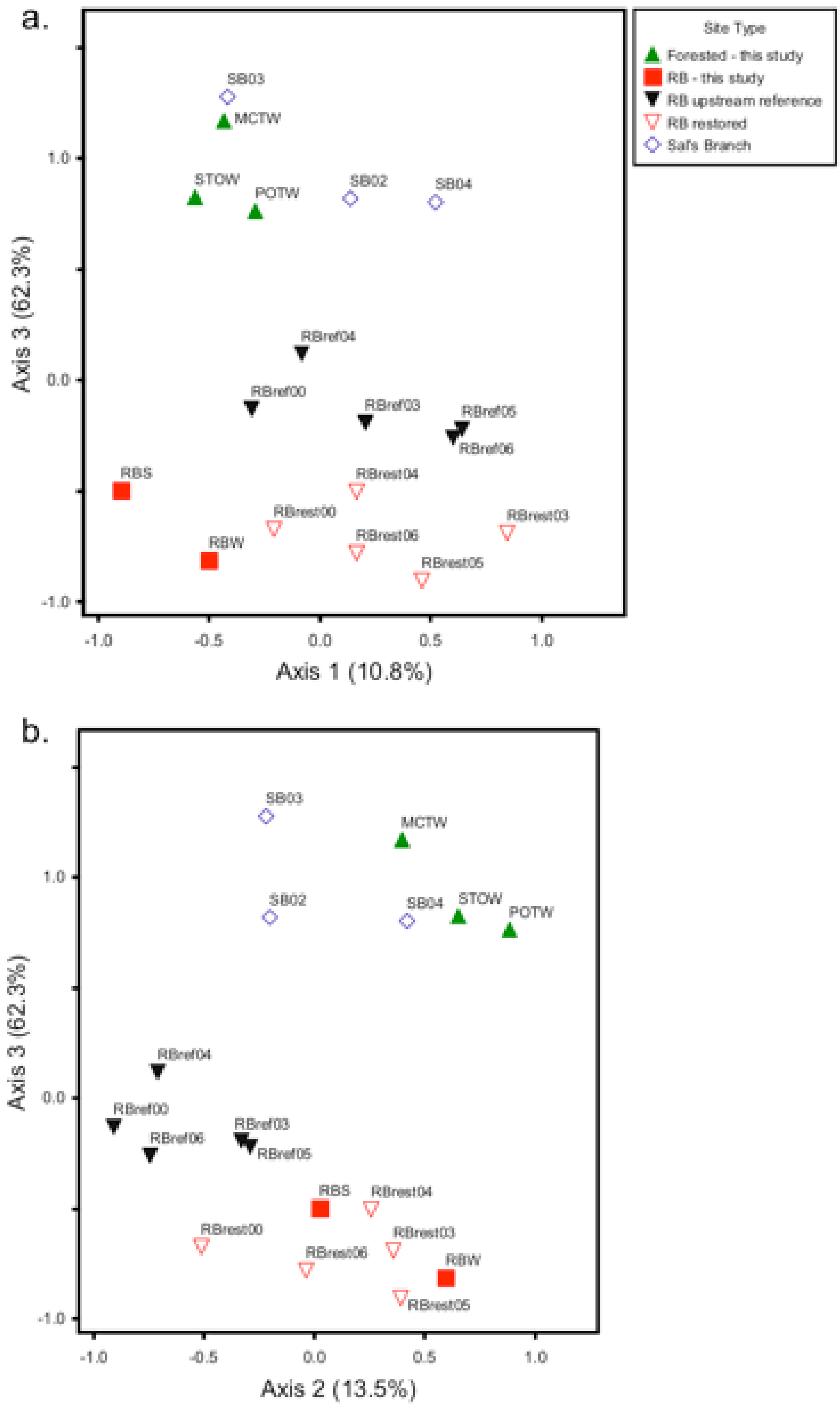


Figure 6. Hydrographs and station map of Upper Mud Creek, Lower Mud Creek, and Mud Creek Tributary.

Figure 7. Two-dimensional representations of a.) axis 3 vs. axis 1 and b.) axis 3 vs. axis 2 of 3-dimensional NMS of all Rocky Branch and forested site data (final stress = 0.09, cumulative r^2 = 0.866). Axis 3 explained the majority of compositional differences (r^2 = 0.623), therefore 2D plots are shown relative to this axis. Site abbreviations: "RBS" = Rocky Branch summer sample, this study; "RBW" = Rocky Branch winter sample, this study; "RBref00" = Rocky Branch monitoring, upstream reference 2000 (pre-restoration); "RBref03" = Rocky Branch monitoring, upstream reference 2003; "RBref04" = Rocky Branch monitoring, upstream reference 2004; "RBref05" = Rocky Branch monitoring, upstream reference 2005; "RBref06" = Rocky Branch monitoring, upstream reference 2006; "RBrest00" = Rocky Branch monitoring, restored 2000 (pre-restoration), "RBrest03" = Rocky Branch monitoring, restored 2003; "RBrest04" = Rocky Branch monitoring, restored 2004; "RBrest05" = Rocky Branch monitoring, restored 2005; "RBrest06" = Rocky Branch monitoring, restored 2006; "MCTW" = Mud Creek Tributary winter; "POTW" = Pot's Branch winter; "STOW" = Stony Creek winter; "SB02" = Sal's Branch 2002; "SB03" = Sal's Branch 2003; "SB04" = Sal's Branch.


a.
Site Type
Forested - this study
RB - this study
RB upstream reference
RB restored
Saf's Branch
SB03
MCTW
STOW
POTW
SB02
SB04
RBref04
RBref00
RBref03
RBref05
RBref06
RBS
RBrest04
RBrest00
RBrest03
RBrest06
RBW
RBrest05
1.0
0.0
-1.0
-1.0
-0.5
0.0
0.5
1.0
Axis 3 (62.3%)
Axis 1 (10.8%)
b.
SB03
MCTW
SB02
SB04
STOW
POTW
RBref04
RBref00
RBref06
RBref03
RBref05
RBS
RBrest04
RBrest00
RBrest03
RBrest06
RBW
RBrest05
1.0
0.0
-1.0
-1.0
-0.5
0.0
0.5
1.0
Axis 3 (62.3%)
Axis 2 (13.5%)

Chapter 3

Stream restoration fails to improve community structure and shows limited correlation between mitigation success criteria and macroinvertebrate community dynamics.

Abstract

Stream restoration has become a common method for rehabilitating degraded ecosystems, and under the auspices of the no net loss provision of the Clean Water Act, has lead to the creation of mitigation banks to provide mitigation credits to public and private development projects that result in stream loss. Currently, however, stream mitigation credits in North Carolina are rewarded based on measures that are considered surrogates for in-stream structure and function, and little information exists on how well biotic communities at restored sites are responding to restoration. Here we present the results of long-term benthic invertebrate monitoring of 13 Natural Channel Design projects implemented in rural, urban, and agricultural watersheds throughout North Carolina. Watershed land use type significantly affected the level of community degradation both pre and post-restoration. When compared with upstream-unrestored sites, invertebrate communities did not show improvement over time in any community structure variable, and in all but one case, differed significantly in species composition from upstream-unrestored sites. These results suggest that restoration is ineffective at restoring biotic community structure, and that local scale restoration may lead to long-term changes in community composition due to restoration activities.

Introduction

Human land use practices have resulted in the widespread degradation of waterways draining the surrounding landscape. Activities such as farming and urban and suburban development lead to geomorphically simple, polluted, poorly functioning streams with lower biological diversity than streams in undisturbed catchments. Agricultural and stormwater runoff and sewer overflows have led to nutrient and pollutant loading (Lenat and Crawford 1994, Carpenter et al. 1998, Bernhardt et al. 2008). Construction, farming and erosion from poorly protected stream banks and hydrological alteration have increased sedimentation (Wolman 1967, Costa 1975, Booth and Jackson 1997). Riparian deforestation has increased due to the proximity of these practices to stream channels (Benstead et al. 2003, Sweeney et al. 2004). These insults lead to poor water quality, unstable habitat, altered trophic structure, and unstable hydrology to which organisms cannot adapt (see Allan 2004 for a thorough review). Ultimately, land use alteration leads to loss of aquatic diversity, ecosystem function, and consequently, ecosystem services (Paul and Meyer 2001, Palmer et al. 2004, Sweeney et al. 2004, Meyer et al. 2005).

The consequences of land use change for stream degradation depend on both the type and extent of land use within the watershed, and the proximity of development to the stream channel (Wang and Kanehl 2003, Moore and Palmer 2005). Watershed urbanization increases impervious surface cover, which accelerates changes in stream structure such as channel incision (deepening of the channel) and channel widening through erosional processes, increased sediment input due to stream erosion and delivery by runoff from the surrounding watershed (Poff et al. 1997, Stepenuck et al. 2002, De Carlo et al. 2004), riparian zone impairment (Sweeney et al. 2004), and increased point and non-point source pollution of fertilizers, and trace metals pollution (De Carlo et al. 2004) from runoff, leaky sanitary sewers, and direct stormwater input (Bernhardt et al. 2008).

The antagonistic relationship between human land use and native biodiversity is a well-established global phenomenon (Blair 1996, Chapin et al. 2000, Germaine and Wakeling 2001, Tilman and Lehman 2001, Clark et al. 2007, Grimm et al. 2008, McKinney 2008). Increases in both agricultural production and the expansion of urban centers have led to global declines in biodiversity (Foley et al. 2005). Aquatic ecosystems are strongly affected by land use patterns (Lenat and Crawford 1994, Sponseller et al. 2001, Allan 2004), and terrestrial land use has led to declines in fishes and aquatic invertebrates (Wang et al. 2001, Wang and Kanehl 2003)

The extent of impervious surface in urban catchments is strongly, inversely correlated with macroinvertebrate species richness (Stepenuck et al. 2002, Morse et al. 2003, Wang and Kanehl 2003) and macroinvertebrate community composition shifts from an assemblage dominated by pollution-sensitive taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT), to one characterized by tolerant taxa such as Chironimidae, Tubificidae, and Oligochaeta (Morse et al. 2003). Agricultural activities in watersheds and riparian zones have similar consequences to those in urban watersheds, although the mechanisms of action may be distinct. Erosion, sedimentation, and channelization result from crop tillage and stream straightening to maximize drainage and cultivation area (Landwehr and Rhoads 2003), and broad, non-point nutrient loading (Lenat and Crawford 1994) and livestock lead to direct, often extensive physical degradation and heavy nutrient loading (Strand and Merritt 1999, reviewed in Belsky et al. 1999).

To alleviate channel incision and bank erosion, water quality degradation, and habitat and biodiversity loss, degraded waterways are often targeted for restoration. Stream restoration or rehabilitation encompasses a variety of strategies by which human impacts are mitigated and previous damage is addressed, with the overarching goal of returning the stream to as close to pre-impacted conditions as possible (National Research Council 1992). Historically, stream restoration implementation has often been site specific and employed for a specific cause, such as local erosion or flooding, or targeted conservation of an endangered or commercially important species (Bash and Ryan 2002, Roni et al. 2002, Doyle and Yates 2010). Post-restoration monitoring is rarely done and when done, monitoring results are rarely made available (Bernhardt and Palmer 2007, Palmer et al. 2007).

Recently, however, stream restoration has fallen under the umbrella of the Clean Water Act (CWA), under which streams are restored for compensatory mitigation (Lave et al. 2008, Doyle and Yates 2010). In North Carolina, the recent widespread implementation of reach-scale stream restoration is largely funded by the need to mitigate for stream loss elsewhere due to development and public works projects (Lave et al. 2008). The Clean Water Act requires no net loss of stream function, for which stream length is currently used as a surrogate. As both public and private development projects must mitigate for any loss of freshwater ecosystems (Lave et al. 2008), this stipulation of the CWA has led to the creation of mitigation banks – entrepreneurial enterprises that restore streams and wetlands to generate mitigation credits available for purchase by developers. This has led to a stream restoration market (Doyle and Yates 2010), comprised of a system of mitigation banks and state financing. In spite of the large amount of money spent, little information is available on the outcome of restoration success. Currently in North Carolina, there are no in-stream biological or functional success criteria. Rather, practitioners monitor vegetation and geomorphology, and equate reasonably stable morphology and adequate live riparian plantings with both biological and functional success (Lave et al. 2008, BenDor et al. 2009).

In addition to mitigation success requirements outlined *a priori*, stream restoration projects are often implemented with the specific goals of water quality improvement and habitat provision (Bernhardt et al. 2007, Sudduth et al. 2007) yet few projects have been adequately evaluated to determine whether these goals are met (Charbonneau and Resh 1992, Palmer et al. 1997, Moerke and Lamberti 2004, Sudduth et al. *in press*, Violin et al. *in press*). Previous studies of stream restoration success have shown limited or mixed geomorphological improvement (Jähnig et al. 2010), and typically show limited (Moerke et al. 2004, Palmer 2010) or no improvement in macroinvertebrate community structure (Jähnig et al. 2010, Violin et al. *in press*). However, because of the individual nature of stream restoration projects, lax or varying monitoring requirements, and the variety of restoration strategies, long-term studies of restoration success are lacking (but see Moerke et al. 2004).

Here we present the results of multi-year macroinvertebrate monitoring from 13 stream restoration projects located throughout North Carolina. Each stream was restored following Natural Channel Design protocols (Rosgen 1994, 1996) and has pre-restoration data and at least 4 years post-restoration data. To our knowledge, this study represents the first that reports analyses from multiple restoration projects utilizing monitoring data collected by the same methods, and possessing both pre-construction, and longitudinal post-restoration invertebrate monitoring data. Our expectations are that 1) successful restoration will result in macroinvertebrate communities whose species richness and biological integrity either equal or exceed upstream-unrestored sites, 2) community composition at restored sites will approach that of upstream sites, particularly where upstream sites are biologically intact, and 3) improvement in biological metrics after successful restoration will occur over time as a restoration sites stabilizes following the extensive stream disturbances often caused by the restoration processes.

Methods

Site selection

The majority of the study sites were chosen from restoration projects primarily managed by the North Carolina Ecosystem Enhancement Program (NCEEP), and implemented to provide compensatory mitigation credits. An additional project was implemented by North Carolina State University. Funding for these projects came from a number of sources including the NCEEP, NC Department of Transportation, NC clean water management trust fund, and the Environmental Protection Agency. Project design, construction, and monitoring were carried out by various consulting firms and university groups. Study sites were selected based on the availability of both pre and post-restoration invertebrate monitoring data and chosen from sites for which invertebrate monitoring was conducted by the

North Carolina State University Water Quality Group. Based on these criteria, we selected 13 stream restoration sites for use in the analysis. Each site had one year of pre-construction data, and between four and eight years of post-restoration monitoring data. At each stream restoration project, for each monitoring year, macroinvertebrate communities were sampled at an upstream-unrestored site above the restoration and at least one sampling location within restored channel. Because the majority of sites had five years of post-restoration data and 5 years is the time monitoring time frame for mitigation purposes, the majority of analyses were carried out for the initial 5-year post-construction monitoring period.

Site Descriptions

Study sites were located throughout North Carolina, and encompassed a number of land use types and drainage basins within the Blue Ridge, Piedmont, and Southeast Plains ecoregions (Griffth et al. 2002, Table 1, Fig. 1), The 13 stream restoration projects studied were all restored using Natural Channel Design (NCD) methods. NCD reconfigures the pattern, profile and dimensions of a degraded channel to emulate an unimpacted ideal (Rosgen 2007). This method utilizes heavy machinery to re-grade and reshape a degraded channel and employs hard structures such as log vanes or cross vanes to control grade, installs root wads to stabilize banks, adds coarse bed material to create riffles, and re-vegetates reconfigured or newly created riparian areas.

The restoration of an unnamed tributary to Bear Swamp Creek in Franklin County, NC restored 1400 linear feet for the NC EEP to provide compensatory mitigation for stream loss in the Tar/Pamlico river basin and was completed in 2002. The project objective was,

> *"to restore habitat and water quality to the restored reach and the Tar-Pamlico River Basin as a whole. By stabilizing the streambed and banks, the restoration will improve water quality by reducing the amount of sediment contributed to the watershed. Exclusion of cattle and establishment of a permanent riparian buffer should further help reduced sediment and nutrient input. The newly established riparian buffer will provide shade, thereby reducing water temperatures, and increase habitat and food for wildlife"* (Ecoscience Corporation 2006).

The High Vista stream restoration project is on County Line creek, located in the French Broad river basin. This project was restored in 2002 and consists of 3500 linear feet (Table 1). Further construction was completed in 2007. All macroinvertebrate monitoring data was collected prior to maintenance construction. According to project planning documents, the purpose of this project was to,

> *"design adjustments to the stream reach that will increase its long-term stability and create a more functional riparian ecological community. The design for the existing stream will adjust geomorphic dimensions, patterns, and profiles. The proposed changes reflect stable conditions of reference reaches and their current geomorphic conditions. Additionally, vegetated buffers will be created that match proximal natural ecological communities found in similar physiographic and climatic regions. The reach will be redesigned to maximize natural design in light of the needs of the golf course and physical constraints within the project area"* (NCDENR-NC Ecosystem Enhancement Program 2009).

Little Pine Creek is a tributary to Brush Creek in Allegheny County, NC, in the Upper New Watershed and was restored in 2001. This restoration project was implemented by the NC EEP and created approximately 1013 linear feet of reconfigured stream from approximately 600 channelized feet. Significant repair work was performed on 1013 linear feet of Little Pine Creek in 2006. The goals of this restoration project were,

> *"streambank re-grading to relieve pressure on the banks during high flows; installation of in-stream structures to stabilize the banks and bank toe; to provide grade control by repositioning riffle areas to stop the channel from incising; and planting of vegetation along disturbed streambanks for long-term bank stability and habitat improvement (HDR 2005a, 2005b)."* (NC Wildlife Resources Commission 2009).

The Hominy Swamp Creek restoration is located in Wilson County, NC within the Conentnea River Basin and restored 2232 linear feet of degraded urban stream in 2001. The objectives of the Hominy Swamp Creek restoration were to,

> *"Establish an stable dimension, pattern, and profile on 2160 linear feet of Hominy Swamp Creek: Improve habitat within Hominy Swamp Creek: Establish a riparian buffer along Hominy Swamp Creek: Incorporate this project into a watershed wide management plan."* (Rummel, Klepper and Kahl 2008).

The Payne Dairy stream restoration project was completed in 2000 and restored 8397 linear feet of Jumping Run Creek located in Alexander County, NC, within the Upper Catawba River Basin. The goals of this restoration project were,

> *"To restore Jumping Run from an altered/degraded stream corridor, including adjacent riparian zones and flood prone areas, to its natural, or referenced stable condition. The ultimate goal of this project is to improve water quality, and the natural function of Jumping Run."* (Kimley-Horn and Associated 2006).

Rocky Branch is a stream located on the urban North Carolina State University campus in Raleigh, NC, within the Upper Neuse River basin. This project, the first of three phases, comprises 3300 linear feet and was restored in 2001. The goals of this restoration project included, *"Restore a stable self-maintaining morphological pattern in the stream channel; Stabilize stream banks using vegetation; Create and improve habitat for fish and aquatic invertebrates; Improve the quality of stormwater entering the creek through restoring and enhancing riparian buffers and establishing stormwater control within the creek's watershed; Provide safe and enjoyable access to the stream and passage through the campus by completing the greenway path adjacent to the creek."* (Doll 2003).

