# AN EXAMINATION OF THE IMPACT OF URBANIZATION ON STREAM BIODIVERSITY AND ECOSYSTEM FUNCTION

by

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# ABSTRACT

ANTHONY JOSEPH ROUX. An Examination of the Impact of Urbanization on Stream Biodiversity and Ecosystem Function. (Under the direction of DR.SANDRA CLINTON)

The "Urban Stream Syndrome" is a term that refers to a group of predictable negative impacts to stream ecosystems due to the alteration of the natural hydrologic regime that is associated with urbanization. These negative impacts include increases in the volume and intensity of stormwater inputs to streams, channel erosion, streambed sedimentation, and nutrient and pollutant concentrations. The impacts of urbanization ultimately degrade the habitat available to the aquatic biota in streams including fish and benthic macroinvertebrate communities. The decline in benthic macroinvertebrate taxa richness due to urbanization has been well documented. However, the impact of the stressors associated with the increased stormwater flashiness to the composition of the benthic macroinvertebrate assemblages' taxa and trait richness and diversity is not well known. For my dissertation, I proposed three research studies designed to improve the understanding of how the increased stormwater volume and intensity due to unmitigated runoff from urban areas impacts the benthic macroinvertebrate assemblages' taxa and trait richness and diversity. To do this, I first examined a 26-year data set to study the impact of land use changes on biodiversity and ecosystem function in stream ecosystems in watersheds that span a gradient of impervious cover (IC) and stream habitat conditions. Next, to better understand the impact of urbanization on biodiversity and ecosystem function, I examined the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity at the watershed scale. Finally, to better understand stormwater impacts on macroinvertebrates assemblages, I took advantage of a natural field experiment to compare macroinvertebrate taxa and trait richness and diversity in 2 adjacent headwater

tributaries that received stormwater runoff through different processes (via stormwater infrastructure versus natural overland and subsurface processes).

Study 1) I investigated: *How do EPT Taxa and Trait Richness and Diversity change with increases in percent IC?* I hypothesized that EPT taxa richness and diversity would decline greater than EPT Trait Richness and diversity with increasing percent IC. I also investigated: *Which Indicators of Hydrologic Alterations (IHA) have greater impacts on EPT taxa and trait richness and diversity?* I hypothesized that the IHA metrics closely associated with storm runoff flashiness would have greater impacts on taxa and trait richness and diversity. EPT taxa richness and diversity significantly declined with increases in percent IC. Trait richness and diversity declined with increases in percent IC, but not to the same extent as taxa richness and diversity. **T**hreshold **I**ndicator **T**axa **AN**alysis (TITAN) identified distinct percent IC thresholds for sensitive EPT taxa between 5 to 10% IC while tolerant EPT taxa increased in abundance beginning around 30% IC. TITAN analysis identified similar thresholds for traits associated with sensitive EPT taxa between 5% to 10% IC while traits associated with tolerant EPT taxa increased in abundance starting at 30% IC. Stream habitat condition declined with increases in percent IC which was positively correlated with the R-B Flashiness Index. Higher shear stress associated with flashy stormwater contributed to the less stable and more homogenous habitats found in urban streams in watersheds with impervious cover greater than 25%. TITAN analysis found distinct thresholds for sensitive EPT taxa between MHAP scores of 120 and 140 (lower end of the partially supporting habitat condition classification) while tolerant EPT taxa began increasing in abundance at MHAP conditions below 100 which are typical habitat scores in urban streams. TITAN analysis showed distinct declines in traits associated with sensitive EPT taxa at MHAP scores at 120 and a second threshold at MHAP scores of 80. Several sensitive

EPT taxa were found to be indicators of streams with percent IC <10%. Several of the more tolerant EPT taxa were found to be indicators of streams with watersheds with percent IC greater than 25%.