The Smith-Austin stream restoration project was completed in 2002 and restored 5367 and 5587 linear feet of Smith and Austin Creeks respectively, and is located in Wake County, NC in the Upper Neuse River Drainage Basin. These two projects share an upstream-unrestored site (on Austin Creek), but were analyzed as separate projects because the two restoration sites are located on separate streams, in proximity to different insults: medium density housing construction and athletic fields on Austin Creek, and completed medium density housing, golf course, and athletic fields on Smith Creek. The goals of this project included,

"1. Establish stable dimension, pattern, and profile along approximately 11,000 linear feet of Smith and Austin Creeks. 2. Improve aquatic habitat with bed variability and the use of in-stream structures in Smith and Austin Creeks. 3. Provide a terrestrial wildlife corridor and refuge in an area that is highly developed for residential and commercial purposes. 4. Establish a forested riparian buffer adjacent to Smith and Austin Creeks. 5. Incorporate this project into a watershed management plan." (Axiom Environmental, Inc. 2008).

2400 linear feet of an unnamed tributary to Lyle Creek, in Catawba County, within the Catawba River Basin were restored for the EEP in 2002. The goals of this project were to,

"1. Restore 2400 linear feet of an unnamed tributary (UT) to Lyle Creek. 2. Enhance the riparian area through planting native species, and 3. Exclude cattle access to the UT to Lyle Creek and 800 linear feet of a second unnamed tributary" (Jordan, Jones, and Goulding, Inc. 2007).

The Price Park stream restoration located in Guilford County, NC restored 1776 linear feet of urban stream on an unnamed tributary to Horsepen Creek within the Upper Cape Fear River Basin. This project was completed in 2001. The goals of this project were as follows,

> *"Provide a stable stream channel that neither aggrades nor degrades, while maintaining its dimension, pattern, and profile with the capacity to transport its watershed's water and sediment load. Reconnect the stream with its floodplain. Improve aquatic habitat with the use of natural material stabilization structures such as root wads, rock vanes, woody debris and a riparian buffer. Provide wildlife habitat and bank stability through the creation of a riparian zone. Incorporate the existing greenway plan into the stream restoration plan."* (KCI Associates 2009).

The Beaver Creek restoration project was completed in 2002 and consists of 4670 linear feet of restored stream on Beaver Creek in Surry County, NC and is located in the Upper Yadkin River Basin. The goals of this restoration project were as follows,

> *"Restore 4,670 linear feet of Beaver Creek (as measured along the thalweg); Provide a stable stream channel that neither aggrades nor degrades while maintaining its dimension, pattern, and profile with the capacity to transport its watershed's water and sediment load; Improve water quality and reduce further property loss by stabilizing eroding stream banks; Reconnect the stream to its floodplain or establish a new floodplain at a lower elevation; Improve aquatic habitat with the use of natural material stabilization structures such as root wads, rock vanes, woody debris and establish a riparian buffer; and Provide aesthetic value, wildlife habitat, and bank stability through the creation or enhancement of a riparian zone."* (Earth Tech 2009).

The Brown Branch restoration project is located in a primarily forested watershed in Caldwell County, NC within the Upper Catawba River basin and consists of 5100 linear feet restored in 2003. The goals of this stream restoration project were,

> *"Goals of the Brown Branch restoration project include the establishment of a dynamically stable plan form; to create cross sectional and profile patterns that will enhance in-stream habitat and water quality, and to improve the functional and aesthetic value of the riparian corridor. The design increased the sinuosity of the channel and incorporated rock and log structures. Structures were put in place to decrease erosive stress on the banks and provide increased aquatic habitat. By creating a range of aquatic niches, the project intends to provide in-stream habitats that may support future trout populations."* (MACTEC 2007).

The Stone Mountain stream restoration consists of 5000 linear feet of restored stream on the East Prong of the Roaring River, located within Stone Mountain State Park in Wilkes County, NC. This project is located in the Upper Yadkin River Basin and was completed in 2000. The goals of this project were,

> *"to develop a stable stream channel with reduced bank erosion, efficient sediment transport, enhanced warm water fisheries, and improved overall stream habitat and site aesthetics."* (NCSU BAE).

Macroinvertebrate data collection

Macroinvertebrate data were collected using either the full scale or Qual-4 versions of the North Carolina Department of Water Quality (NC DWQ) semi-quantitative collection protocol (NC DWQ 2006), depending on catchment size. This sampling protocol is conducted so that sampling effort is proportional to stream size and drainage area and consistent among study sites. Each Qual-4 sample consisted of one 2-3 min, 1 m^2, 1 mm mesh kick net sample from a characteristic riffle, one 500 mm mesh D-sweep net of stream marginal habitats such as root mats and bank vegetation, an approximately 500 g leaf pack sample collected from rock or snag habitats, and visual assessments of habitats not easily sampled with the above methods (e.g., large rocks or logs). The full-scale protocol utilizes the same collection methods, but consists of two kick nets, 3 sweep nets, visual assessments, and 3 fine mesh rock-log wash epifaunal samples. Samples were field-sorted and specimens were preserved in 95% ethanol. Non-chironomid taxa were identified at 45x to the lowest possible taxonomic level, typically species. Chironomidae were slide mounted in CMC-10 medium (Master's Chemical Co., Wood Dale, IL.), and identified at 400x magnification to genus or species. Following the NC DWQ protocol, we classified taxa as abundant (>10 individuals), common (3-9 individuals), or rare (1-2 individuals).

Landscape analyses

We acquired the 1/3" digital elevation model for study counties from the USGS Seamless Server and calculated flow direction and accumulation analysis using the ArcHydro extension of ArcGIS. Streams were defined based on a 1000 pixel threshold, and watersheds were delineated for all sites. Land use and impervious surface cover within study watersheds were analyzed based on the USGS 2001 National Land Cover Dataset (NLCD), and Impervious Surface Cover dataset (Homer et al. 2004). NLCD was reclassified into six categories: developed, forest, agriculture, pasture, crops, and natural, and for each watershed we calculated the percent of each land use type and percent impervious surface cover. Percent forested catchment was used as a predictor variable in subsequent analyses. Based upon land use characteristics in each study watershed, we classified sites as rural, agricultural, or urban.

Data analyses

Macroinvertebrate community metrics

For each study site, at each time point, for both upstream-unrestored and restored locations, we calculated total species richness (Total S) and richness of the orders Ephemeroptera, Plecoptera, and Trichoptera, (EPT S) as a measure of pollution-sensitive taxa richness. Biotic Index (BI) was calculated to quantify overall macroinvertebrate community pollution tolerance. BI was calculated as a weighted mean of taxa tolerance values relative to their abundance (NC DWQ 2006); higher BI values indicate a more pollution-tolerant assemblage. Taxon tolerance values were taken from the NC DWQ benthos standard operating protocol (Lenat 1993, NC DWQ 2006). Taxa for which BI information was not available were infrequent, and were excluded from the BI calculations. Within monitoring year, we calculated Bray-Curtis dissimilarity (Bray and Curtis 1957) of log(x+1) transformed abundance data to assess differences in community composition between paired upstream-unrestored and restored sampling sites. For projects where there was more than one sampling location within the restored reach, we utilized data from only the most downstream sampling location in order to capture the overall effect of the reach-scale restoration.

Initial macroinvertebrate community structure

To determine the initial level of macroinvertebrate community degradation at each study location, we used ordinary least squares regression to quantify the effects of catchment development on macroinvertebrate species richness, EPT richness, and biotic index. Because catchment development encompasses a variety of development types with disparate effects, percent forested watershed was used as a measure of catchment impairment as it represents the un-impacted state for all study locations.

Pre and post-restoration macroinvertebrate community structure

To evaluate differences in macroinvertebrate community structure among catchment types at upstream-unrestored and restored study sites both before and after restoration, and to test for pre vs post-restoration macroinvertebrate community differences within site type, we used two-way repeated measures ANOVA with site type and restoration status (pre vs post) as factors, using upstream or restored species richness, EPT richness, and biotic index as response variables in separate analyses. Post-restoration data were from either the fifth monitoring year or the last post-restoration year for which data were available (post-restoration year four for Rocky Branch). Where the overall effect of site type was significant, we used Bonferroni multiple comparison test to determine differences between pairs of site types.

Community response time lags

We analyzed macroinvertebrate community metrics and land use data to determine if macroinvertebrate communities were improving with time since restoration, and to evaluate whether community composition became more similar between restored sites and upstream-unrestored sites over time.

For each paired upstream and restored sample, we calculated the restoration response for each community metric (S, EPT S, and BI) by subtracting the value of the metric at the upstream-unrestored sampling site from the value at the restored site for the pre-restoration year and the five year post-restoration monitoring period. The response values indicate whether macroinvertebrate community structure is improved by restoration. For EPT richness and total richness, a positive value indicates higher richness at restored sites compared to upstream-unrestored conditions. For biotic index, a positive value indicates a more tolerant macroinvertebrate assemblage at restored sites relative to upstream. We used two way repeated measures ANOVA to evaluate the effects of monitoring year and site type on the restoration response of aggregate community metrics and upstream-restored Bray-Curtis dissimilarity. Sites with missing data were excluded from the analyses.

In addition to longitudinal effects, we evaluated species compositional similarity between restored and upstream-unrestored sites, using relativized, log(x+1) transformed abundance data in paired upstream and restored sampling sites. We analyzed community patters with blocked multi-response permutation procedure (MRPP) (Zimmerman 1985, Meilke and Berry 2001, PC Ord v. 6, McCune and Grace 2006), blocked by monitoring year, using Euclidean distance and median block alignment (Zimmerman 1985). As these analyses were carried out within individual restoration projects, all available monitoring data was used in this analysis and varied by site from 5-8 years, including pre-restoration data.

Results

Pre-restoration macroinvertebrate community structure at both upstream and reference sites were correlated with the amount of forested watershed (Table 2,

Fig. 2). Upstream and restored species richness were both positively correlated with percent forested watershed (r^2=0.46, P<0.05; r^2=0.33, P<0.05 respectively) as were upstream and restored EPT richness (r^2=0.63, P<0.01; r^2=0.53, P<0.01 respectively). Biotic index was inversely correlated (higher biotic index indicates a more tolerant macroinvertebrate community) with forested watershed for both upstream and restored locations (r^2=0.51, P<0.01; r^2=0.40, P<0.05) (Table 2, Fig. 2).

Two-way repeated measures ANOVA of pre and post restoration macroinvertebrate community structure at both upstream and restored locations revealed differences among site types and within site type when comparing pre and post restoration (Table 4, Fig. 3). Species richness, EPT richness, and biotic index differed significantly among site types at upstream-unrestored and restored locations both before restoration and at the end of the five-year monitoring period. Biotic index tended to increase from rural-agricultural-urban, while total richness and EPT richness tended to degrease from rural to agricultural to urban sites. Time (pre vs post restoration) was a significant predictor of upstream and restored species richness and restored EPT richness, which showed significant post-restoration declines (Table 4, Fig. 3),

Annual restoration responses of biotic index, EPT richness, and total species richness revealed differing community responses among sites and community metric. Repeated measures ANOVA revealed no significant effect of time on the restoration responses of species richness, EPT richness, or biotic index, however individual restoration projects differed significantly in their restoration responses

(Table 5, Figs. 4-5). There was no effect of site type or interactive effect of site type and monitoring year on the restoration response of any community metric.

Repeated measures ANOVA of Bray-Curtis dissimilarity showed no effect of site type or time since restoration on macroinvertebrate compositional similarity between upstream and restored species composition, however as with aggregate community metrics, sites differed significantly in their response (Tables 5-6).

In blocked MRPP analyses, species composition at upstream and restored sites within individual restoration projects differed significantly in species composition at all sites, with the exception of one urban site, Hominy Swamp (Table 6).

Discussion

We used four measures of macroinvertebrate community structure (total richness, EPT richness, biotic index, and Bray-Curtis dissimilarity) to assess the success of 13 restoration projects throughout North Carolina. The correlation of pre-restoration biotic index, EPT richness, and total species richness with the amount of intact watershed agrees with a large body of previous work on the role of catchment land use in degrading macroinvertebrate communities (Allan et al. 1997, Stepenuck et al. 2002, Violin et al. *in press*). Higher percent forested watershed was associated with higher species richness and community sensitivity (Table 2, Fig 2). The significant difference in macroinvertebrate community structure among site types pre-restoration indicates that watershed land use affects macroinvertebrate community structure, and that watershed development is important. The lack of post-restoration similarity among site types indicates that catchment-based effects are not ameliorated by restoration. (Table 4, Fig. 3).

The significant declines in total species richness seen pre vs post restoration at both upstream-unrestored sites and restored sites (Fig. 3) may be due to increased development in rural catchments since restoration, but are also likely due in large part to drought conditions that affected North Carolina in 2001 and from 2007-2008. The decline in restored EPT richness within rural sites may be due to poor restoration design. However, the apparent, albeit non-significant, decline in this metric at rural upstream sites indicates that catchment development or post-restoration drought conditions are more likely explanations (Fig. 3).

Evaluating macroinvertebrate communities in restored stream reaches with respect to upstream-unrestored locations allows project evaluation with respect to watershed conditions. This method can account for things like drought, increased catchment degradation, changes in macroinvertebrate communities due to annual differences or differences in annual sampling time. Annual restoration responses of biotic index, EPT richness and total richness at each site illustrate that generally, assemblages are more tolerant at restored locations when subtracting out non-restoration effects however these results did not always coincide with other aggregate measures of community integrity (Fig. 4). As biotic index is a measure of pollution tolerance across all taxa, it is probably the best indicator of the ability of stream restoration to improve water quality. Less striking patterns among restoration projects are seen for both total taxa richness and EPT richness (Fig. 4). Total richness is the least predictable and reliable restoration response, since degradation may lead to species replacement with more tolerant taxa, without affecting species counts.

Restoration itself is a catastrophic disturbance to already impaired stream ecosystems (Tullos et al. 2009) and as a result, we expect time lags between restoration implementation and community recovery. However, time since restoration was not a significant predictor of restoration response for any of the aggregate community metrics assessed, or Bray-Curtis dissimilarity. This demonstrates that in spite of restoration efforts, macroinvertebrate communities in restored reaches are not improving over time with respect to initial conditions or when accounting for catchment processes. The non-significant effect of site type, but highly significant inter-site effects demonstrates that catchment land use did not predict restoration outcome (Table 5, Figs. 4-5). Over time, we would expect restoration to significantly affect community response, but the time scale of this response is yet unknown. The five-year post-restoration interval outlined for mitigation purposes is inadequate to detect a longitudinal response, and it appears that monitoring should be continued beyond the initial 5-year period.

In direct contrast with the goal of emulating reference conditions, results of species compositional analyses indicate that restoration is not having any measurable effect, and may alter community composition. Blocked MRPP revealed that for all but one site, composition at upstream and downstream sites differed significantly. These results, coupled with the non-significant response of Bray-Curtis dissimilarity indicates that within restored reaches, pre-restoration degradation may have been responsible for differences in community structure, however post-restoration these effects are at least partially replaced by local site differences due to restoration activities. The one site with statistically similar upstream and restored communities, Hominy Swamp, is located in a heavily urbanized watershed. At this restoration site, restoration may be having a positive effect, or watershed effects due to urban land use common at both sampling sites are superseding any restoration effect.

Potential restoration successes

Based on our analyses, the success of most restoration projects is equivocal at best. The majority of restored reaches had more tolerant communities than their upstream-unrestored sites. Species richness and EPT richness responses were mixed, and as previously stated, sites did not improve significantly with time. However, several projects showed some level of consistent improvement. Within urban sites, Hominy swamp had mixed restoration responses for both biotic index and EPT richness, but similar community composition to the upstream-unrestored site. Rocky Branch restoration response values were consistently positive for total species richness and EPT richness. However, the Rocky Branch upstream-unrestored site is located in the same highly urbanized watershed, the improvements were minimal, and both upstream and restored sites have very low sensitive taxa richness and high biotic indicies. One rural project, Brown Branch, demonstrated primarily positive restoration responses for both EPT richness and total richness, however had consistently more tolerant communities than the upstream-unrestored site. Furthermore, for both Rocky Branch and Brown Branch, upstream-unrestored and restored compositional similarity showed no improvement, and restored communities were statistically distinct from their respective upstream-unrestored sites.

Suggestions for the practice

As with all ecological restorations, stream restoration methodology should reflect the "first do no harm" principle (Leopold 1948, Palmer et al. 2005) and should aim to accomplish restoration goals with minimal intervention (Palmer 2010). Natural channel design involves major changes to the stream, one of which is riparian canopy loss due to restoration activities (Sudduth et al. *in press*, Violin et al. *in press*). This may fundamentally change the thermal regime (Tait et al. 1994, Caissie 2006) and food web structure (Hawkins et al. 1982, 1983) and inhibit colonization by terrestrial adults (Briers and Gee 2004, Collier et al. 1997, Harrison and Harris 2002), the effects of which may persist for many years, until the canopy matures. Additionally, Natural Channel Design introduces sediment both during restoration, and potentially over the long term from structure failures due to high flows and channel migration (Shields et al. 1995, Bernhardt and Palmer 2007) which can further impair the community and prevent the establishment of species sensitive to fine sediment.

Examples of limited success are presented herein (e.g. Brown Branch), however it is impossible to determine if equal success was achievable by simply removing proximate insults and using minimally invasive approaches, such as riparian zone protection and rehabilitation only. Previous work has shown that restored sites tend to be comprised of opportunistic species capable of quick colonization and persistence in unstable environments (Tullos et al. 2009) and a more minimalist approach, specifically those that minimize canopy loss or sediment introduction might ameliorate compositional shifts seen at most sites (Violin et al. *in press*).

This study highlights both the importance of collecting monitoring data, but also the great need for standardized monitoring and reporting protocols. We were able to demonstrate that restoration was not improving community structure over time, that catchment land use leads to community differences, and that restoration itself lead to distinct macroinvertebrate communities. However there wasn't enough consistent habitat data or any functional data available to attempt to decipher potential proximate causes of these patterns, or to evaluate whether restoration was in fact successfully restoring habitat. Furthermore, while upstream-unrestored sites in rural and many agricultural watersheds serve as examples of unimpacted channels within the drainage basin, in more developed catchments, particularly urbanized watersheds, upstream-unrestored sites may be heavily impacted and may, at best represent the average watershed insult. To truly ascertain restoration success above degraded conditions in these channels, it is necessary to have macroinvertebrate data from minimally impacted stream reaches within the same sub-basin for comparison.

Stream mitigation and restoration success criteria

Given the expense involved in stream restoration, and the results presented herein, it is necessary to reframe restoration goals, and reevaluate methods and mitigation requirements. Stream mitigation credits in North Carolina are currently awarded for geomorphological and riparian measures used as surrogates for in-stream ecological structure and function (Lave et al. 2008). The results presented herein show that regardless of mitigation success, biological communities are showing little, if any improvement following stream restoration, and in the majority of cases, restored communities are compositionally distinct from upstream-unrestored sites, regardless of catchment type.

Undoubtedly, stream restoration success from a mitigation standpoint should incorporate in-stream measures of biological and functional recovery. Additionally, site selection, restoration methodology, goals and restoration success criteria should be based on expectations underpinned by catchment land use (Palmer et al. 2005, Bernhardt and Palmer 2007). Continued water pollution and hydrological disturbance should be expected to impair recovery under current urban reach scale restoration methodology (Booth 2005). However, designing restorations to specifically address these factors (Walsh et al. 2005a), or maximizing restoration for a specific ecosystem function (e.g. N retention) rather than habitat or biotic community recovery may actually improve downstream water quality (Groffman and Crawford 2003, Grimm et al. 2005). Even under the best multi-scale oriented efforts, biological recovery in urban watersheds may be minimal (Bernhardt and Palmer 2007). Conversely, in highly forested watersheds with local-scale, limited stream degradation from historic land use practices, local-scale restoration may effectively ameliorate negative impacts.

Conclusions

Within the chosen study sites, Natural Channel design is having little, if any positive effect on macroinvertebrate community strucuture. Independent of watershed type, the aggregate community metrics biotic index, total species richness, and EPT richness showed no improvement over time. This suggests that restoration is not mitigating processes determining community tolerance and richness in restored reaches, irrespective of watershed land use type. Compositional similarity did not change over time, and communities at paired upstream-unrestored and restored sites were generally compositionally distinct, suggesting that *a priori* local differences between upstream and restored conditions either persisted post-restoration, or were exacerbated by restoration activities. These results point to a need to reevaluate restoration methods in North Carolina, and suggest that the current mitigation success criteria of riparian live stems/acre and minimal channel migration are not adequate surrogates for in stream community dynamics.

Collaborator acknowledgements: Dave Penrose collected and identified the majority of the invertebrate samples. David Palange and Kayleigh Somers performed GIS analyses, Emily Bernhardt provided guidance with regard to analyses and interpretation.

Table 1. Study site characteristics. Drainage basins are based on USGS 8-digit HUC codes. Ecoregional designations are EPA level IV (Griffith et al. 2002). Ag. = agricultural catchment.

Site	County	Drainage basin	Eco-region	Year restored	Linear feet	Percent forested watershed	Site Type
Austin Creek	Wake	Upper Neuse	45f	2002	5587	53.6	Urban
Beaver Creek	Surry	Upper Yadkin	45e	2002	4670	54.3	Rural
Brown Branch	Caldwell	Upper Catawba	45b	2003	5100	99.8	Rural
High Vista	Henderson/ Buncombe	Upper French Broad	66j	2002	3500	35.8	Ag.
Hominy Swamp Creek	Wilson	Conentnea	65m	2001	2232	13.1	Urban
Little Pine Creek	Allegheny	Upper New	66c	2001	1013	33.2	Ag.
Murphy Farm – UT Bear Swamp Creek	Franklin	Upper Tar	45f	2002	1400	43.6	Rural
Payne Dairy	Alexander	Upper Catawba	45b	2000	8397	30.1	Ag.
Price Park	Guilford	Haw River	45b	2001	1776	34	Urban
Rocky Branch	Wake	Upper Neuse	45f	2001	3300	0.02	Urban
Smith Creek	Wake	Upper Neuse	45f	2002	5367	53.6	Urban
Stone Mountain	Wilkes	Upper Yadkin	45e	2000	5000	94.2	Rural
UT Lyle Creek	Catawba	Upper Catawba	45b	2002	1940	29.6	Ag.

Table 2: Results from least-squares linear regression analyses of pre-restoration macroinvertebrate metrics at upstream-unrestored and restored sampling sites (*P* value significance codes: *0.05, **0.01).

Metric	r^2	F
Upstream species richness	0.46	9.33*
Upstream EPT richness	0.63	18.8**
Upstream biotic index	0.51	11.27**
Restored species richness	0.33	5.51*
Restored EPT richness	0.53	12.4**
Restored biotic index	0.40	7.72*

Table 3: Mean values (± SE) of pre and post-restoration macroinvertebrate community metrics for both upstream and restored sampling sites in rural (R), agricultural (A), and urban (U) watersheds.

	Upstream			**Restored**		
Metric	R	A	U	R	A	U
Pre-restoration						
Biotic index	3.9±0.56	4.37±0.24	6.89±0.37	4.92±0.64	5.87±0.69	6.640.33
EPT richness	33±6	17.8±1.8	6.2±2.2	31±7.4	14.5±5.5	5.2±1.8
Species richness	69±10.7	40.8±2.6	28.8±4.3	70.5±12.4	44.3±8.2	26.6±3.6
Post-restoration						
Biotic index	3.75±0.14	3.81±0.34	6.49±0.64	5.04±0.42	5.68±0.48	6.84±0.29
EPT richness	24.8±5.6	18.8±4.7	6±2.3	21.3±7.2	13.3±6	4.2±1.6
Species richness	48±10.3	33.5±7.8	24.4±4.4	44. ±9.8	31.3±10.0	23.4±3.0

Table 4. F ratio and *P* values of two-way repeated measures ANOVA analyzing the effect of site type and restoration year on macroinvertebrate community metrics.

	Site Type		Restoration year (pre or post)		Site type: restoration year		Site	
Metric	F	*P*	F	*P*	F	*P*	F	*P*
Upstream species richness	7.163	0.01	7.622	0.02	1.675	0.24	3.212	0.04
Restored species richness	5.571	0.02	9.242	0.01	5.771	0.02	2.907	0.053
Upstream EPT richness	10.34	0.004	2.089	0.18	2.73	0.11	5.93	0.005
Restored EPT richness	5.153	0.03	5.421	0.04	2.725	0.11	10.44	0.0005
Upstream biotic index	15.5	0.0009	4.294	0.065	15.5	0.66	7.137	0.0023
Restored biotic index	4.191	0.05	0.0543	0.82	0.3361	0.72	5.952	0.005

Table 5. Results of two-way repeated measures ANOVA with site type and restoration year as factors. The response variables, in single response models are the annual restoration response values of total species richness, EPT richness, and biotic index, and annual upstream-restored Bray-Curtis dissimilarity.

	Site Type		Restoration year		Site type: Restoration year		Site	
Restoration response	F	*P*	F	*P*	F	*P*	F	*P*
Species richness	0.9281	0.44	0.395	0.85	1.313	0.26	3.275	0.009
EPT richness	2.54	0.15	0.645	0.67	0.903	0.54	3.622	0.005
Biotic index	3.632	0.08	1.263	0.30	1.211	0.32	9.328	<0.0001
Bray-Curtis index	2.758	0.13	2.758	0.08	0.8251	0.61	5.177	0.0004

Table 6. A statistic and P values from blocked MRPP analyses. The A statistic is a measure of effect size and $P<0.05$ indicates a significant difference in macroinvertebrate community composition between paired upstream-unrestored and downstream restored sampling sites.

Site	A	*P*
Austin Creek	0.095	0.009
Beaver Creek	0.039	0.017
Brown Branch	0.088	0.008
High Vista	0.08	0.0084
Hominy Swamp Creek	0.0066	0.24
Little Pine Creek	0.021	0.015
Murphy Farm – UT Bear Swamp Creek	0.098	0.0084
Payne Dairy	0.068	0.002
Price Park	0.031	0.041
Rocky Branch	0.088	0.016
Smith Creek	0.055	0.016
Stone Mountain	0.066	0.003
UT Lyle Creek	0.038	0.009

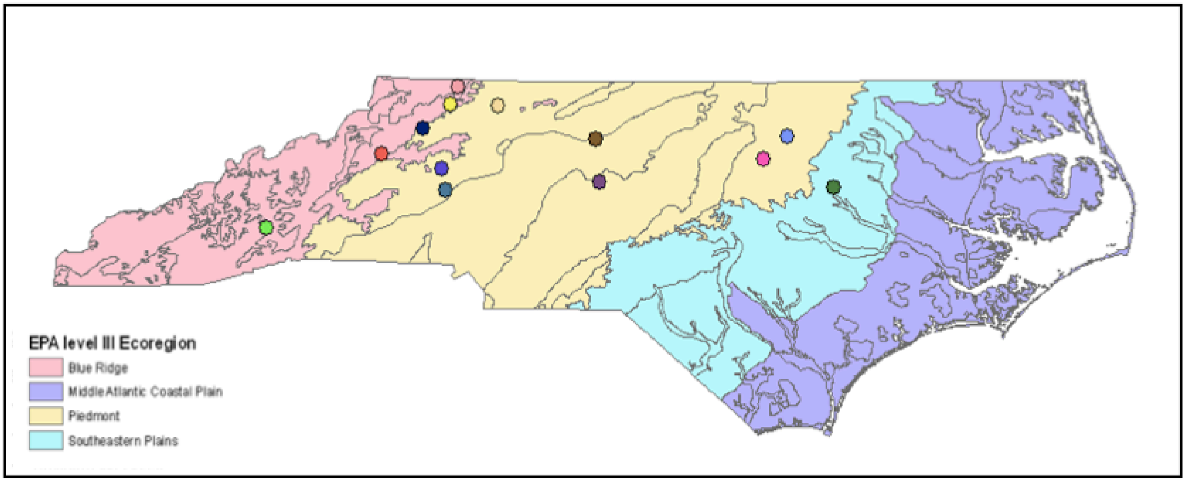


Figure 1. Study site locations.

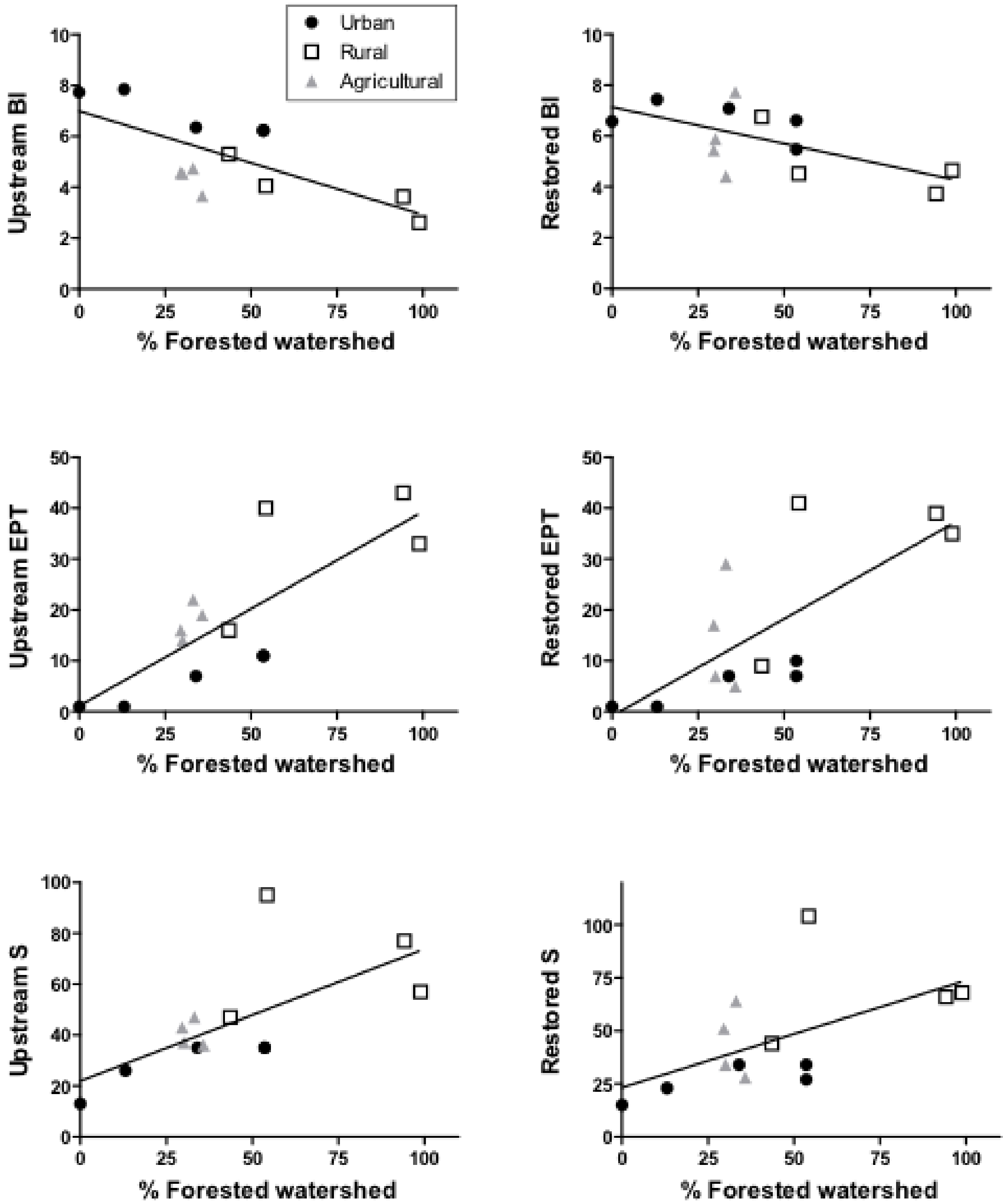


Figure 2. Least squares linear regression of pre-restoration macroinvertebrate community metrics at both upstream-unrestored and restored sampling sites vs percent forested watershed. R^2 and P values given in Table 2.

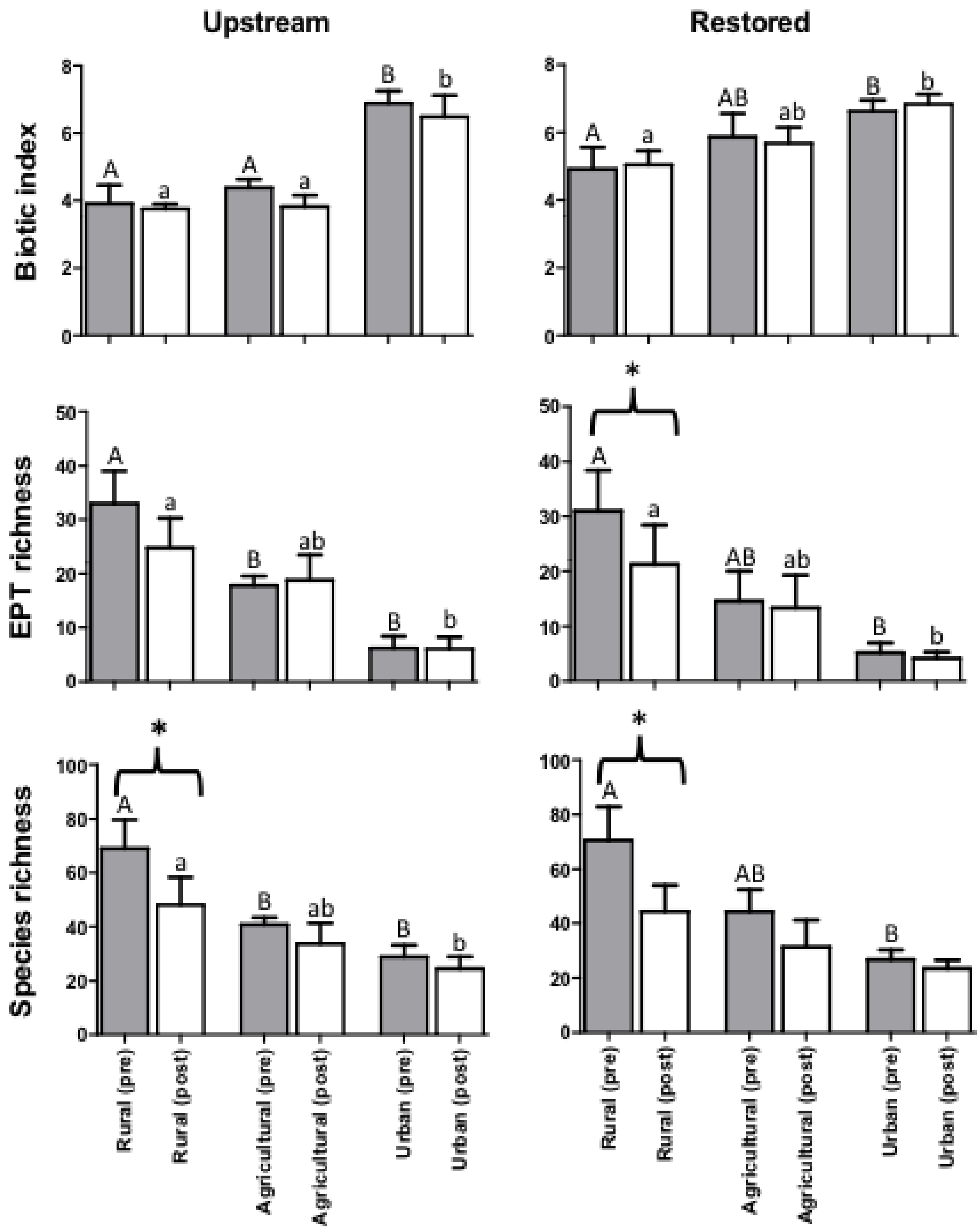


Figure 3. Mean values of macroinvertebrate biotic index, EPT richness, and total species richness (± SE) both pre-restoration and post-restoration for upstream-unrestored and restored sites for rural, agricultural, and urban restoration sites (two-way ANOVA, *P*<0.05). Pairwise differences among stream types indicated by capital letters for pre-restoration metrics and lower-case letters for post-restoration (Bonferroni's multiple comparison test, α=0.05). Asterisks indicate significant within-metric differences pre and post-restoration (Bonferroni's multiple comparison test, α=0.05).