Study 2) I investigated: *How do taxa and trait richness and diversity respond to decreases in stream habitat condition*? I hypothesized that the decrease in total taxa richness and diversity would be greater than the decrease in total trait richness and diversity with decreasing habitat diversity. I also investigated: *How are taxa and traits distributed within and between the microhabitats found in streams*? I hypothesized that similar traits are found among the aquatic insect taxa residing in similar microhabitats within the same stream. Streams with fully supporting habitat conditions were more similar to each other than to partially supporting and impaired streams when described by taxa abundance; however, fully supporting and partially supporting streams were more similar when described by trait abundance. As MHAP scores declined, habitat diversity variability increased in the partially supporting and impaired sites. I found that both taxa richness and diversity declined at a greater rate than trait richness and diversity along an in-stream habitat diversity gradient. Trait richness and diversity changed very little with changes in habitat condition. The impaired streams generally had lower habitat diversity than both the partially supporting and supporting streams. Taxa richness was positively correlated with pools, runs, backwater, leaf packs, and riffles, while trait richness was positively correlated with runs, small wood, and riffles. Taxa diversity was positively correlated with pools and leaf packs while trait diversity was positively correlated with runs, leaf packs, small wood, and riffles. Riffles, leaf pack, large wood, and runs were important microhabitats for taxa richness while undercut banks were important for taxa diversity and habitat diversity and small wood were important for trait richness. The taxa and traits found in riffles and leaf packs were more similar than taxa and traits found in undercut banks and root wads.

Study 3) I investigated: *Do the patterns of taxa and trait richness and diversity differ between the 2 tributaries receiving stormwater from different sources*? and *Do the carbon sources available to benthic macroinvertebrate assemblages differ between these 2 tributaries*? I hypothesized that both taxa and trait richness and diversity would be lower in the stormwater impacted (TI) tributary than in the forested (TF) tributary due to the increased stormwater runoff**.** I hypothesized that the carbon sources available to benthic macroinvertebrate food webs would be altered in the TI tributary with changes in food sources. The habitat condition in the TI tributary, which had higher predicted shear stress than the TF tributary for the same storm event, was impaired and had EMAHP scores significantly lower than in the TF tributary. Taxa richness and diversity were significantly higher in the TF tributary than in the TI tributary. Trait richness was also significantly higher in the TF tributary. Trait diversity was not significantly different between tributaries. The summer carbon $\delta^{13}C$ values of the periphyton and leaf pack food sources were distinctly different in the TF tributary while they were very similar and closer to allochthonous source $\delta^{13}C$ values in the TI tributary. The carbon $\delta^{13}C$ values of the collector-gatherers, herbivore-scrapers, predators, and shedders were closer to the carbon $\delta^{13}C$ values of the periphyton in both tributaries. In the winter, the carbon $\delta^{13}C$ values of the periphyton and leaf pack food sources were distinctly different in both tributaries. All the FFG trait richness except for the collector-gatherer were similar between tributaries. The collector-gatherer richness was significantly greater in the TF tributary. Collector-gatherers have been found to be the most abundant benthic macroinvertebrate feeding groups in impaired urban streams like TI.

Improving restoration design is necessary to restore the benthic macroinvertebrate community in urban streams. This study's results show that unmitigated stormwater will have significant negative effects on the benthic macroinvertebrate assemblages. Addressing the sources of hydrologic alterations as part of a stream restoration plan may lead to more successful restoration of an urban stream aquatic ecosystem. Another improvement to stream restoration design to improve the benthic macroinvertebrate community would be to expand the habitat improvement designs to increase the diversity of habitats available to aquatic biota.

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# DEDICATION

I would like to dedicate this dissertation to the memory of my parents, Joseph Alexander Roux and Claire Marie Milot Roux. I regret that they both passed away before I could finish my journey towards my Doctoral degree. My mother was my biggest cheerleader and always believed that I could complete this dissertation.