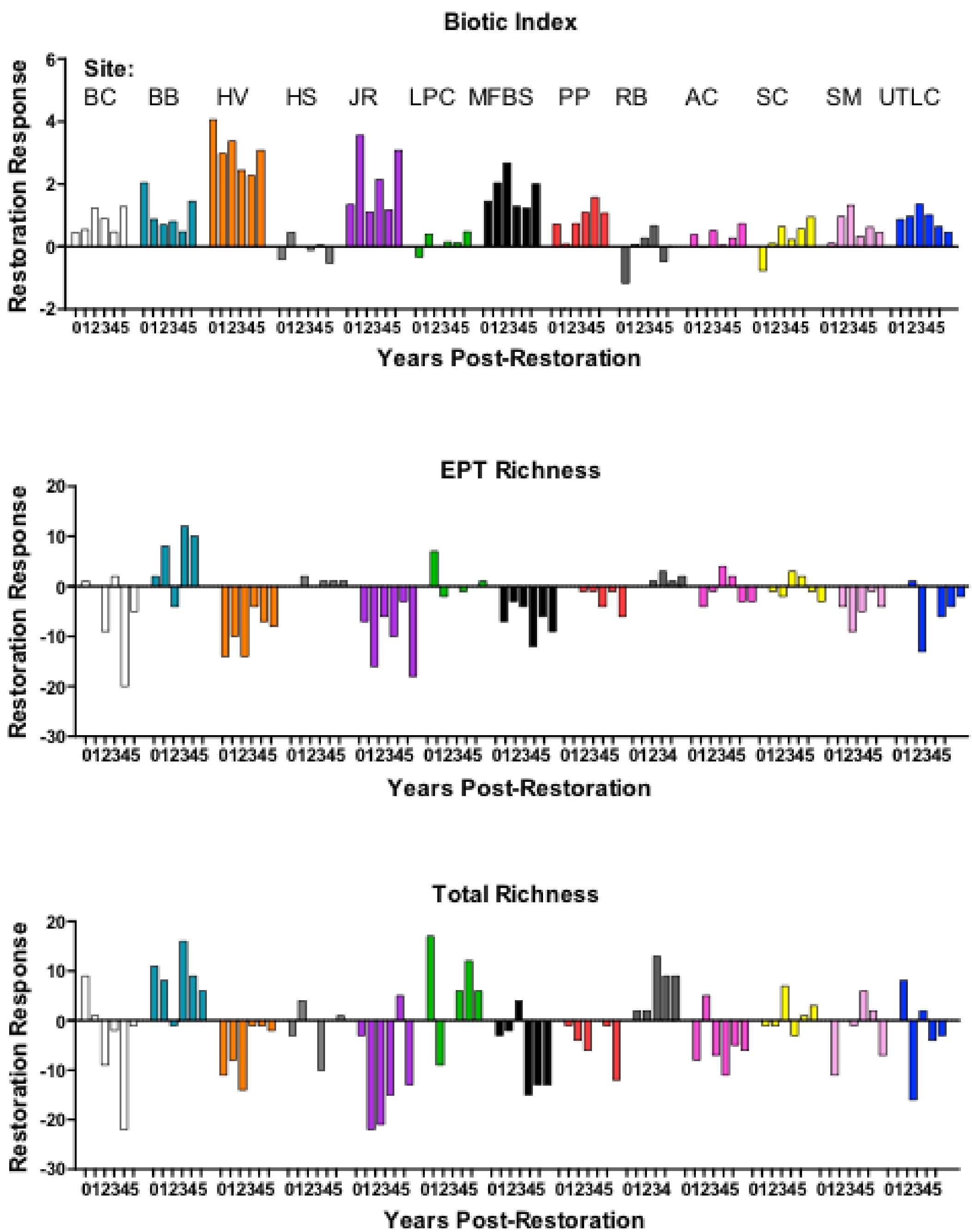


Figure 4. Annual restoration responses of macroinvertebrate biotic index, EPT richness, and total species richness. For biotic index, a positive value indicates a more tolerant community at restored sites. For EPT and total species richness, positive values indicate higher richness at restored sites.

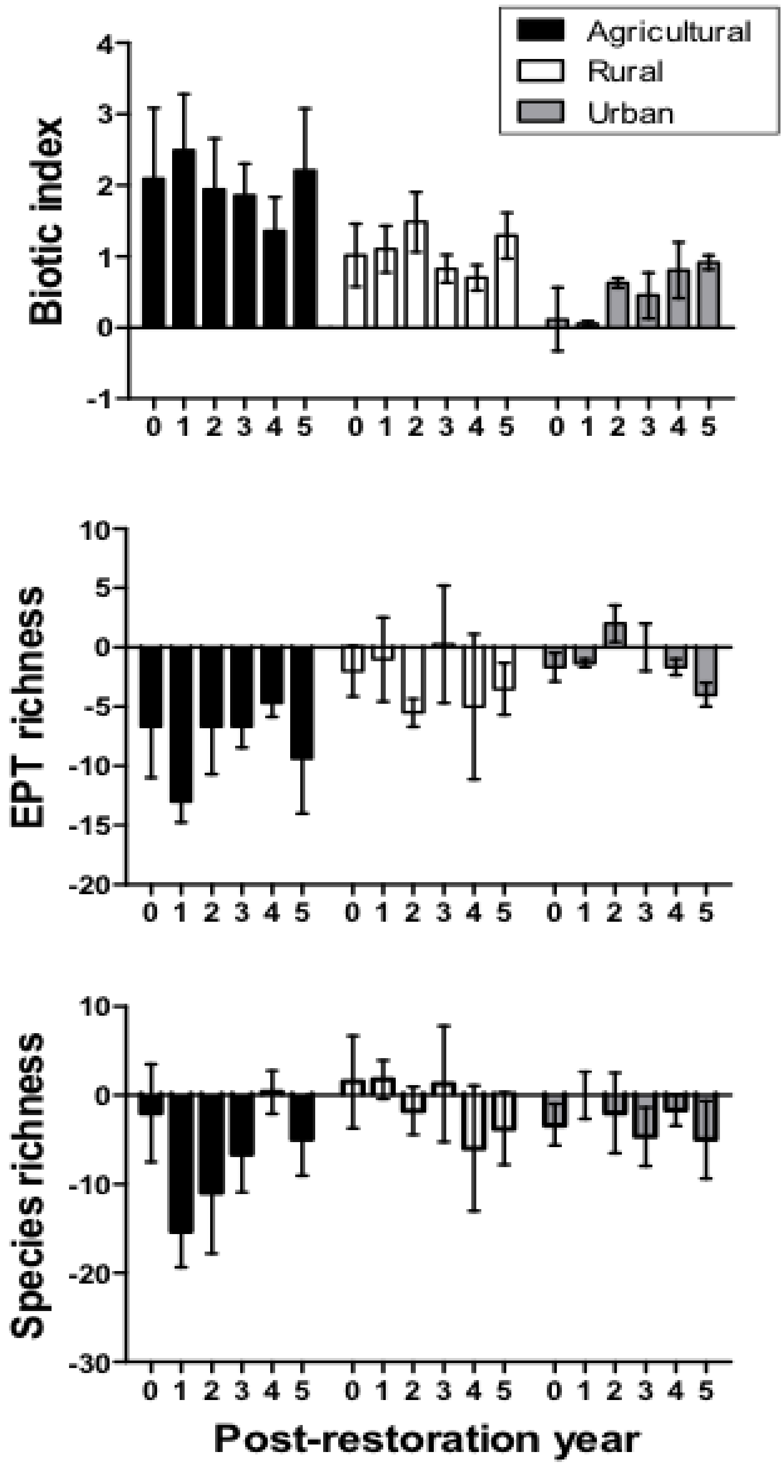


Figure 5. Mean restoration responses (± SE) by site type for each restoration monitoring year for biotic index, EPT S, and total S. Post-restoration year 0 means are pre-restoration means.

Chapter 4

Identifying factors affecting macroinvertebrate community composition on an urbanization gradient: a structural equation modeling approach.

Abstract

Catchment urbanization degrades draining streams by altering hydrology, water quality, habitat, thermal regime, biotic community structure and ecosystem function. As part of a multi-faceted research effort investigating landscape characteristics, microbial community structure and function, water quality, pollution, thermal regime, benthic organic matter dynamics, and invertebrate community structure, we analyzed macroinvertebrate compositional data from streams on a forested-agricultural-urban gradient throughout the Piedmont region of North Carolina. These data were used to identify pathways through which urbanization affects macroinvertebrate taxonomic composition. We used Non-metric Multidimensional Scaling to identify gradients in species composition due to urbanization, and used NMS axis scores as response variables in subsequent analyses. Using a structural equation model approach, we developed a model of macroinvertebrate community composition based on prior knowledge of factors that influence macroinvertebrate community structure, and identified the specific pathways through which urbanization and other landscape-scale variables affect macroinvertebrate species composition. The final structural equation model agreed with the data and retained variables related to landscape characteristics, stream water quality, thermal regime, hydrology, habitat, and soil characteristics. Of these, hydrologic flashiness and water quality variables were the most important predictors of community composition on an urban gradient. Developing models that untangle the complex network of pathways through which urbanization degrades macroinvertebrate community structure and identify the primary

mechanisms responsible has the potential to facilitate better management and restoration strategies.

Introduction

Human population growth will occur predominantly in urban centers, and therefore an increasing proportion of the world's freshwater ecosystems will become impacted by urban factors (United Nations 2008). Continuing urban development in catchments is leading to further degradation of ecosystem structure and function in urban watersheds, and aquatic community impairment is associated with urban development across a range of taxa (Wang et al. 1997, Paul and Meyer 2001, Wang et al. 2001, Roy et al. 2003a, Cuffney et al. 2005, Roy et al. 2005, Cuffney et al. 2010).

The suite of physical, biological, and functional impairments common to streams in urban watersheds has been labeled the "Urban Stream Syndrome" (Walsh et al. 2005b). Characteristics of the urban stream syndrome are unstable hydrology due to impervious surface cover and direct stormwater inputs (Walsh et al. 2005a, Bernhardt et al. 2008), channel incision and simplification (Shields et al. 2003, Niezgoda and Johnson 2005, Sudduth and Meyer 2006), habitat homogenization (FISRWG 1998, Malmqvist et al. 2002, Malmqvist and Rundle 2002, Walsh et al. 2005b), thermal stress (Pluhowski 1970, LeBlanc et al. 1997, Krause et al. 2004), and increased nutrient and pollutant concentration (Groffman and Crawford 2003, Grimm et al. 2005, Meyer et al. 2005).

Macroinvertebrate species richness and community composition are controlled by processes acting at multiple spatial scales (Poff et al. 1997), and can be profoundly affected by urban insults. Regional processes such as climate and biogeography define the regional species pool (Heino et al. 2003, Vinson and Hawkins 2003), and landscape and regional processes such as dispersal (Palmer et al. 1996, Malmqvist 2002, Smith et al. 2009) and local scale characteristics such as surficial geology and local scale soil properties (Walsh et al. 2001), habitat structure, hydrologic disturbance, productivity, and biotic interactions (Downes et al. 1998, Malmqvist 2002, Heino et al. 2003) define community composition at the local scale.

Urbanization induces landscape and local scale changes that alter macroinvertebrate community compositon (Roy et al. 2003a, Walsh et al. 2005a, Violin et al. *in press*) and increase community tolerance (Morse et al. 2003, Violin et al. *in press*). Many studies have quantified the effects of urbanization on macroinvertebrate community structure. Fragmentation of the riparian zone and surrounding landscape affects aquatic insect dispersal (Wiens 2001) and riparian vegetation removal exposes adult invertebrates to predators and thermal stress (Briers and Gee 2004, Collier and Smith 2000, Smith and Collier 2005). Macroinvertebrate richness, diversity, and community tolerance are inversely correlated with both total impervious surface cover (Morse et al. 2003, Roy et al. 2003a) and connected impervious surface cover (Walsh et al. 2001, Stepenuck et al. 2002, Moore and Palmer 2005). Urbanization also induces macroinvertebrate community changes due to stream water chemistry (Roy et al. 2003), sediment particle size (Roy et al. 2003, Violin et al. *in press*), hydrology (Walsh et al. 2001, Walsh et al. 2005a, Cuffney et al. 2010), sedimentation (Minshall 1984, Roy et al.

2003a), and metal pollution (Sloane and Norris 2003). Additionally, many of these studies have employed multivariate approaches and/or developed multimetric urbanization indices (Walsh et al. 2001, Roy et al. 2003, Sloane and Norris 2003, Cuffney et al. 2005, 2010) to determine a suite of predictors that explain community patterns. In spite of this, the mechanistic pathways by which catchment urbanization degraded stream ecosystems and their relative importance to other factors need further examination.

As an alternative to classic univariate and multivariate approaches, structural equation modeling allows simultaneous evaluation of multiple pathways and the comparison of direct and indirect effects of predictor variables on a response variable of interest (Grace 2006, Grace and Keeley 2006). This method relies on *a priori* knowledge of the system in question to choose model parameters and allows the evaluation of competing models (Grace 2008), and is adept at untangling complex networks of interactions. SEM compares patterns in the model to patterns in the data using covariance matricies, and maximum likelihood estimation (Bollen 1989), and at times yields different results than linear multivariate approaches (Grace and Keeley 2006). Within stream ecosystems, using a structural equation model approach can distinguish among multiple pathways in a complex network (Grace 2008). This process allows the identification of landscape and historical variables such as watershed urbanization and underlying geology external to the stream and riparian environment. We can then simultaneously model pathways through variables within the stream ecosystem (e.g. water chemistry or hydrological variables) to the response variable of interest (e.g. invertebrate species composition).

We collected macroinverterbrate community data from 50 sites as part of a multi-faceted research effort investigating landscape characteristics, microbial community structure and function, water quality, pollution, thermal regime, benthic organic matter dynamics, and invertebrate community structure in 78 streams on a forested-agricultural-urban gradient in the Piedmont region of North Carolina to identify mechanistic pathways through which urbanization affects various aspects of ecosystem structure and function (e.g. organic matter dynamics). This study focuses on macroinvertebrate community results; data collected for other areas of this study were used as predictor variables in community analyses. The goals of this study were to 1. Use a structural equation modeling framework to identify variables that influence community composition that respond to urbanization, and 2. Compare the importance of urban variables to non-urban determinants of community structure.

Methods

Study sites

Sites were selected to capture a forested to agricultural to urban gradient. Candidate sites were identified from previous research sites or through GIS analysis to identify watersheds with suitable land use characteristics. Of 100 initial candidate sites, 78 were chosen for inclusion in this study following site visits on May 19,

2009. Sites were assayed for a large suite of biological, physical, and functional metrics, and companion papers focus on different aspects of the data collected.

Landscape characterization

We calculated a number of land use metrics to evaluate the effects of the type, extent, and spatial arrangement of land use using the 2005 land use/land cover imagery (Sexton et al., *in review*) and GIS software (ArcGIS v.9.3, ESRI, Redlands, CA, USA, see Somers et al. *in prep* for complete GIS landscape and soil characterization methods and calculated variables). For each study stream, we calculated mean levels of development, agriculture, and forested cover. We computed hydrologic flow paths to the stream, and used these to calculate effective development (dev50-dev2000), effective impervious area (wtd50-wtd2000), and effective buffer (buf50-buf2000) at seven different spatial scales (50m, 100m, 250m, 500m, 1000m, 1500m, and 2000m, Appendix A). Effective development within a given proximity to the stream was calculated as the mean number of developed cells weighted by distance to the stream. Effective impervious area at each spatial scale (50-2000m) was calculated as the mean developed cells weighted by stream proximity development intensity, and intervening land cover between the stream and impervious landscape features. To quantify the effect of riparian buffer on stream channels, we calculated the effective buffer as the mean forested cells for the entire stream network, weighted by channel proximity. We also calculated the percent of impervious catchment directly connected to the stream as the percent development within 100m of the stream channel divided by the total watershed area.

To approximate the effects of roads and traffic on streams, for each watershed, we determined road density, inverse weighted traffic volume, and mean stream crossings. Road density was calculated as meters road/hectare watershed. We estimated traffic volume as mean traffic volume per area of watershed, weighted by stream proximity. Road-stream crossings were calculated as average road and stream intersections per km.

Landscape soil data were taken as the average over 0 to 100 cm depth over the entire watershed, and were obtained from the USDA Soil Data viewer. For each watershed, we calculated soil % clay, % sand, and % silt. Additionally, we calculated K factor, a measure of landscape-level soil erodiblilty, soil organic matter content, soil pH, and soil cation exchange capacity, a measure of the soil's ability to adsorb and exchange cations.

Habitiat sampling

We quantified habitat metrics at 54 of the study sites (see Somers et al. *in prep* for in-depth habitat characterization methods). Habitat variables were measured over 100m of each study reach. To quantify habitat complexity, we determined the number of habitat transitions by identifying the longitudinal boundaries of riffle, run, and pool habitat of within each study reach. We calculated the minimum, maximum, mean, coefficient of variation (CV), and standard error of wetted width and depth for the reach, based on cross section profiles taken at each transition, and additional cross sections spaced every 10 m. We calculated percent canopy cover at three random cross sections (based on spherical densiometer measurements (Lemmon 1957), and performed reach-scale pebble counts (Wolman 1954).

Thermal Regime

Stream water temperature data were collected from 69 of the study sites, every 10 minutes, from May 20, 2009 to June 10, 2009. We used stream water temperature data to calculate several metrics representing different aspects of the in-stream thermal regime (see Somers et al. *in prep* for detailed methods), including mean, maximum, minimum temp, and diel temperature range. To assay potential developmental constraints, and possible thermal stress, we calculated cumulative degree days using the double triangle method (Sevacherian et al. 1977)

Hydrologic disturbance

We used geomorphological and thermal proxies to estimate hydrologic disturbance for each stream channel (see Somers et al. *in prep* for detailed methods). We measured channel incision at the three random cross sections at which we measured canopy cover. Greater channel incision is correlated with greater hydrologic disturbance (Booth 1990). Additionally, we calculated the absolute value of maximum temperature change (storm change) between 10 minutes during a 24 hr. period surrounding a major storm (5/28/09 11 am to 5/29/09 11 am). Higher maximum stream temperature change over a small time period during a summer storm results from the fast delivery of warm water from hot impervious surface cover directly to the stream channel. This temperature change should be proportional to the amount of urban cover in the watershed and therefore serve as a proxy for urban hydrology.

Water quality

In stream water quality was assayed by measuring stream carbon, nutrient and ion concentrations. We measured the concentrations of total organic carbon (as non-purgable organic matter, NPOC) total nitrogen (TN), nitrate-N (NO_3^-) ammonium-N (NH_4^+), dissolved organic nitrogen (DON), and digested total N. Phosphorus species measured were phosphate (PO_4^{3-}), and digested total P (digTP). In addition to nutrient concentrations, we measured bromide (Br^-), sulfate (SO_4^{2-}), and chloride concentrations (Cl^-). Nitrate concentrations were measured with a Dionex ICS-2000 ion chromatograph with an AS-18 column (Dionex Corporation, Sunnyvale, CA, USA). Ammonium was measured following Holmes et al (1999) and analyzed on a fluorometer (Turner Designs, Sunnyvale, CA, USA). TN and NPOC were measured on a Shimadzu TOC analyzer with a nitrogen module (Shimadzu Scientific Instruments, Columbia, MD, USA). (See Sudduth et al. *in prep* for complete water quality methods and measured variables).

Stream sediment function

To assay stream sediment function, we determined ash free dry mass (AFDM) and substrate induced respiration (SIR). AFDM was measured as the proportion of a dry sediment that is organic, and was measured by drying replicate sediment samples at 60^{o}C, weighing them, and then combusting them at 400^{o}C to burn off the organic content. AFDM was then calculated as the mass loss due to combustion divided by total dry mass. SIR is a measure of total sample microbial activity (and therefore biomass), and was measured using a SIR assay (See Sudduth et al. *in prep* for complete sediment methods).

Metal contamination

We measured sediment concentrations of nine trace metals, including silver (Ag), aluminum (Al) Arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn). To measure heavy metal concentrations, we re-sieved sediment subsamples (1 mm mesh size), dried them at 60°C for 48hrs, and digested 1 g samples in triplicate using standard EPA protocols (EPA method 3050B, USEPA 1996). Trace metals were quantified by inductively coupled plasma-mass spectrometry (Perkin-Elmer Elan 6000 ICP-MS, Perkin-Elmer, Waltham, MA, USA) (See Wang et al. *in prep* for in depth trace metal methods). To account for differences in sediment organic matter content, we normalized trace metal concentration by AFDM (Liu et al. 2003, Loring 1991).

Macroinvertebrate sampling

Macroinvertebrates were sampled once at 50 of the 78 study sites between May and August 2009 using the North Carolina Department of Water Quality Qual 4 semi-quantitative protocol (NC DWQ 2006). This sampling protocol is designed to assess macroinvertebrate diversity in small streams (drainage area <7.8 km^2) and is conducted so that sampling effort is consistent among study sites. Each sample consisted of one 2-3 min, 1 m^2, 1 mm mesh kick net sample from a characteristic riffle, one 500 μm mesh triangular sweep net of stream marginal habitats such as root mats and bank vegetation, an approximately 500 g leaf pack sample collected from rock or snag habitats, and visual assessments of habitats not easily sampled with the above methods (e.g., large rocks or logs). Samples were field-sorted and specimens were preserved in 95% EtOH. Chironomidae were identified to family, all other specimens were identified in the lab at 45x to genus level, or the lowest possible taxonomic level (Smith 2001, Merritt et al. 2008). Based on the number of each taxon collected, we classified taxa as abundant (≥50 individuals), common (49-10 individulas), few (9-3 individuals), rare (2 individuals), or single (1 individual).

Exploratory data analysis

Landscape, soil, habitat, water quality, hydrology, metal contaminants, and sediment function variables were used as potential predictor predictors in analyses of macroinvertebrate community structure. Of the 50 sites sampled for macroinvertebrates, we excluded 11 due to missing predictor variable data from other categories, because subsequent analyses and structural equation modeling require a suite of predictor variables with no missing data. Analyses were carried out on the remaining 39 sites. We used landscape characteristics to define the 39 sites as urban (>50% development), suburban (>15% development, less than 70% forest), forested (>70% forest), agriculture (>50% fields), and mixed rural (< 15% development, not fitting any other category).

We determined total species richness and richness of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). We used least squares linear regression to determine if these community structure measures were responding to urban development. To visualize site type differences in macroinvertebrate community composition and to calculate ordination axis scores for all study sites for subsequent structural equation modeling, we used non-metric multidimensional scaling (NMS) to ordinate sites in species space (PC-Ord v.6, McCune and Mefford 2006). We excluded taxa present in less than 5% of samples prior to analysis.

Ordination was carried out using Bray-Curtis dissimilarities of square root transformed taxon abundance values. Solutions were obtained from 500 runs (250 randomized, 250 with real data) using random starting coordinates. Results were rotated to principle components, such that NMS axis 1 had the highest explanatory power, and explanatory power decreased with successive axes, and all NMS axes were orthogonal. We created joint plots by incorporating a second matrix of the functional, thermal, physical, water quality, pollution, and landscape variables collected as part of this study. We set a minimum r^2 of 0.10 to identify geomorphological and functional parameters correlated with macroinvertebrate community structure at different sites. To identify candidate predictor variables for subsequent use in structural equation models, we determined the correlation of each NMS environmental variable with NMS axes. In addition to correlating predictor variables within NMS, we determined Pearson's correlation coefficients between environmental variables and site axis scores for both NMS axes using the cor2m function in the ecodist package (v. 1.2.3, Goslee and Urban 2010). To evaluate whether or not we had a good response variable for our model, we used least squares linear regression determined whether or not our NMS axes responded to urban development.

To investigate statistical differences in community composition due to site type, we performed permutation multivariate analysis of variance (perMANOVA, Anderson 2001) using the adonis function in the vegan package (v. 1.17-10, Okasnen et al. 2011) in R (v. 2.12.2, R Development Core Team 2011). PerMANOVA compares variation due to the predictor (site type) to random variation based on permutations. We used Bray-Curtis dissimilarity of square root transformed taxon abundances, and calculated the pseudo F statistic based on 999 permutations.

SEM model development

We used an exploratory model-building approach (Grace 2008) to develop a structural equation model capable of explaining observed macroinvertebrate compositional patterns among our study sites. We used the axis site scores from the NMS analysis as simultaneous response variables to create an observed variable structural equation model (Grace 2006, 2008). We defined the pathways in the structural equation model based on a priori knowledge of possible mechanisms by which urbanization can influence macroinvertebrate community structure (Fig. 1). Landscape and soil variables such as % watershed development and soil % clay were treated as exogenous variables, which are not affected by other variables within the model. Water chemistry, pollution, thermal, hydrological, habitat, organic matter, and invertebrate measures were treated as endogenous variables, which respond to both exogenous and other endogenous variables (Grace 2006). A complete list of potential SEM predictor variables is given in Appendix A.

We began by creating three separate structural equation models using only historical and landscape variables as model predictors. These models contained no proximate pathways, and are those we seek to improve explanation of by including endogenous variables. To initially parameterize our full structural equation model, we utilized results from the Pearson product-moment correlation and NMS matrix correlation analyses to identify metrics through which urbanization and other landscape factors might affect macroinvertebrate community structure. Based on the presence of strong positive or negative correlations with community composition, we initially included at least one variable from each area of our conceptual model: landscape, soil, habitat, metals, water quality, temperature, hydrology, and dispersal (Fig. 1). We also included additional metrics from each group where we felt appropriate based on prior knowledge (for instance, when we felt that two variables within the same group may have different mechanistic effects).

Because predictor variables within groups may be highly correlated (e.g. measures of weighted or total development at different spatial scales), the original variables chosen may not be the best representative of that group. We substituted parameters within category to evaluate model fit with different measurements of water chemistry, etc. and compared explanatory power and statistical agreement with the data among models with different predictors. Additionally, because of differing analytical approaches, variables with high Pearson correlations may be found to be unimportant in the SEM, and other variables added based on prior knowledge may be retained.

SEM analyses were performed using AMOS modeling software (v16, IBM, SPSS, Armonk, New York, USA). We began with a fully saturated model (all possible pathways are present) and used maximum likelihood estimation to simultaneously evaluate all pathways and overall model fit. Goodness of fit was based on X^2 statistical tests, and a P value >0.05 indicates that the model is a good fit with the data. We removed non-significant pathways until all remaining individual paths were significant ($P<0.1$), statistical agreement with the data was achieved ($P>0.05$), and further path exclusion resulted in either a large increase in X^2 and decrease in P value, or a large loss in explanatory power. Models with different predictor variables were evaluated by comparing explanatory power of the, X^2, p values, and information criteria measures (e.g. AIC). The final model chosen maximized explanatory r^2 for the model response while minimizing X^2 and information criteria).

Results

A total of 109 invertebrate taxa were collected from 39 sites analyzed. After excluding sites with missing predictors and excluding rare taxa, 66 taxa were left for use in subsequent analyses. Both total species richness and EPT richness were inversely correlated with catchment urbanization (r^2=0.016, P<0.05, and r^2=0.29, P<0.001 respectively, Fig. 3). NMS ordination resulted in a two dimensional solution (final stress = 20.3), and explained 75.8% of compositional variation. Axis 1 and axis 2 explained 54.8% and 21% of compositional variation among sites respectively (Fig. 2). Species correlations and overlaying environmental variables revealed that axis 1 was the primary axis of urbanization (Tables 1-3, Fig. 2). Specifically, this axis was negatively correlated with tolerant gastropods, odonates, and dipterans and positively correlated with sensitive Ephemperoptera, Plecoptera, Trichoptera, Megalopteran, and Elmidae taxa (Table 3). Environmental variables revealed a gradient of high catchment development, hydrologic flashiness, metal contamination, soils with high plasticity and cation exchange capability, to higher minimum stream width, watershed field, and canopy cover. Axis 2 explained substantially less variation than axis 1 and was poorly defined by environmental variables. Based on species correlations, this axis appears to represent habitat and functional feeding gradients of collector-gathers, burrowers, and predators that prefer depositional or fine-grained substrate to filter feeders that prefer more stable large cobbles and bedrock substrates (Table 3).

Joint plots revealed that this axis was partially explained by an inverse correlation with higher d16 (the 16th percentile particle size, in mm), and stream water arsenic and chloride concentrations (Tables 1-2, Fig. 2). There was substantial variation in the taxa correlations with axis 2. Few tolerant species were negatively correlated with axis 2, and a number of sensitive taxa were weakly negatively correlated with this axis. Several taxa were positively correlated with this axis, and there was no discernable tolerance pattern (Table 3). Axis 1 site scores were significantly inversely correlated with urban development (r^2= 0.21, P<0.01), however there was no relationship between urban development and NMS axis 2 (Fig. 3).

Convex hulls, which define the ordination space occupied by different site types, revealed that urban sites were negatively associated with axis 1, while other site types showed both positive and negative correlations. Urban streams did not vary widely on axis 2, all other site types showed substantial variation (Fig. 2). Permutation MANOVA revealed a significant effect of site type on macroinvertebrate composition ($F_{1,37}$ = 1.86, P=0.033).

The initial three exogenous variable models all statistically agreed with our data, but provided low predictive power (Fig. 4). The soil variable model explained

9% and 6% of axes 1 and 2 respectively (X^2 =0.212, P=0.645). The urbanization only model explained 21% of axis 1, and no variance of axis 2 (X^2 = 0.025, P=0.874). The mean forest cover model explained 8% of axis 1 and 3% axis 2 (X^2=0.087, P=0.645). Among individual paths, only the effects of urban development and mean forest on axis 1 were significant at α=0.1 level.

The full, observed variable SEM was stable and stastically fit the data (X^2 = 76.3, P=0.121, Fig. 5). The model retained indicator variables from the habitat (d16 (mm) and % canopy cover), thermal (degree days), pollution (mean [Pb] μg/g C), water quality (mean [total N] (mg/L) and mean [$SO^{2-}{}_4$] (mg/L)), hydrology (storm change (^{o}C)), landscape (% forested watershed and % developed watershed), and history (soil % clay and K factor) conceptual pathways and explained 72% of NMS axis 1 compositional variation and 43% of axis 2 (Fig. 5).

Catchment development had a total negative effect on both NMS axes, with standardized total effects (STE) = -0.363 and -0.015 for axes 1 and 2 respectively, but did not directly predict either NMS axis (Table 4). Urbanization effects were mediated through both direct and indirect correlations with endogenous variables, with positive total effects on degree days (STE=0.325), mean $SO^{2-}{}_4$ (STE=0.681), total N (STE=0.185), mean Pb (STE=0.746), storm change (STE=0.629) (Tables 4-5, Fig. 5). Mean forested catchment had a positive total effect on axis 1 (STE = 0.238), which included direct and indirect effects, and a slightly negative indirect total effect on axis 1 (STE =-0.017) (Tables 4-5, Fig. 5). Forested catchment indirectly positively predicted d16 (STE = 0.324), canopy cover (STE=0.582), and total N (STE=0.039), and negatively mediated and temperature range (STE=-0.263), degree days (STE=-

0.122) through its positive effect on degree days. The exogenous soil variables soil % clay (positive effect) and K factor (negative effect) directly predicted axis 1 scores (Tables 4-5, Fig. 5), but did not affect any other pathways.

The single strongest predictor for both NMS axes was mean $SO^{2-}{}_{4}$ concentration, which was inversely correlated with both axes, and therefore also inversely correlated with sensitive taxa on axis 1 (Table 4, Fig. 3). The exogenous predictor variable catchment development was the sole predictor of mean $SO^{2-}{}_{4}$ within the model, which was highly significant; in addition the standardized path coefficient indicates a strong effect (Fig. 5). The endogenous variable storm change, a measure of hydrologic flashiness, also negatively predicted axis 1. Positive direct correlations with axis 1 were observed for d16, total N, and degree days (Fig. 5). In addition to the negative effect of $SO^{2-}{}_{4}$, axis 2 score was directly predicted by positive effects of total N, degree days, and Pb (Fig. 5). Indirect effects of canopy, temperature range, and degree days were observed for both axes (Table 5).

Discussion

Community differences among land use types have been demonstrated for a number of studies (Walsh et al. 2001, Violin et al. *in press*, Violin Ch. 3), and were confirmed for both richness and compositional measures (Figs. 2-3). NMS analyses revealed that delineating sites based on species composition resulted in a primary explanatory axis (axis 1) that was defined by an urbanization gradient. This was further confirmed by the inverse correlation between axis 1 and urban development (Fig. 3). The low species-axis correlations observed are likely due to the standard removal of rare taxa that may define the tail ends of environmental gradients, but could also be due to random noise, and the substantial overlap of non-urban sites on both axes. Additionally, the two-dimensional NMS stress value was on the high side of the acceptable range (McCune and Grace 2006).

The initial structural equation models containing only exogenous predictors did not explain much of the compositional variation seen on either axis (Fig. 4). However, the final structural equation model defined the importance of the four exogenous predictors through intermediate variables. We were able to determine the influence of urbanization on community composition relative to historical and other landscape factors, and determine the relative importance of the multitude of pathways by which urbanization degrades stream communities. Based on path coefficients and the variation explained, macroinvertebrate community composition appears to be most strongly controlled by underlying geology, stream water chemistry, hydrology, and temperature, of which the latter three respond strongly to urbanization.

Stream water $SO^{2-}{}_4$ concentrations in urban catchments may result from atmospheric deposition from vehicle exhaust and power plant emissions (Sprague 2007), stormwater runoff and sewage associated pollutants (Rose 2002), and precipitation (Rosemond et al 1992), Additionally, $SO^{2-}{}_4$ can be a soil constituent that leaches into stream water and affects water chemistry (Astrom and Bjorklund 1995), however these soil types are uncommon in North Carolina (Buol 2003). Previous work has shown increased $SO^{2-}{}_4$ in base flow and storm waters in urban watersheds (Rose 2002, Schoonover et al. 2005), and stream acidification is associated with higher SO^{2-}_4 (Rosemond et al. 1992). Within the SEM, the linkage between SO^{2-}_4 and storm water and sewage indicate an that SO^{2-}_4 may indicate an urban hydrology and contaminant effect, and its role in stream acidification suggests that it may be a surrogate for stream pH, which was not measured. Anthropogenic stream acidification has been shown to decrease macroninvertebrate species richness (Rosemond et al. 1992, Petrin et al 2008). The exogenous predictor variable catchment development was the sole predictor of sulfate within the model, which was highly significant; in addition the standardized path coefficient indicates a strong effect (Fig. 5).

The role of both soil % clay and K factor indicate a strong effect of underlying catchment geology on community composition, as reported elsewhere (Walsh et al. 2001, Bonada et al. 2007). The positive correlation between soil % clay and axis 1 suggests soils containing non-plastic clays, since soil plasticity was negatively correlated with axis 1 in both correlation analyses. High plastic clay content is characteristic of soils such as those found in the Triassic basin, (Traverna et al. 2004), which are generally more susceptible to erosion. This is further confirmed by the negative relationship of axis 1 with soil erodibility (K-factor), which is characteristic of both Triassic basin and high silt soils. The negative effect of soil erodibility on axis 1 scores, and thus it's inverse correlation with sensitive taxa is not at all surprising, since erosion and associated fine sediment introduction impair macroinvertebrate communities (Wood and Armitage 1997, Roy et al 2003).

Several model pathways confirm effects documented by previous research. The negative effect of storm change, an indication of hydrologic flashiness, agrees with previous work demonstrating the major effect of hydrology on stream invertebrates (Resh et al. 1988, Lake 2000), and that urbanization leads to hydrologic disturbance in draining streams (Walsh 2005a, Bernhardt 2008), resulting in habitat degradation and sensitive taxa loss.

Axis 1 scores were positively correlated with percent forested catchment, which is correlated with macroinvertebrate community integrity (Roy et al. 2003). However the direct positive connection between forested watershed and sensitive taxa indicates unresolved proximate mechanisms for this relationship. The positive effects in the structural equation model of forested watershed on axis 1 scores mediated through d16 and canopy cover, and indirect negative effects on degree days and temperature range (Table 5) indicate that in addition to unresolved landscape effects, forested watershed protects habitat and moderates the thermal regime.

The positive effect of d16 on community structure is also expected, as a higher d16 indicates less fine sediment in the stream channel. The positive effect of canopy is likely due to reduced thermal stress and maintenance of the characteristic food web (Sweeney et al. 2004). Canopy cover did have a slight indirect negative indirect effect on axis 1 scores, but total effect was positive (STE=0.299, Tables 4-5). Based on the path diagram, this negative effect results from the positive effects of temperature range on degree days, and degree days on site scores for both NMS axes, in spite of their negative association with % canopy cover. Indeed, the indirect correlation between canopy and degree days is negative (-0.21).

The positive effects of degree days and total N were surprising, as higher degree days can indicate thermal stress and in previous work has been shown to be positively correlated with urbanization (Violin et al. *in press*), and eutrophication has long been know to impair aquatic communities (Bernhardt et al. 2008). There are several possible explanations for these patterns. Firstly, the relationship between both degree days and total N and macroinvertebrate community compositon may not be linear. Higher total N that is sub-eutrophication may represent higher resource availability, which seems plausible given the positive effect of degree days on total N (Fig. 5). In addition to a potential effect on resources, higher degree days that are below thermal stress levels may indicate longer development time. Bivariate modeling of both total N and degree days with axis 1 scores using quadratic function did not produce a positive correlation (data not shown), however it is possible that including these higher order terms in the SEM would have different results. Secondly, it was necessary to exclude 11 sites from our analysis, and it is possible that including those sites would lead to a broader range in both total N and degree days, and better quantify the pattern. Lastly, it is possible that the positive effect of total nitrogen is due in part to the ordination of the agricultural sites positively on axis 1. Clearly, the roles of degree days and total N are complex, and deserving of further study.

Axis 2 scores were not well explained by the structural equation model. In addition to similar positive correlations with total N and degree days, and a strong negative correlation with $SO^{2-}{}_4$ seen with axis 1, axis 2 scores were strongly positively predicted by mean Pb (Fig. 5), which was predicted solely by catchment urbanization. Tolerant taxa were both positively and negatively correlated with axis 2 (Table 3), therefore it is possible that the structural equation model is capturing the responses of a few taxa to metal pollution, hydrology, pH, or resource or development gradients. Urban sites did not separate well along axis 2, while other site types showed wide variation in axis 2 scores (Fig. 2), Given this, and the habitat and functional feeding gradients on axis 2, it is likely that compositional variationon is due to mechanisms not well-examined as part of this study, rather than urban processes which were the focus of this work.

Model caveats

Stream macroinvertebrates are a highly diverse group of organisms, which display a wide range of responses to many environmental variables. We employed a model building, rather than confirmatory approach (Grace 2008), because while we could hypothesize mechanistic pathways based on prior knowledge, it was difficult to know the relative importance of our conceptual categories and what specific variables would be retained in the model for each category beforehand. While the presented structural equation model adequately fits the data, the identified paths are by no means exhaustive, and it is possible that other models may equal or exceed the explanatory power of the one presented. Thus we consider the paths discussed here as hypotheses for future testing, preferably with data from distinct sites, to evaluate the strength of our model.

This model also suggests further research questions that need to be addressed. For example, further explanation of the role of $SO^{2-}{}_4$ in community degradation is needed, as is further defining the role of soil properties, specifically soil % clay in invertebrate community composition.

Conclusions and Application

Urbanization is a complex phenomenon and affects aquatic ecosystems through myriad pathways, and is not the only factor controlling aquatic invertebrate community composition. Using a structural equation modeling approach, we were able to simultaneously parse the effects of four exogenous predictor variables: soil % clay, K factor, watershed development, and forested watershed, into proximate pathways, and to determine the importance of urbanization to community composition, relative to other factors.