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| | |
|---|---|
| CMSWS | Charlotte-Mecklenburg Storm Water Services |
| EPT | Ephemeroptera, Plecoptera, Trichoptera |
| FFG | Functional Feeding Group |
| IC | Impervious cover |
| IHA | Indicators of Hydrologic Alteration |
| LULC | Land use land cover |
| MHAP/EMHAP | Mecklenburg habitat assessment protocol/Enhanced MHAP |
| NCDEQ | NC Department of Environmental Quality |
| NLCD | National land cover data set |
| R-B INDEX | Richards-Baker Flashiness Index |
| TF | Torrence Creek Forested Tributary |
| TI | Torrence Creek Stormwater Impacted Tributary |
| TITAN | **T**hreshold **I**ndicator **T**axa **AN**alysis |
| TV | Tolerance Value (pollution) |
| USGS | US Geological Survey |

# CHAPTER 1: INTRODUCTION

Biodiversity, a measure of the variety of organisms present in an ecosystem, has been shown to be correlated to changes in ecosystem function in terrestrial communities (Naeem et al. 1994; Tilman 1997; Cardinale 2012). For example, Tilman (1997) found that changes in plant biodiversity impacted ecosystem function by impacting soil nutrients and plant species productivity. While the relationship between biodiversity and ecosystem function has been well studied in terrestrial plant communities, the relationship between biodiversity and ecosystem function in stream ecosystems is not well documented (Bêche and Statzner 2009). Vannote et al. (1980) introduced a model known as the River Continuum Concept (RRC) that described how a stream ecosystem changes in various ecosystem functions such as functional feeding group (taxa traits) and taxa composition as the stream increases in size from small first and second order headwater streams to large fifth to seventh order streams and small rivers. The percent of each functional feeding group (collector-filterer, collector-gatherer, herbivore-scraper, predator, and shredder) within the benthic macroinvertebrate community changes as the primary energy source changes with stream order.

As a forested or rural watershed becomes developed and more urban, a predictable set of negative impacts to the stream channel geomorphology, hydrology and biota generally occurs. These negative impacts to urban streams due to the increased stormwater inputs associated with development have been referred to as “the Urban Stream Syndrome” (Walsh et al. 2005). Impervious cover (IC) as low as 5% in a watershed has been shown to have negative effects on benthic macroinvertebrate taxa diversity and biomass (Schueler 1994; Center for Watershed Protection 2003). However, the impact of urbanization on the relationship between ecosystem function and diversity in urban streams is not well understood (Wenger et al. 2009).

I examined the impact of urbanization on benthic macroinvertebrate biodiversity and ecosystem function by integrating the following 3 studies:

## 1.1 IMPACT OF LAND USE CHANGES OVER A PERIOD OF 26 YEARS ON BENTHIC MACROINVERTEBRATE DIVERSITY AND FUNCTION IN PIEDMONT STREAMS IN NORTH CAROLINA

The Charlotte-Mecklenburg Storm Water Services (CMSWS) Water Quality program has been monitoring benthic macroinvertebrates in the streams in Charlotte and Mecklenburg County since 1994. As an employee of CMSWS, I have overseen the Mecklenburg County Biological Laboratory including the benthic macroinvertebrate monitoring program. This long-term data set presents a unique opportunity to study the impact of land use changes on biodiversity and ecosystem function in stream ecosystems in watersheds that span a gradient from low to high percent impervious cover (IC). I chose to focus on the Ephemeroptera, Plecoptera, and Trichoptera (EPT) aquatic insect orders as they represent the most sensitive taxa to environmental degradation including changes in physical and water chemistry characteristic of stream ecosystems (Lenat 1993; Barbour et al. 1999; Cuffney et al. 2010). I examined how taxa and traits change in richness and diversity along a gradient of percent IC, a surrogate measure of urban development. As alterations of the natural hydrologic flow regime is one of the more significant impacts of development, I looked at the relationships between alterations in the natural flow regime in streams, storm flashiness, and the taxa and trait richness and diversity using the Indicators of Hydrologic Alterations (IHA) Richards-Baker Flashiness Index (R-B Index) metrics.

## 1.2 EVALUATION OF THE RELATIONSHIP BETWEEN STREAM HABITAT QUALITY AND TAXA RICHNESS AND TRAITS IN PIEDMONT STREAMS IN NORTH CAROLINA

Urban streams are presented with numerous problems that are largely due to the alteration of the natural hydrologic regime (Walsh et al. 2005). Numerous researchers have shown that stream restorations that just address geomorphological stream channel characteristics without taking into consideration stream functional traits and the ecological requirements of the benthic macroinvertebrates fail to stimulate the recovery of the benthic macroinvertebrate community (Palmer et al. 2005; Sudduth et al. 2011). To better inform restoration design and implementation, I investigated the relationship between stream habitat quality and benthic macroinvertebrate taxa and trait richness and diversity by evaluating 30 streams in the Piedmont, North Carolina spanning a gradient of good to poor habitat quality. I also examined the distribution of taxa and traits among microhabitats within the same stream. Understanding the preferences of taxa and associated traits for specific microhabitats may improve stream restoration designs to enhance habitat diversity by including less commonly restored microhabitats that are important in supporting diverse benthic macroinvertebrate assemblages.

### 1.3 IMPACT OF STORMWATER ON BENTHIC MACROINVERTEBRATE DIVERSITY AND STREAM ECOSYSTEM FUNCTION IN A PIEDMONT STREAM IN NORTH CAROLINA

To better understand stormwater impacts on benthic macroinvertebrates assemblages, I examined the impact that stormwater runoff had on urban headwater stream ecosystem function. This study took advantage of a natural field experiment consisting of two adjacent tributaries alike in all aspects except stormwater where one tributary receives stormwater from a residential development via storm drain infrastructure while the other tributary receives stormwater via more natural overland and subsurface processes. I quantified the impact of unmanaged stormwater runoff on stream channel morphology, hydrology, stream benthic macroinvertebrate taxa and trait richness and diversity, ecosystem function, and an urban stream food web in a Piedmont stream. Changes in biodiversity can impact ecosystem function by altering trophic interactions within the local ecosystem's food web (Loreau et al. 2001; Thébault and Loreau 2006; Thompson et al. 2012).

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# CHAPTER 2: EVALUATION OF 26 YEARS OF LAND USE CHANGE ON BENTHIC MACROINVERTEBRATE DIVERSITY AND FUNCTION IN PIEDMONT STREAMS IN NORTH CAROLINA.

## 2.1 ABSTRACT

The "Urban Stream Syndrome" is a term that refers to a group of predictable negative impacts to stream ecosystems due to the alteration of the natural hydrologic regime associated with urbanization including increases in the volume and intensity of stormwater inputs to streams, channel erosion, streambed sedimentation, and nutrient and pollutant concentrations. These negative impacts of urbanization ultimately degrade the habitat available to the aquatic biota in streams including fish and benthic macroinvertebrate communities. The decline in benthic macroinvertebrate taxa richness due to urbanization has been well documented. However, the impact to the composition of the benthic macroinvertebrate assemblages' taxa and trait richness and diversity is not well known.

Charlotte-Mecklenburg Storm Water Services has been monitoring the benthic macroinvertebrates since 1994. This unique long-term data set presents the opportunity to study the impact of land use changes on biodiversity and ecosystem function in stream ecosystems in watersheds that span a gradient of impervious cover and stream habitat conditions. In this study, I examined the influence of land use changes, as seen through changes in percent impervious cover (percent IC), on stream benthic macroinvertebrate biodiversity and function in Piedmont streams in North Carolina. I investigated: 1) How do EPT taxa and trait richness and diversity change with increases in percent IC? I hypothesized that the decline in EPT taxa richness and diversity would be greater than the decline in EPT trait richness and diversity with increasing percent IC. 2) How will individual traits respond to increases in percent IC? I hypothesized that individual trait responses would not be uniform and would vary by traits. 3) How will the FFG

traits change with increases in percent IC? I hypothesized that there would be a decline in shredder richness and an increase in herbivore-scraper richness in response to increases in percent IC. 4) Which Indicators of Hydrologic Alterations have greater impacts on EPT taxa and trait richness and diversity? I hypothesized that the IHA metrics more closely associated with the flashiness of a storm event would have greater impacts on taxa and trait richness and diversity.