This model enabled us to explain a substantial amount of the role of urban development, a sweeping concept with numerous effects, such that the direct links from catchment urbanization to either NMS axis were not retained. This is beneficial to management and restoration strategies. Catchment urbanization itself is not easily addressed; therefore understanding its primary mechanisms of action may allow managers and restoration designers to address those variables specifically.

Current restoration methods intended to improve aquatic community structure, namely through habitat improvement, were found to be of minor importance in our model. In fact, within the study sites, d16 is as much an indicator of fine sediment in the channel as it is of habitat quality. Other metrics that better define habitat quality; for example median particle size, or habitat complexity were not significant model predictors. In contrast, riparian canopy, which is positive predictor of sensitive taxa presence in the model, is often actively removed during restoration. Additionally, hydrology and water quality, two variables with strong negative effects on axis 1 score, and therefore sensitive taxa presence, are rarely addressed by current urban restoration methodology. This suggests that current restoration methodology does not address factors responsible for community structure. Therefore, further development and utilization of more mechanistic models of urbanization may facilitate better management and restoration strategies.

Collaborator acknowledgements: Emily Bernhardt and Dean Urban coordinated the project and provided invaluable guidance on data analysis and SEM model development. Elizabeth Sudduth was responsible for benthic organic matter analyses, Si-Yi Wang was responsible for microbial analyses and with Brooke Hassett was responsible for nutrient and pollutant analyses. Kayleigh Somers performed landscape and soil GIS analyses, and was responsible for all temperature data. Joe Adrigola collected macroinvertebrate samples.

Table 1. Pearson correlation coefficients between environmental variables and NMS axes 1 and 2 significant at the *P*=0.1 level. Variables in bold were retained in the final structural equation model. Dev250-dev1500 are measures of effective development within x meters of the stream channel. Wtd50-wtd1500 are measures of effective imperviousness within x meters of the stream channel.

Metric	NMS Axis 1
Dev1000	-0.5543899
Dev500	-0.5533359
Dev1500	-0.5464661
Dev250	-0.5392338
Dev2000	-0.5346771
Wtd500	-0.5161024
Soil CEC	-0.5160084
Wtd1000	-0.5133803
Dev100	-0.5085105
Wtd1500	-0.4997304
Wtd250	-0.4962978
Dev50	-0.4936143
95 ws development	-0.4825983
85 ws development	-0.4776875
Soil plasticity	-0.4643666
% connected ISC (PIC)	-0.4603771
Wtd100	-0.4562355
05 ws development	-0.4556271
Mean [Cd] μg/g C	-0.4544986
Wtd50	-0.4405379
Storm change (^oC)	-0.4359047
Mean SO_4^{2-} mg/L	-0.335785
Trafidw	-0.3104517
Mean [Pb] μg/g C	-0.3000643
Mean incision	-0.2793542
Mean [As] μg/g C	-0.2772676
Mean [Zn] μg/g C	-0.274682
Mean [Al] μg/g C	-0.2729555
K factor	-0.269201
SIR mg CO_2/ gdm/min	-0.2577401
Rds	-0.2127919
Minimum depth (m)	0.2247902
Mean [Cu] μg/g C	0.2251209
Mean [Ni] μg/g C	0.2251209
Mean width (m)	0.2721177
Forested watershed	0.283788
Mean Field	0.3302696
Minimum width (m)	0.3357075
Canopy (% cover)	0.3784003

Metric	NMS Axis 2
Mean [Cl-] mg/g C	-0.3205533
D16 (mm)	-0.319204
Mean [As] μg/g C	-0.3172604
Mean [Br] μg/g C	-0.3083499
AFDM (g)	-0.3081388
SIR mg CO_2/ gdm/min	-0.2709584
K factor	-0.2525685
Mean [digTP] mg/L	-0.2470469
Mean [NPOC] mg/L	-0.222012
Max width	0.2106281
Mean [NO_3-N] mg/L	0.22281
Length (m)	0.2532058
Canopy CV	0.2968644

Table 2. Environmental variables from NMS joint plots correlated with axes 1 and 2, r^2 cutoff: α=0.1. Dev250-dev1500 are measures of effective development within x meters of the stream channel. Wtd1000-wtd1500 are measures of effective imperviousness within x meters of the stream channel.

Metric	Axis 1 r	Axis 1 r^2
dev1000	-0.554	0.307
dev500	-0.553	0.306
dev1500	-0.546	0.299
dev250	-0.539	0.291
wtd500	-0.516	0.266
Soil CEC	-0.516	0.266
wtd1000	-0.513	0.264
wtd1500	-0.5	0.25
95 ws development	-0.483	0.233
85 ws development	-0.478	0.228
Soil plasticity	-0.464	0.216
% connected ISC (PIC)	-0.46	0.212
05 ws development	-0.456	0.208
Mean [Cd] µg/g C	-0.454	0.207
Storm change (°C)	-0.429	0.184
Mean SO_4^{2-} mg/L	-0.336	0.113
Mean field (% ws)	0.33	0.109
Min width (m)	0.336	0.113
Canopy (% cover)	0.378	0.143

Metric	Axis 2 r	Axis 2 r^2
[Cl-] mg/L	-0.321	0.103
d16	-0.319	0.102
[As] µg/g C	-0.317	0.101

Table 3. Correlations coefficients and r^2 values for individual taxa with NMS ordination axes. R^2 cutoff: α=0.05.

	NMS Axis 1 r	NMS Axis 1 r^2
PHYSELLA	-0.55	0.303
MENETUS	-0.359	0.129
ISCHNURA	-0.316	0.1
HIRUDINEA	-0.265	0.07
PSEUDOLIMNO.	-0.234	0.055
TRIAENODES	0.225	0.051
PSEPHENUS	0.261	0.068
DUBIRAPHIA	0.263	0.069
PERLESTA	0.264	0.069
HEXATOMA	0.273	0.074
STENACRON	0.287	0.082
HETEROCLOEON	0.289	0.084
STENELMIS	0.325	0.106
CORYDALUS	0.371	0.137
MACCAFFERTIUM	0.385	0.148
SIMULIUM	0.412	0.169
HYDROPSYCHE	0.421	0.177
NIGRONIA	0.421	0.177
MACRONYCHUS	0.454	0.206
ISONYCHIA	0.474	0.224

	NMS Axis 2 r	NMS Axis 2 r^2
HYALELLA	-0.631	0.399
SIALIS	-0.63	0.397
HYDROPORUS	-0.54	0.292
NEUROCORDULIA	-0.454	0.206
ENALLAGMA	-0.424	0.18
HELICHUS	-0.371	0.137
DUBIRAPHIA	-0.345	0.119
OLIGOCHAETA	-0.313	0.098
PERLESTA	-0.312	0.097
TIPULA	-0.262	0.069
SOMATOCHLORA	-0.256	0.066
STENONEMA	-0.238	0.057
DIPLECTRONA	-0.235	0.055
MENETUS	-0.234	0.055
PROSIMULIUM	0.252	0.063
MACRONYCHUS	0.263	0.069
SIMULIUM	0.3	0.09
HYDROPSYCHE	0.498	0.248

Table 4. Standardized total effects for SEM model variables.

	Soil % clay	K factor	Forest cover (%)	2005 dev. (%)	Canopy	Temp range (°C)	Degree days
Canopy	0	0	0.582	0	0	0	0
Temp range (°C)	0	0	-0.263	0	-0.453	0	0
Degree days	0	0	-0.122	0.325	-0.21	0.465	0
D16 (mm)	0	0	0.324	0	0	0	0
SO_4^{2-} (mg/L)	0	0	0	0.681	0	0	0
Total N (mg/L)	0	0	0.039	0.185	0.068	-0.15	0.568
[Pb] ug/L	0	0	0	0.746	0	0	0
Storm change (°C)	0	0	0	0.629	0	0	0
NMS Axis 1	0.296	-0.266	0.238	-0.363	0.299	0.044	0.433
NMS Axis 2	0	0	-0.017	-0.015	-0.029	0.064	0.385

	D16 (mm)	SO_4^{2-} (mg/L)	Total N (mg/L)	[Pb] (μg/g C)	Storm change (°C)
Canopy	0	0	0	0	0
Temp range (°C)	0	0	0	0	0
Degree days	0	0	0	0	0
D16 (mm)	0	0	0	0	0
SO_4^{2-} (mg/L)	0	0	0	0	0
Total N (mg/L)	0	0	0	0	0
[Pb] μg/g C	0	0	0	0	0
Storm change (°C)	0	0	0	0	0
NMS Axis 1	0.199	-0.446	0.38	0	-0.318
NMS Axis 2	0	-0.631	0.277	0.389	0

Table 5. Standardized indirect effects for SEM model variables. Endogenous variables without indirect effects are not shown.

	Soil % clay	K factor	Forest cover (%)	2005 dev. (%)	Canopy	Temp range (°C)	Degree days
Canopy	0	0	0	0	0	0	0
Temp range (°C)	0	0	-0.263	0	0	0	0
Degree days	0	0	-0.122	0	-0.21	0	0
D16 (mm)	0	0	0	0	0	0	0
SO_4^{2-} (mg/L)	0	0	0	0	0	0	0
Total N (mg/L)	0	0	0.039	0.185	0.068	0.264	0
[Pb] µg/g C	0	0	0	0	0	0	0
Storm change (°C)	0	0	0	0	0	0	0
NMS Axis 1	0	0	0.238	-0.363	-0.02	0.044	0.216
NMS Axis 2	0	0	-0.017	-0.015	-0.029	0.064	0.158

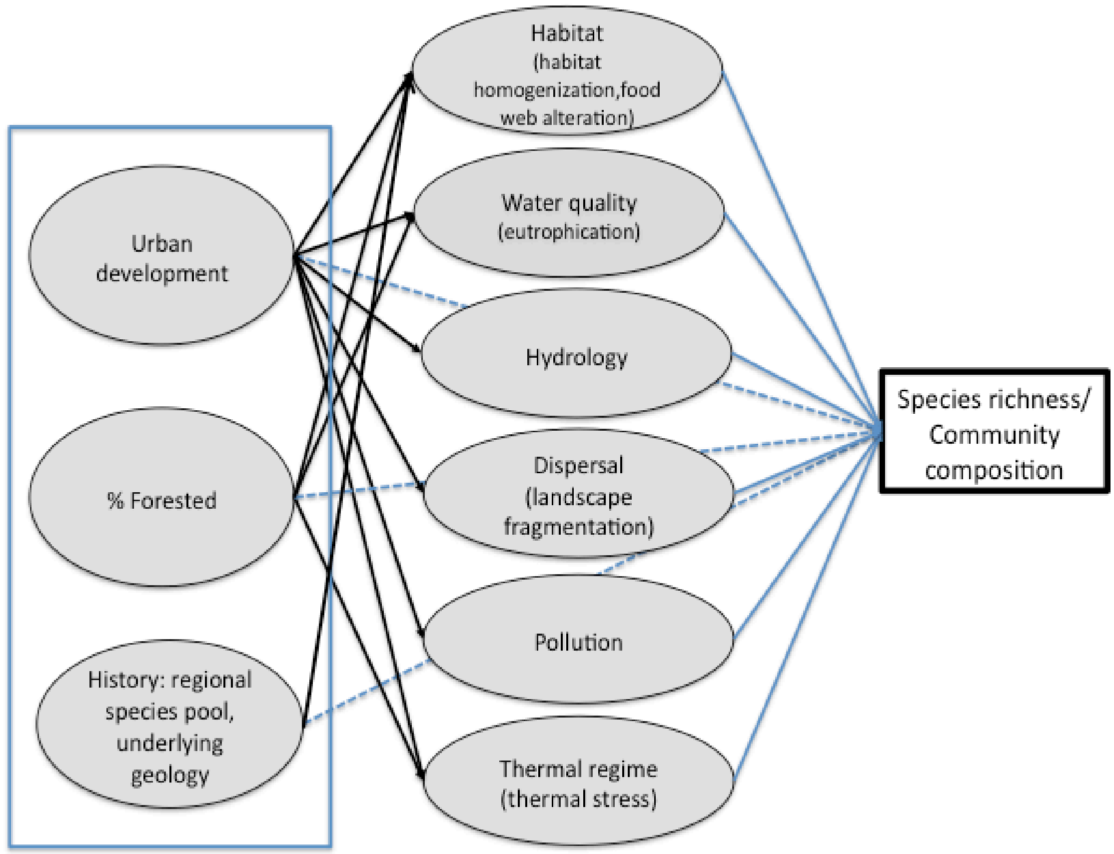


Figure 1. Conceptual model of macroinvertebrate community structure.

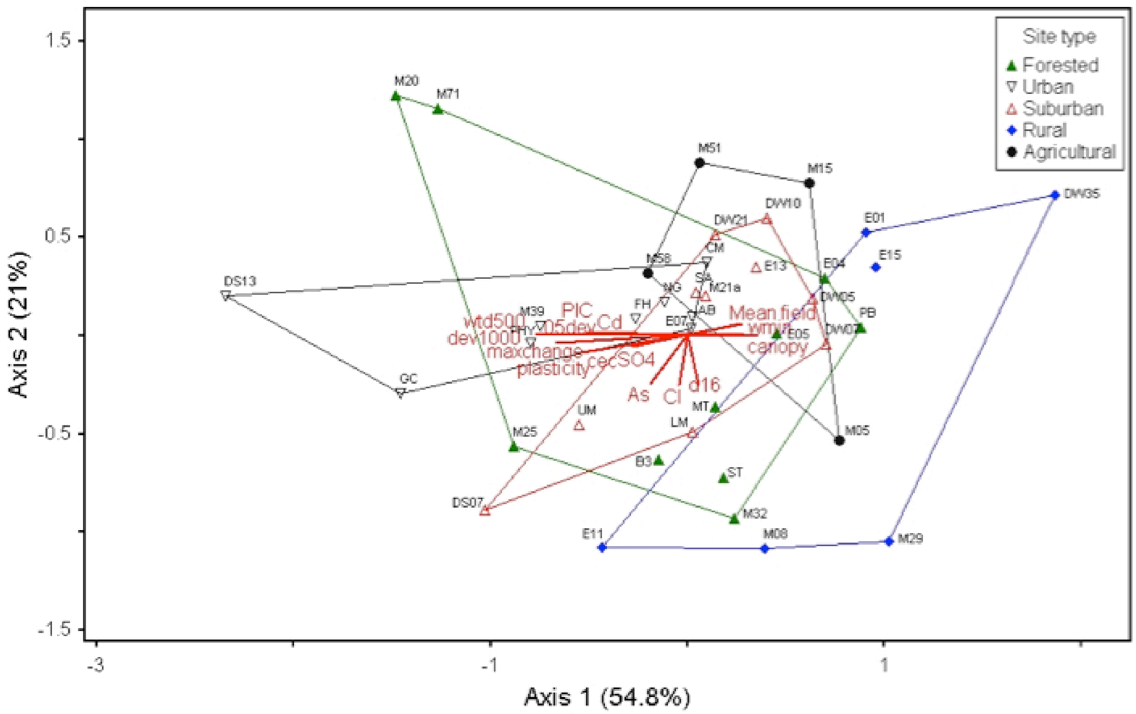


Figure 2. Joint plot of NMS 2-dimensional ordination of sites in species space with overlaying variables. For simplicity, effective development (dev250-1500) and effective imperviousness (wtd500-1500) are each represented in the joint plot by the single variable from each group which explains the most variation. Polygons represent convex hulls that define the ordination space occupied by each site type.

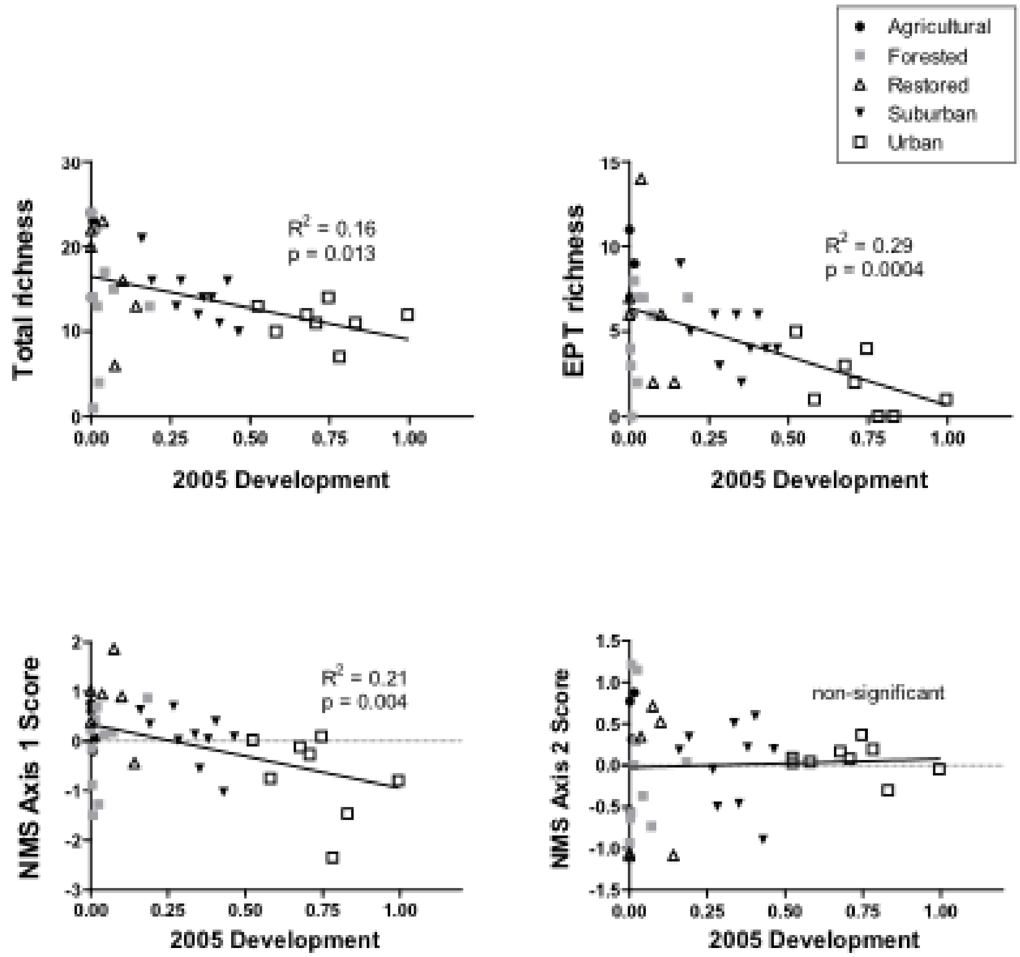


Figure 3. Least squares linear regression of total richness, EPT richness, NMS axis 1 scores, and NMS axis 2 scores vs. 2005 urban development.