EPT taxa and trait richness and diversity were negatively impacted by alterations of the natural hydrologic regime due to urbanization. Taxa richness and diversity significantly declined with increases in percent IC, a surrogate measure of urban development. Trait richness and diversity declined with increases in percent IC, but not to the same extent as taxa richness and diversity. **T**hreshold **I**ndicator **T**axa **AN**alysis (TITAN) analysis identified distinct percent IC thresholds for sensitive EPT taxa between 5 to 10% IC while tolerant EPT taxa increased in abundance beginning around 30% IC. TITAN analysis identified similar thresholds for traits associated with sensitive EPT taxa between 5 to 10% IC while traits associated with tolerant EPT taxa increased in abundance starting at 30% IC. Stream habitat condition (as measured by MHAP scores) declined with increases in percent IC which was positively correlated with the R-B Flashiness Index. Higher shear stress associated with flashy stormwater contributed to the less stable and more homogenous habitats found in urban streams in watersheds with impervious cover greater than 25%. TITAN analysis found distinct thresholds for sensitive EPT taxa between MHAP scores of 120 and 140 (lower end of the partially supporting habitat condition classification) while tolerant EPT taxa began increasing in abundance at MHAP conditions below 100 which are typical habitat scores in urban streams. TITAN analysis showed distinct declines in traits associated with sensitive EPT taxa at MHAP scores at 120 and a second threshold at MHAP scores of 80. Several sensitive EPT taxa were found to be indicators of streams with percent IC <10%. These were among the EPT taxa the TITAN analysis identified as declining in abundance at percent IC as low as 5%. Several of the more tolerant EPT taxa were found to be indicators of streams with watersheds with percent IC greater than 25%. These taxa were also the EPT taxa identified by the TITAN analysis as increasing in abundance when the percent IC rose above 30%.

## 2.2 INTRODUCTION

As population growth and development transforms watersheds from rural to more urban settings, the percent impervious cover of these watersheds increases, limiting the amount of precipitation that naturally infiltrates into the soil while increasing the amount of surface water runoff. Walsh et al. (2005) described a group of commonly occurring negative impacts due to the increased stormwater inputs to streams associated with urbanization as “the Urban Stream Syndrome.” Stream channels are degraded by the increased volume and intensity of stormwater runoff. Sediment from eroding stream banks and adjacent development cover the streambed, degrading the habitats of the benthic macroinvertebrate communities which are a fundamentally important component of stream ecosystems. Along with changes in hydrology and channel geomorphology, nutrient, and pollutant concentrations in the stream increase. Percent impervious cover (IC) has been shown to be a good surrogate for measuring urban development (Schueler 1994; Brabec et al. 2002; Shuster et al. 2005; Schueler et al. 2009) as well as correlated with declines of aquatic biota (Center for Watershed Protection 2003; Morse et al. 2003; Wenger et al. 2008). Impervious cover as low as 5% in a watershed has been shown to have negative effects on benthic macroinvertebrate taxa diversity and biomass (Schueler 1994; Paul and Meyer 2001; Stepenuck et al., 2002; Center for Watershed Protection 2003; Morse et al., 2003; Ourso and Frenzel 2003; Wenger et al., 2008; Cuffney et al., 2010). The biological communities in streams (algae, benthic macroinvertebrates, and fish) respond to urbanization by shifting to communities dominated by pollution tolerant taxa through the loss of sensitive taxa (Coles et al., 2012).

### 2.2.1 BIODIVERSITY AND FUNCTIONAL DIVERSITY

Biodiversity, a measure of the variety of organisms present in an ecosystem, has been shown to be correlated to changes in ecosystem function in terrestrial communities (Naeem et al., 1994; Tilman 1997; Naeem 1998; Tilman 2001; Cardinale et al., 2006a; Cardinale et al., 2006b; Cadotte et al., 2011; Cardinale 2012). Tilman et al. (1997) found that changes in plant diversity in a grassland-savanna community impacted the ecosystem function of the grassland community by impacting the soil N concentration and grass taxa productivity. They found that the loss or addition of taxa with unique functional traits may impact the overall production of the community. While the relationship between biodiversity and ecosystem function has been well studied in terrestrial plant communities, the relationship between biodiversity and ecosystem function in stream ecosystems is not well documented (Bêche and Statzner 2009; Wenger et al., 2009).