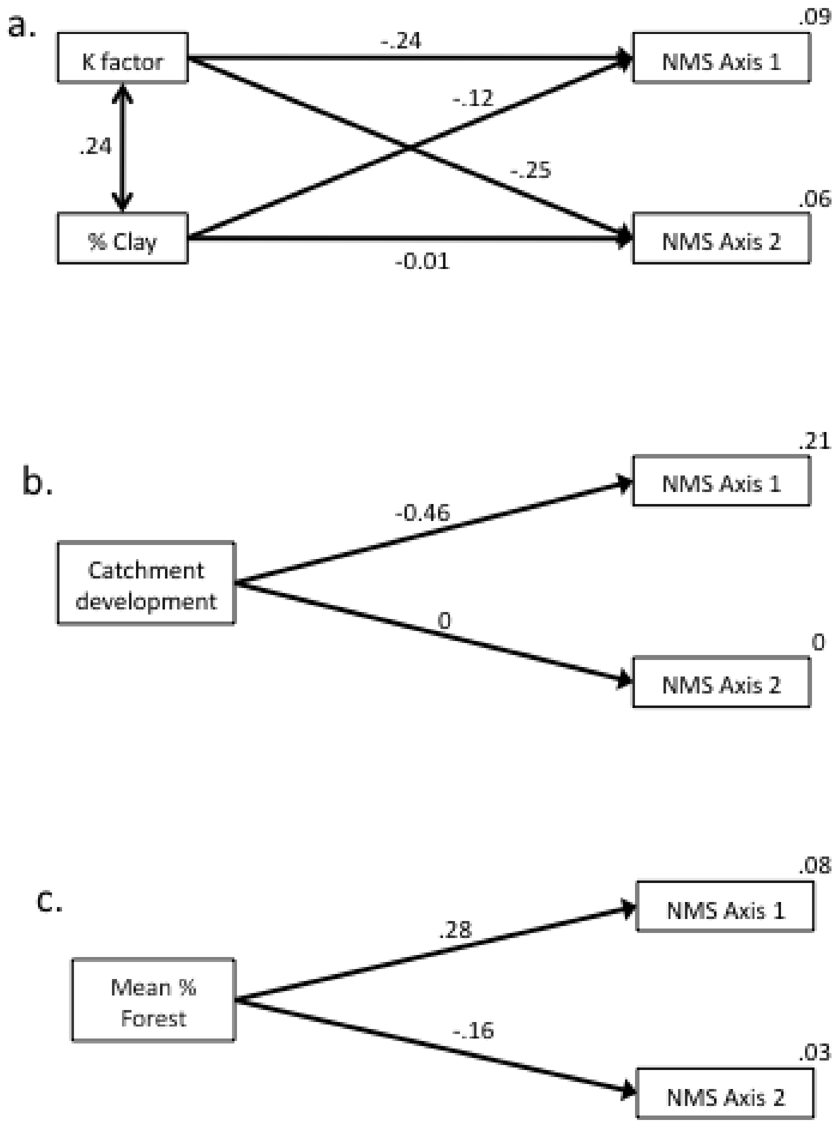


Figure 4. Exogenous variable structural equation models of NMS axes 1 and 2. Numerical values associated with errors are standardized regression weights. Values associated with variable boxes indicate the total variation explained for each response variable.

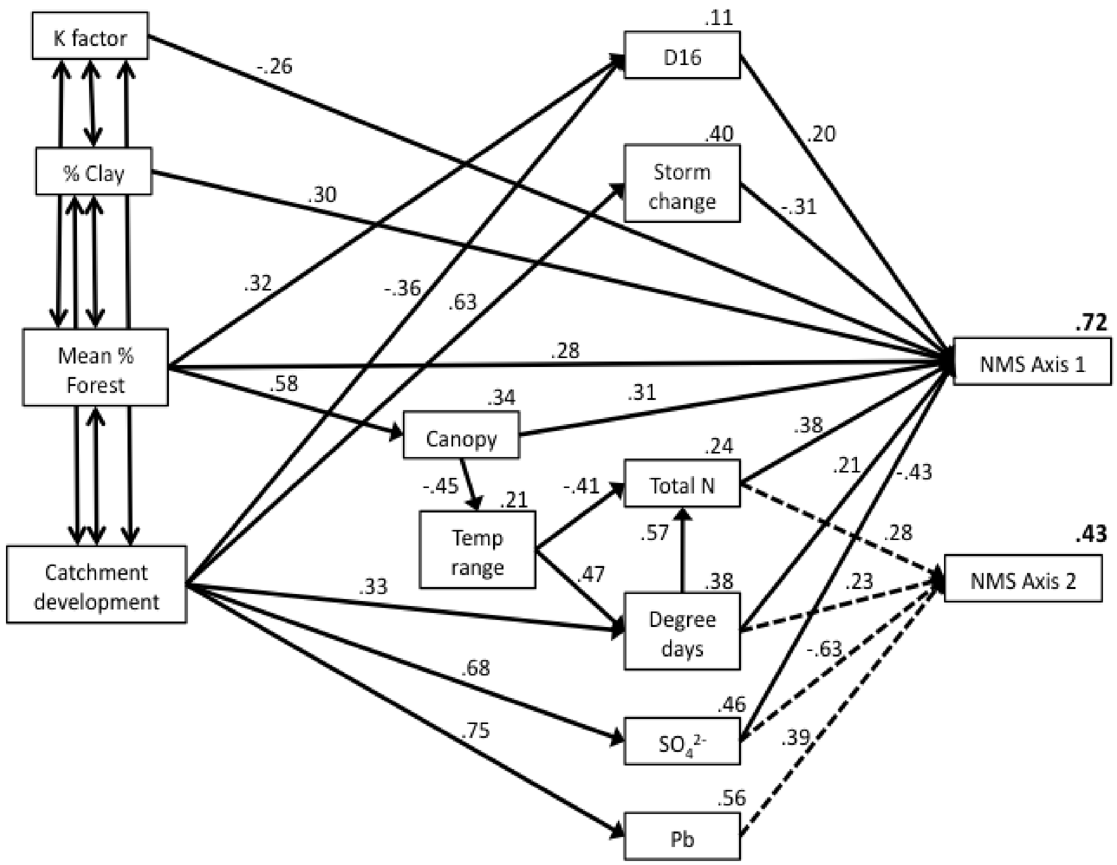


Figure 5. Final structural equation model predicting macroinvertebrate species composition defined by site scores for 2 dimensional NMS axes. Dashed lines indicate direct effects to NMS axis 2 response. Numerical values associated with errors are standardized regression weights. Values associated with variable boxes indicate the total variation explained for each endogenous variable. Final model X^2=76.3, P= 0.121

Chapter 5

Conclusions

Human land use practices have resulted in the widespread degradation of waterways draining the surrounding landscape. The consequences of land use change for aquatic biodiversity are well studied, and the extent to and mechanisms by which land use change leads to community degradation depend on the type and intensity of land use, and the proximity of development to the stream channel (Wang and Kanehl 2003, Moore and Palmer 2005). Land use induced degradation encompasses changes to hydrology, geomorphology, water chemistry, ecosystem function, and biotic community integrity. Stream restoration attempts to combat these influences, but there has been little evaluation of whether common practices are also best practices. As land use continues to impair stream structure and function and restoration continues to be used to attempt mitigation of these influences, it is critical to assess the benefits and drawbacks of current stream restoration approaches. In addition, stream restoration provides an opportunity to assess the mechanisms of stream degradation. Improved understanding of these processes may foster more effective stream protection and restoration efforts.

In a comparison of restored urban streams with urban and forested endpoints (Chapter 2, Violin et al. *in press*), restoration failed to improve any measure of habitat or macroinvertebrate community structure over impaired urban channels, in spite listing habitat rehabilitation and biotic community improvement. Additionally, restored macroinvertebrate community composition was more similar to urban degraded streams than forested channels. Further analysis of a forested site with suitable habitat, but degraded invertebrate communities, demonstrated urban hydrological signals can be propagated far downstream, even within forested stream reaches, and that habitat may be necessary, but is not sufficient for community recovery (Palmer et al. 2010), since urban hydrological signals may degrade communities without impairing habitat (Doyle et al. 2005). These results suggest that watershed level hydrologic processes, which are not addressed by reach scale restoration, are controlling physical and biological structure, and degrading restoration efforts.

In a broad scale study of restoration patterns (Chapter 3), results showed that in streams restored to mitigate for stream loss elsewhere due to development, independent of watershed type, Natural Channel Design restoration methods had little, if any positive effect on any measure of macroinvertebrate community structure over the five year post-restoration monitoring time frame, even when accounting for watershed effects. For the majority of restoration projects, invertebrate communities at restored sites were compositionally distinct from upstream reference sites, and community dissimilarity did not decrease over time. These results suggest that watershed effects are overwhelming local-scale restoration improvements, that restoration is not mitigating the factors responsible for community degradation, or that the five-year post restoration monitoring period is insufficient for community recovery. Additionally, the persistent dissimilarity of most restored communities when compared with upstream reference sites suggests that differing habitat characteristics persist between between restored and upstream references reaches, either due to enduring pre-restoration differences or restoration-induced changes in local conditions.

The structural equation model developed in chapter four identified variables that control community composition in streams on a forested-urban gradient, and identified proximate mechanisms that are responsible for degradation in urban environments. Urbanization had a strong, negative effect on macroinvertebrate community composition, which was mediated primarily through increased hydrologic flashiness, decreased water quality, and potentially through lower pH. Percent forested catchment was positively correlated with sensitive taxonomic composition both directly and through indirect effects on habitat, thermal regime, water quality, and potentially resource availability. Additionally, underlying geology directly affected taxonomic composition through soil composition and erodibility pathways.

The results from the three studies presented herein advance our understanding of the effects of urbanization on stream communities, and the potential of reach scale restoration to ameliorate watershed-scale degradation in catchments of differing land use types. The results from chapters two and three demonstrate that restoration does little to improve macroinvertebrate community structure regardless of catchment type. The mechanistic pathways identified from the structural equation model developed in chapter four suggest that several of the primary pathways by which urbanization leads to sensitive taxa are ones which are not currently addressed by restoration. Interestingly, a main focus of reach scale restoration is habitat improvement, however in addition to the absence of evidence that reach scale restoration improves habitat in urban streams, structural equation modeling suggested that habitat was of minor importance in predicting community composition in urban catchment, when compared with the effects of hydrology and water quality. Within rural and agricultural catchments, there was insufficient habitat data to determine if restoration was successfully restoring habitat in non-urban channels, and if this had any role in the poor community responses observed.

Together, these results demonstrate the limited utility of Natural Channel Design reach-scale restoration to combat watershed scale degradation. Given the expense of restoration, particularly in urban watersheds, and the extensive disturbance caused by restoration itself that may actually inhibit community recovery, it is important to reevaluate and redefine current restoration methodology and success criteria. Stream restoration should not cause further harm to an already degraded channel (Palmer et al. 2005), and should seek to restore degraded reaches with the least invasive methods possible (Palmer et al. 2010). Natural channel design extensively disturbs stream already impaired stream ecosystems (Tullos et al. 2009). Canopy removal due to construction activities can lead to long-term changes in the thermal regime (Tait et al. 1994, Caissie 2006) and food web structure (Hawkins et al. 1982). Additionally, natural channel design introduces sediment both during restoration, and potentially over the long term from structure failures due to high flows and channel migration (Shields et al. 1995, Bernhardt and Palmer 2007), which can inhibit community recovery.

Stream mitigation credits in North Carolina are currently awarded for geomorphological and riparian measures used as surrogates for in-stream ecological structure and function (Lave et al. 2008). The results presented in chapters two and three suggest that independent of restoration success from a mitigation perspective, restoration is not improving aquatic communities and thus the current success criteria are poor surrogates for in-stream community improvements, and the no net loss of function provision of the Clean Water Act.

If stream restoration and stream mitigation are to be changed to better facilitate actual in-stream community improvements, then restoration methodology, monitoring, and the criteria for awarding of mitigation credits must be altered. One of the more overwhelming conclusions of this body of work, consistent with previous research, is that local scale stream restoration will not be successful unless catchment based changes in hydrology are instituted, particularly in urban catchments. Specifically, protecting streams from directly-routed runoff and storm water that lead to frequent, short duration, high intensity floods that dislodge organisms, scour habitat, and carry nutrients and pollutants that simultaneously degrade water quality, and sediment pulses that further degrade habitat.

Natural Channel Design is a costly and invasive restoration method that through its implementation can fundamentally alter restored stream ecosystems by removing canopy and reducing water infiltration in the riparian zone due to heavy machinery compacting soil (Tullos et al. 2006). There is no evidence to suggest that Natural Channel Design is superior to stream restoration or rehabilitation strategies that solely reduce or remove the primary sources of aquatic degradation, for example by redesigning storm water systems (Walsh et al. 2005a), simply fencing out cattle, or solely revegetating riparian areas or exposed banks. It is also possible that a combination of these approaches with active in channel improvements may prove best. Starting with or building upon a minimalist approach and monitoring restoration projects to determine what methods are working is a better strategy for successful restoration than completely revamping an entire stream channel, and if successful, trying to tease apart successful components afterward. At the very least, this approach has less potential to further degrade the local stream ecosystem, even if restoration fails.

Undoubtedly, stream mitigation success should incorporate in-stream measures of biological and functional recovery, however it may be necessary to define success criteria based on catchment land use (Palmer et al. 2005, Bernhardt and Palmer 2007). It is important to realize that even under the best multi-scale oriented restoration efforts; biological recovery in urban watersheds may be minimal (Bernhardt and Palmer 2007). In contrast, local scale restoration may effectively ameliorate stream degradation in forested watersheds with local-scale, limited stream degradation from historic land use practices.

In summary, the work presented here suggests the need for a change in current stream methodology. Natural channel design is not effectively restoring degraded macroinvertebrate communities, and modeling efforts suggest that the primary pathways through which degradation occur are not addressed by this restoration method. Employing evidence-based restoration strategies which address mechanisms shown to predict stream ecosystem structure and function, and minimizing further degradation using the least invasive methods possible will lead to better restoration outcomes and aquatic ecosystem preservation.

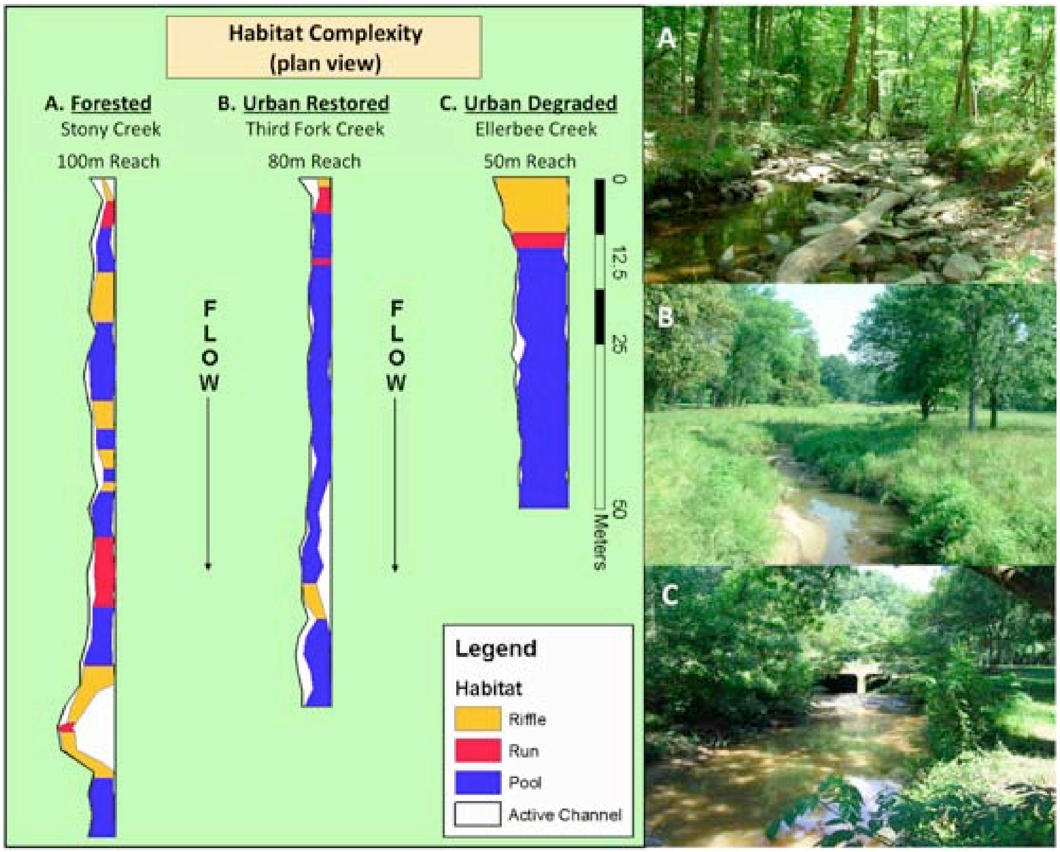
APPENDIX 2A

Appendix 2A. Study site UTM coordinates.

Site	Easting	Northing
Walnut Creek Tributary	634059.78180200000	224414.00363699900
Cemetery Creek	643519.15532300000	227025.39682299900
Third Fork Creek	617447.82504999900	247242.18925800000
Goose Creek	620051.56214000000	248697.20929200000
Lower Mud Creek	612227.07800700000	250097.04197500000
Mud Creek Tributary	612177.33430100000	250053.51623199900
Ellerbe Creek	618959.82658700000	251977.47655900000
Pot's Branch	631365.28550799900	235052.15745299900
Rocky Branch	638733.15191799900	225743.55909400000
Sandy Creek	613969.17513200000	247959.85438400000
Stony Creek	603615.71002000000	252347.58727300000
Upper Mud Creek	612488.23246700000	250594.47904000000

APPENDIX 2B

Appendix 2B. Example habitat maps and photos of study site block 1.



APPENDIX 2C

Appendix C. Study site species lists.