A healthy stream ecosystem has been described as having a high diversity of taxa (Hynes 1970; Cummins 1974; Allan and Castillo 2007). Each taxon possesses traits that reflect adaptations to the specific microhabitats where they are found (Poff 1997; Poff et al., 2006a; Menezes et al., 2010; Cummins 2016; Alahuhta et al., 2019). Taxa traits have been used to characterize the functional composition of benthic macroinvertebrate communities (Poff 1997; Statzner et al., 1988; Usseglio-Polatera et al., 2000; Statzner et al., 2001; Miller et al., 2010) as well as the stability and resiliency of those communities (Statzner et al., 2004; Bêche and Statzner 2009; Verberk et al., 2013). Vannote et al. (1980) introduced a model known as the River Continuum Concept (RRC) that described how a stream ecosystem changes in various ecosystem functions such as functional feeding approaches (taxa traits) and taxa composition as the stream increases in size from small first and second order headwater streams to large fifth to seventh order streams and small rivers. The percent of each functional feeding group (collector-filterer, collector-gatherer, herbivore-scraper, predator, and shredder) within the benthic macroinvertebrate community changes as the primary energy source changes with stream order.

Recent work has shown that taxa traits can provide insights into the factors that influence the composition of benthic macroinvertebrate assemblages (Menezes et al., 2010; Statzner and Beche 2010), including identifying the stressors driving changes in assemblage composition observed along environmental gradients (Lange et al., 2014; Berger et al., 2018; Castro et al., 2018; Monk et al., 2018; Marques et al., 2019; Ntloko et al., 2021).

Poff et al. (2006a; Table 2.1) described 20 benthic macroinvertebrate traits grouped into 4 major categories: Life History (rate of development), Mobility (ability to fly, crawling rate), Morphology (size, shape, armoring), and Ecology (habitat preference, thermal preference, feeding preference).

**Table 2.1.** Aquatic insect traits with abbreviations used in this study (Poff 2006a)

| **Trait Group** | **Trait** | **Trait state** | **Abbreviation** |
|---|---|---|---|
| **Life History** | Voltinism | Multivoltine | MV |
| | | Semivoltine | SV |
| | | Univoltine | UV |
| | Development | Fast seasonal | FSE |
| | | Nonseasonal | NSE |
| | | Slow seasonal | SSE |
| | Synchronization emergence | Poorly synchronized | PSY |
| | | Well synchronized | WSY |
| | Adult Life Span | Long | LLF |
| | | Short | SLF |
| | | Very short | VSLF |
| | Adult ability to exit | Absent | EAB |
| | | Present | EPR |
| | Survive desiccation | Absent | SAB |
| | | Present | SPR |
| **Mobility** | Female Dispersal | High | HDIS |

| | | | |
|---|---|---|---|
| | | Low | LDIS |
| | Flying strength | Strong | SFLY |
| | | Weak | WFLY |
| | Occurrence Drift | Abundant | ADRF |
| | | Common | CDRF |
| | | Rare | RDRF |
| | Max crawling rate | High | HCW |
| | | Low | LCW |
| | | Very low | VLCW |
| | Swim ability | None | NSW |
| | | Strong | STSW |
| | | Weak | WKSW |
| **Morphology** | Attachment | Both | BATT |
| | | None | NATT |
| | | Some | SATT |
| | Armoring | Good | GARM |
| | | None | NARM |
| | | Poor | PARM |
| | Shape | Not Streamlined | NOSTR |
| | | Streamlined | STR |
| | Respiration | Gills | GIL |
| | | Plastron, spiracle | PLA |
| | | Tegument | TEG |
| | Size at maturity | Large (>16 mm) | LG |
| | | Medium (9-16 mm) | MD |
| | | Small (<9 mm) | SM |
| **Ecology** | Rheophily | Depositional and Erosional | DE |
| | | Depositional only | DEP |
| | | Erosional | ERO |
| | Thermal Preference | Cold stenothermal | CLD |
| | | Cool eurythermal | COL |