Walnut Creek Tributary (Abbott Restoration)

Order	Family	Taxon
Coleoptera	Dryopidae	*Helichus spp.*
	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Stenelmis* spp.
	Hydrophilidae	*Enochrus* spp.
Decapoda	Cambaridae	
Diptera	Chironomidae	*Chironomus* spp.
		Conchapelopia gr.
		Cricotopus infuscatus gr.
		Cricotopus vieriensis gr.
		Cryptochironomus spp.
		Dicrotendipes neomodestus
		Eukiefferiella brevicalcar gr.
		Orthocladius robacki
		Orthocladius clarkei gr.
		Phaenopsectra spp.
		Polypedilum aviceps
		Polypedilum fallax
		Polypedilum illinoense gr.
		Polypedilum scalaneum
		Rheocricotopus robacki
		Rheotanytarsus spp.
		Tanytarsus spp.
	Culicidae	*Anopheles* spp.
	Ephydridae	
	Simuliidae	*Simulium* spp.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Baetidae	*Baetis flavistriga*
Odonata	Calopterygidae	*Calopteryx* spp.
	Coenagrionidae	*Argia* spp.
Trichoptera	Hydropsychidae	*Diplectrona modesta*
		Hydropsyche betteni
Subclass Oligochaeta		

Cemetery Creek

Order	Family	Taxon
Coleoptera	Dytiscidae	*Copelatus* spp.
Decapoda	Cambaridae	
Diptera	Chironomidae	*Ablabesmyia mallochi*
		Chironomus spp.
		Conchapelopia gr.
		Cricotopus infuscatus gr.
		Cricotopus varipes gr.
		Cricotopus vieriensis gr.
		Cricotopus/Orthocladius sp. 51
		Cryptochironomus fulvus
		Cryptochironomus spp.
		Eukiefferiella brevicalcar gr.
		Natarsia sp. A
		Orthocladius (Euorthocladius)
		Orthocladius obumbratus gr.
		Paratendipes spp.
		Polypedilum aviceps
		Polypedilum flavum
		Polypedilum halterale gr.
		Polypedilum illinoense gr.
		Potthastia longimanus
		Tanytarsus spp.
	Tipulidae	*Hexatoma* spp.
		Limnophila spp.
		Tipula spp.
Ephemeroptera	Baetidae	*Baetis propinquus*
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
		Hydropsyche betteni
Subclass Oligochaeta		

Goose Creek

Order	Family	Taxon
Coleoptera	Hydrophilidae	*Helophorus* spp.
Diptera	Chironomidae	*Chironomus* spp.
		Conchapelopia gr.
		Cricotopus infuscatus gr.
		Polypedilum illinoense gr.
		Psectrotanypus dyari
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Odonata	Gomphidae	*Progomphus obscurus*
Subclass Oligochaeta		

Lower Mud Creek

Order	Family	Taxon
Amphipoda	Gammaridae	*Gammarus* spp.
Coleoptera	Dryopidae	*Helichus* spp.
	Elmidae	*Stenelmis* spp.
	Psephenidae	*Ectopria nervosa*
		Psephneus herricki
Decapoda	Cambaridae	
Diptera	Chironomidae	*Ablabesmyia mallochi*
		Conchapelopia gr.
		Diplocladius cultriger
		Natarsia sp. A
		Natarsia spp.
		Orthocladius clarkei gr.
		Parametriocnemus lundbecki
		Polypedilum halterale gr.
		Polypedilum illinoense gr.
		Procladius spp.
		Pseudochironomus spp.
		Tanytarsus spp.
		Thienmaniella spp.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Ameletidae	*Ameletus lineatus*
	Baetidae	*Baetis flavistriga*

	Heptageniidae	*Stenacron interpunctatum*
Isopoda	Asellidae	*Caecidotea* spp.
Odonata	Calopterygidae	*Calopteryx* spp.
	Corduliidae	*Somatochlora* spp.
Plecoptera	Capniidae	*Allocapnia* spp.
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
Subclass Oligochaeta		

Mud Creek Tributary

Order	**Family**	**Taxon**
Amphipoda	Gammaridae	*Gammarus* spp.
Coleoptera	Dryopidae	*Helichus* spp.
	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Stenelmis* spp.
	Psephenidae	*Psephneus herricki*
	Ptilodactylidae	*Anchytarsus bicolor*
Decapoda	Cambaridae	
Diptera	Chironomidae	*Chironomus* spp.
		Diplocladius cultriger
		Eukiefferiella claripennis gr.
		Microtendipes spp.
		Parachaetocladius spp.
		Parametriocnemus lundbecki
		Phaenopsectra spp.
		Tanytarsus spp.
		Tvetenia bavarica gr.
	Dixidae	*Dixa* spp.
	Simuliidae	*Simulium* spp.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Ameletidae	*Ameletus lineatus*
	Baetidae	*Baetis intercalaris*
		Centroptilum spp.
	Ephemerellidae	*Ephemerella dorothea*
		Eurylophella temporalis

		Eurylophella verisimilsis
	Heptageniidae	*Stenonema ithaca*
		Stenonema modestum
	Leptophlebiidae	*Paraleptophlebia* spp.
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Isopoda	Asellidae	*Caecidotea* spp.
Odonata	Cordulegasteridae	*Cordulegaster maculata*
	Gomphidae	*Ophiogomphus* spp.
		Stylogomphus albistylus
Plecoptera	Capniidae	*Allocapnia* spp.
	Chloroperlidae	*Haploperla brevis*
	Nemouridae	*Amphinemura* spp.
	Perlidae	*Eccoptura xanthenes*
		Perlesta placida
	Perlodidae	*Isoperla bilineata*
Trichoptera	Hydropsychidae	*Diplectrona modesta*
	Limnephilidae	*Ironoquia punctatissima*
	Rhyacophilidae	*Ryacophila ledra*
	Uenoidae	*Neophylax consimilis*
Subclass Oligochaeta		

Ellerbe Creek

Order	**Family**	**Taxon**
Coleoptera	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Ancyronyx variegatus*
		Stenelmis spp.
	Haliplidae	*Peltodytes* spp.
	Hydrophilidae	*Tropisternus* spp.
Decapoda	Cambaridae	
Diptera	Chironomidae	*Ablabesmyia mallochi*
		Chironomus spp.
		Conchapelopia gr.
		Cricotopus infuscatus gr.
		Cricotopus varipes gr.
		Dicrotendipes neomodestus

		Nanocladius spp.
		Natarsia sp. A
		Orthocladius (Euorthocladius)
		Orthocladius clarkei gr.
		Orthocladius obumbratus gr.
		Polypedilum aviceps
		Polypedilum halterale gr.
		Polypedilum illinoense gr.
		Tanytarsus spp.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Baetidae	*Baetis flavistriga*
	Caenidae	*Caenis* spp.
Amphipoda	Hyalellidae	*Hyalella* spp.
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
	Planorbidae	*Helisoma anceps*
Odonata	Coenagrionidae	*Argia* spp.
		Enallagma spp.
		Ischnura spp.
	Gomphidae	*Progomphus obscurus*
Rhynchobdellida	Glossiphoniidae	*Placobdella* spp.
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
		Hydropsyche betteni
	Rhyacophilidae	*Ryacophila ledra*
Veneroida	Corbiculidae	*Corbicula fluminea*
Subclass Oligochaeta		

<u>Pot's Branch</u>

Order	**Family**	**Taxon**
Amphipoda	Gammaridae	*Gammarus* spp.
Coleoptera	Dryopidae	*Helichus* spp.
	Elmidae	*Dubiraphia* spp.
		Stenelmis spp.
	Psephenidae	*Psephneus herricki*

Decapoda	Cambaridae	
Diptera	Chironomidae	*Ablabesmyia mallochi*
		Dicrotendipes neomodestus
		Larsia spp.
		Microtendipes spp.
		O. (Euorthocladius) Type III
		Orthocladius clarkei gr.
		Orthocladius obumbratus gr.
		Polypedilum fallax
		Polypedilum flavum
		Potthastia longimanus
		Rheocricotopus robacki
		Tanytarsus spp.
	Dixidae	*Dixa* spp.
	Simuliidae	*Simulium* spp.
	Tipulidae	*Hexatoma* spp.
		Tipula spp.
Ephemeroptera	Baetidae	*Baetis flavistriga*
		Baetis pluto
	Caenidae	*Caenis* spp.
	Heptageniidae	*Stenacron interpunctatum*
		Stenonema femoratum
Megaloptera	Corydalidae	*Corydalus cornutus*
		Nigronai serricornis
Odonata	Aeshnidae	*Boyeria vinosa*
	Calopterygidae	*Calopteryx* spp.
	Coenagrionidae	*Argia* spp.
	Gomphidae	*Ophiogomphus* spp.
		Stylogomphus albistylus
Plecoptera	Nemouridae	*Amphinemura* spp.
	Perlidae	*Perlesta placida*
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
		Diplectrona modesta
	Philopotamidae	*Chimarra* spp.
	Rhyacophilidae	*Rhyacophila ledra*
	Uenoidae	*Neophylax* spp.

<u>Rocky Branch</u>

Diptera	Chironomidae	*Conchapelopia* gr.
		Cricotopus infuscatus gr.
		Eukiefferiella brevicalcar gr.
		Orthocladius clarkei gr.
		Polypedilum aviceps
		Polypedilum illinoense gr.
	Simuliidae	*Simulium* spp.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Baetidae	*Baetis flavistriga*
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Odonata	Calopterygidae	*Calopteryx* spp.
	Coenagrionidae	*Argia* spp.
		Enallagma spp.
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
		Hydropsyche betteni
Subclass Oligochaeta		

Sandy Creek

Order	**Family**	**Taxon**
Coleoptera	Dryopidae	*Helichus* spp.
	Elmidae	*Stenelmis* spp.
	Haliplidae	*Peltodytes* spp.
	Hydrophilidae	*Tropisternus* spp.
Decapoda	Cambaridae	Cambaridae
Diptera	Ceratopogonidae	*Palpomyia* (complex)
	Chironomidae	*Ablabesmyia mallochi*
		Chironomus spp.
		Conchapelopia gr.
		Cricotopus bicinctus
		Cricotopus infuscatus gr.
		Cricotopus varipes gr.
		Dicrotendipes neomodestus
		Diplocladius cultriger
		Natarsia sp. A
		Natarsia spp.

		Orthocladius (Euorthocladius)
		Orthocladius clarkei gr.
		Orthocladius obumbratus gr.
		Polypedilum aviceps
		Polypedilum fallax
		Polypedilum flavum
		Polypedilum illinoense gr.
		Polypedilum scalaneum
		Psectrotanypus dyari
		Rheotanytarsus spp.
		Tanytarsus spp.
	Culicidae	*Anopheles* spp.
	Tipulidae	*Hexatoma* spp.
		Tipula spp.
Ephemeroptera	Caenidae	*Caenis* spp.
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Odonata	Aeshnidae	*Aeshna umbrosa*
	Calopterygidae	*Calopteryx maculata*
		Calopteryx spp.
	Coenagrionidae	*Argia sedula*
		Argia spp.
		Enallagma spp.
		Ischnura spp.
	Gomphidae	*Progomphus obscurus*
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
	Limnephilidae	*Pycnopsyche* spp.
Veneroida	Corbiculidae	*Corbicula fluminea*
	Pisidiidae	*Sphaerium* spp.
Subclass Hirudinea		
Subclass Oligochaeta		

Stony Creek

Order	**Family**	**Taxon**
Amphipoda	Gammaridae	*Crangonyx* spp.
		Gammarus spp.

Coleoptera	Dryopidae	*Helichus* spp.
	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Stenelmis* spp.
Decapoda	Cambaridae	Cambaridae
Diptera	Chironomidae	*Conchapelopia* gr.
		Hydrobaenus spp.
		Natarsia sp. A
		Orthocladius robacki
		Orthocladius obumbratus gr.
		Parametriocnemus lundbecki
		Polypedilum aviceps
		Polypedilum illinoense gr.
		Procladius spp.
		Rheocricotopus spp.
		Tanytarsus spp.
		Tvetenia bavarica gr.
		Zavrelimyia spp.
	Simuliidae	*Prosimulium* spp.
		Simulium spp.
	Tipulidae	*Antocha* spp.
		Pseudolimnophila spp.
		Tipula spp.
Ephemeroptera	Baetidae	*Acentrella* spp.
		Baetis bimaculatus
		Centroptilum spp.
		Plauditus dubius
	Caenidae	*Caenis* spp.
	Ephemeridae	*Hexagenia* spp.
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Isopoda	Asellidae	*Caecidotea* spp.
Megaloptera	Sialidae	*Sialis* spp.
Odonata	Corduliidae	*Somatochlora* spp.
Plecoptera	Beloneuria	*Beloneuria* spp.
	Nemouridae	*Shipsa rotunda*
	Perlodidae	*Isoperla bilineata*
		Isoperla spp.
	Nemouridae	*Amphinemura* spp.
	Perlidae	*Perlesta placida*

Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
	Limnephilidae	*Ironoquia punctatissima*
		Pycnopsyche guttifer
	Rhyacophilidae	*Rhyacophila ledra*
Veneroida	Corbiculidae	*Corbicula fluminea*
	Pisidiidae	*Sphaerium* spp.
Subclass Oligochaeta		

<u>Third Fork Creek</u>

<u>Order</u>	<u>Family</u>	<u>Taxon</u>
Decapoda	Cambaridae	Cambaridae
Diptera	Chironomidae	*Chironomus* spp.
		Conchapelopia gr.
		Cricotopus infuscatus gr.
		Cricotopus vieriensis gr.
		Cryptochironomus fulvus
		Cryptochironomus spp.
		Natarsia sp A
		Polypedilum illinoense gr.
	Tipulidae	*Tipula* spp.
Ephemeroptera	Baetidae	*Baetis flavistriga*
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Odonata	Aeshnidae	*Aeshna umbrosa*
	Coenagrionidae	*Argia* spp.
		Enallagma spp.
		Ischnura spp.
Trichoptera	Hydropsychidae	*Cheumatopsyche* spp.
Subclass Hirudinea		
Subclass Oligochaeta		

<u>Upper Mud Creek</u>

Order	**Family**	**Taxon**
Amphipoda	Gammaridae	*Gammarus* spp.
Coleoptera	Dytiscidae	*Hydroporus* spp.
	Elmidae	*Stenelmis* spp.
Decapoda	Cambaridae	Cambaridae
Diptera	Chironomidae	*Ablabesmyia mallochi*
		Chironomus spp.
		Conchapelopia gr.
		Cryptochironomus spp.
		Dicrotendipes modestus
		Dicrotendipes neomodestus
		Dipocladius cultriger
		Glyptotendipes spp.
		Microtendipes spp.
		Natarsia spp.
		Parametriocnemus lundbecki
		Paratendipes spp.
		Phaenopsectra spp.
		Polypedilum aviceps
		Polypedilum illinoense gr.
		Polypedilum scalaneum
		Psectrocladius spp.
		Tanytarsus spp.
	Culicidae	
	Empidade	
	Tipulidae	*Pseudolimnophila* spp.
		Tipula spp.
Gastropoda (Pulmonata)	Physidae	*Physella* spp.
Odonata	Calopterygidae	*Calopteryx* spp.
	Libellulidae	*Libellula* spp.
Veneroida	Pisidiidae	*Sphaerium* spp.
Subclass Oligochaeta		

APPENDIX 4A

Appendix 4A. Landscape, soil, water quality, hydrology, pollution, sediment function, temperature variables that are potential predictors for macroinvertebrate SEM….

Variable	**Description**
Trans	Number of habitat transitions per stream reach
Xdepth	Mean thalweg depth in m
Dmin	Minimum thalweg depth in m
Dmax	maximum thalweg depth in m
Dcv	CV of depth
Dsem	Standard error of depth
Xwidth	Mean wetted width in m
Wmin	Minimum wetted width in m
Wmax	Maximum wetted width in m
Wcv	CV of width
Wsem	Standard error of width
Can	% canopy cover (measured with spherical densiometer)
Cancv	CV of canopy cover
Canop_tm	Canopy cover estimated using 2008 NAIP air photos
Length	Reach length (m)
Inc_x	Mean channel incision (calculated as channel depth (bank height to streambed) at thalweg divided by bankfull width) calculated from 3 random cross-sections
Inc_SD	Standard deviation of channel incision
Inc_CV	CV of channel incision
Rds	Road density (m/ha); total road length in watershed divided by watershed area
Trafidw	Inverse-distance weighted traffic; Mean traffic volume per area of watershed, weighted by distance to stream
Rdst	Average road and stream intersections per km
Dev50	Effective Development within 50m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev100	Effective Development within 100m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev250	Effective Development within 250m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev500	Effective Development within 500m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev1000	Effective Development within 1000m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev1500	Effective Development within 1500m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Dev2000	Effective Development within 2000m of stream; Mean developed cells weighted by distance to nearest stream for each watershed
Wtd50	Effective impervious area within 50m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest

	stream
Wtd100	Effective impervious area within 100m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest stream
Wtd250	Effective impervious area within 250m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest stream
Wtd500	Effective impervious area within 500m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest stream
Wtd1000	Effective impervious area within 1000m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest stream
Wtd1500	Effective impervious area within 1500m of stream weighted by development; Mean developed cells weighted by approximate imperviousness based on developed class and distance to nearest stream
Buf50	Effective buffering area in watershed within 50m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf100	Effective buffering area in watershed within 100m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf250	Effective buffering area in watershed within 250m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf500	Effective buffering area in watershed within 500m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf1000	Effective buffering area in watershed within 1000m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf1500	Effective buffering area in watershed within 1500m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
Buf2000	Effective buffering area in watershed within 2000m of stream; Mean forested cells weighted by distance to nearest stream for each watershed
85dev	Mean Development in 1985; % of developed land in the watershed
95dev	Mean Development in 1995; % of developed land in the watershed
05dev	Mean Development in 2005; % of developed land in the watershed
Mean forest	Mean Percentage of forested land in the watershed
Mean field	Mean Percentage of field land in the watershed
PIC	Percent impervious connected; Percent of watershed that is imperviousness directly connected to the stream

Mead Cd	Mean Cadmium corrected for Carbon (mg Cd/g C)
Mean Pb	Mean Lead corrected for Carbon (mg Pb/g C)
Mean Ag	Mean Silver corrected for Carbon (mg Ag/g C)
Mean Zn	Mean Zinc corrected for Carbon (mg Zn/g C)
Mean Cu	Mean Copper corrected for Carbon (mg Cu/g C)
Mean Ni	Mean Nickel corrected for Carbon (mg Ni/g C)
Mean As	Mean Arsenic corrected for Carbon (mg As/g C)
Mean Al	Mean Aluminum corrected for Carbon (mg Al/g C)
Mean Cr	Mean chromium corrected for Carbon (mg Cr/g C)
D16	Diameter of 16th percentile particle (mm)
D50	Diameter of 50th percentile particle (mm)
D84	Diameter of 84th percentile particle (mm)
DD	Degree days. Overall heat of a stream, calculated using double triangle method and a base temperature of 0 degrees Celsius
Xtemp	Average temperature of stream (^{o}C)
Xmin	Average daily minimum temperature of stream (^{o}C)
Xmax	Average daily maximum temperature of stream (^{o}C)
Xrange	Average diel range (^{o}C)
Storm change	Maximum absolute value of temperature change between 10 minutes during 5/28/09 11 am to 5/29/09 11 am (precipitation measured 5/28/09 1 pm to 3 pm; 5/28/09 9 pm to 11 pm)
Mean NH_4	Average of ammonium-N in mg/L
Mean Cl^-	Average of chloride in mg/L
Mean $SO_{2-}{}^{4}$	Average of sulfate in mg/L
Mean Br-	Average of bromide in mg/L
Mean $NO_3{}^-$	Average of nitrate-N in mg/L
Mean PO_4	Average of phosphate-P in mg/L
Mean NPOC	Average of non purgable organic carbon in mg/L
Mean TN	Average of total nitrogen in mg/L
Mean digTN	Average of unfiltered water sample digested using a persulfate digest and analyzed for total nitrogen; mean value of 2 reps reported here
Mean digTP	Average of unfiltered water sample digested using a persulfate digest and analyzed for total phosphorus; mean value of 2 reps reported here
SIR	measurement of the total microbial activity in a sediment sample in mg CO2 / g-drymass /min
AFDM	proportion of sediment, by mass, that is organic
%clay	Soil % clay, averaged over 0-100 cm for the entire watershed
%sand	Soil % sand, averaged over 0-100 cm for the entire watershed
%silt	Soil % silt, averaged over 0-100 cm for the entire watershed
kfac	Soil K factor, a measure of soil erodibility, averaged over 0-100 cm for the entire watershed
om	Soil organic matter, averaged over 0-100 cm for the entire watershed
ph	Soil pH, averaged over 0-100 cm for the entire watershed
plasticity	Soil plasticity, averaged over 0-100 cm for the entire watershed
cec	Soil cation exchange capacity, averaged over 0-100 cm for the entire watershed

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