| | | | |
|---|---|---|---|
| | | Warm eurythermal | WRM |
| | Habit | Burrow | BRW |
| | | Climb | CLB |
| | | Cling | CLG |
| | | Sprawl | SPL |
| | | Swim | SWM |
| | Trophic Habit | Collector-filterer | CF |
| | | Collector-gatherer | CG |
| | | Herbivore-scraper | HB |
| | | Predator | PD |
| | | Shredder | SH |

## 2.2.2 ALTERATIONS IN THE NATURAL FLOW REGIME

The natural hydrologic flow regime is impacted by watershed development (Konrad et al., 2002; Konrad and Booth, 2005; Walsh et al., 2005; Brown et al., 2009; Cuffney et al., 2010; Walsh et al., 2012). As percent impervious cover increases, larger percentages of each storm event reach stream channels through overland flow over impervious surfaces and through storm drain infrastructure, resulting in rapid water level increases and decreases following a storm event (urban stormwater flashiness) (Schueler 1994; Walsh et al., 2001; Brabec et al. 2002; Konrad and Booth, 2005; Roy et al., 2005; Walsh et al., 2005; Schueler et al., 2009; Booth et al., 2016). Along with the increased volume, there is increased erosive flow that erodes stream banks and scours the stream bed, eventually disconnecting the stream channel from the floodplain (Walsh et al., 2005; Coleman et al., 2011; Cappiella et al., 2012) as the homogenization of habitat in urban streams (Segura and Booth 2010). A number of studies have shown that the degradation of the benthic macroinvertebrate community in urban streams is related to the alterations in the natural hydraulic flow patterns observed in urban watersheds (Jones and Clark 1987; Clausen and Biggs 1997; Wang et al., 1997; Paul and Meyer 2001; Roy et al., 2001; Bunn and Arthington 2002; Morse et al., 2003; Roy et al., 2003a; Roy et al., 2003b; Allan 2004; DeGasperi et al., 2009; Kennen et al., 2010; Coleman et al., 2011; Kannan et al., 2018; Monk et al., 2018). Coleman et al. (2011) found that fish and benthic macroinvertebrate communities in Ohio streams were negatively impacted by the increased volume, frequency, and flashiness of urban stormwater runoff. Holomuzki and Biggs (2000) showed that high stream flows that are capable of mobilizing bed material can also dislodge and cause mortality to benthic macroinvertebrates. However, the degree to which the increased stormwater flow intensity and volume from urban areas impacts benthic macroinvertebrate assemblage's trait richness and diversity as well as stream ecosystem function is not well documented (Walsh et al. 2001; Roy et al. 2003; Gage et al. 2004; Wenger et al. 2009; Cuffney et al. 2010; O'Driscoll et al. 2010; King and Baker 2011; Schmera et al. 2017). These researchers report that most studies of the effects of urbanization on streams and benthic macroinvertebrate assemblage focus on the collective impact of increased stormwater runoff on taxa richness (Walsh et al. 2005) and do not specifically address the impact of stormwater on benthic macroinvertebrate taxa trait diversity and ecosystem function.

Richter et al. (1996) introduced a series of metrics to assess the alterations to the natural hydrologic regime referred to as the Indicators of Hydrologic Alteration (IHA). These metrics incorporate 33 parameters, organized into 5 groups, that statistically characterize alterations to the natural flow regime (Table 2.2). The IHA metrics were originally designed to measure the impact of impoundments on the flow regime. Recent work has suggested that the IHA metrics may be useful in assessing the degree of alteration of the natural flow patterns that are associated with the urbanization process (Kiesling 2003; Konrad and Booth 2005; Poff et al., 2006; DeGasperi et al., 2009; Poff and Zimmerman 2010; Turner and Stewardson 2014; Kannan et al., 2018). Olden and Poff (2003) examined many different hydrologic flow metrics and concluded that the IHA metrics covered all the major aspects of the flow regime.

**Table 2.2**. Hydrologic metrics used in the Indicators of Hydrologic Alteration (IHA) (adapted from Richter et al., 1996; Kiesling 2003). The asterisks indicate the metrics used in this study.

| **IHA Statistics Group** | **Regime Characteristics** | **Hydrologic attributes** |
|---|---|---|
| Group 1: Magnitude of monthly water conditions | Magnitude<br>Timing | Mean for each calendar month (median in this application) |
| Group 2: Magnitude and duration of annual extreme water conditions | Magnitude<br>Duration | * Annual minimums of 1-day means<br>* Annual maximums of 1-day means<br>Annual minimums of 3-day means<br>Annual maximums of 3-day means<br>Annual minimums of 7-day means<br>Annual maximums of 7-day means<br>Annual minimums of 30-day means<br>Annual maximums of 30-day means<br>* Annual minimums of 90-day means<br>* Annual maximums of 90-day means<br>* Base flow index |
| Group 3: Timing of annual extreme water conditions | Timing | * Julian date of each annual 1-day maximum<br>* Julian date of each annual 1-day minimum |
| Group 4: Frequency and duration of high- and low-flow pulses | Magnitude<br>Frequency<br>Duration | * Number of high-flow pulses each year<br>* Number of low-flow pulses each year<br>* Mean duration of high-flow pulses in each year<br>* Mean duration of low-flow pulses in each year |
| Group 5: Rate and frequency of water-condition changes | Frequency<br>Rate of change | Means of all positive differences between consecutive daily means |

| | | |
|---|---|---|
| | | Means of all negative differences between consecutive daily means<br>* Rise Rate<br>* Fall Rate |

Another measure of the alteration of the natural hydrologic regime due to urbanization is the Richards-Baker Flashiness Index (R-B Index; Baker et al., 2004). A storm event in an urbanized area can result in a stream storm hydrograph that has a relatively high peak discharge and rapid rising and receding limbs (Poff et al., 1997). This type of urban storm hydrograph is referred to as “flashy”. Mogollon et al. (2016) found that the R-B Index was a very effective metric to evaluate changes in streamflow over time due to impacts of urbanization. DeGasperi et al. (2009) found significant correlations between the R-B Index and total impervious cover and concluded that the R-B Index was an excellent metric to detect changes in stormwater runoff due to urbanization.

## 2.3 STUDY OBJECTIVES AND RESEARCH QUESTIONS

Benthic macroinvertebrate communities in urban streams face many challenges in maintaining diversity and function including stormwater volume and intensity, stormwater pollutants, changing climate conditions, and wastewater collection system failures. While it is well established that the urbanization process negatively impacts the biodiversity of benthic macroinvertebrates in urban streams, the impact on individual taxa, and on the diversity of taxa traits is not well known (Wenger et al., 2009). The Charlotte-Mecklenburg Storm Water Services (CMSWS) has been collecting benthic macroinvertebrate community data since 1994. The CMSWS benthic macroinvertebrate data can be expressed as taxa and trait richness and diversity. This unique long-term data set presents the opportunity to study the impact of land use changes on biodiversity and ecosystem function in stream ecosystems in watersheds that span a gradient of impervious cover and stream habitat conditions.

In this study, I examined the influence of land use changes, as seen through changes in percent impervious cover (percent IC), on stream benthic macroinvertebrate biodiversity and function in Piedmont streams in North Carolina.

**Q2.1. How do EPT Taxa and Trait Richness and Diversity change with increases in percent IC?** I hypothesized that the decline in EPT Taxa Richness and diversity would be greater than the decline in EPT Trait Richness and diversity with increasing percent IC.

**Q2.2. How will individual traits respond to increases in percent IC?** I hypothesized that individual trait responses would not be uniform and would vary by traits.

**Q2.3 How will the FFG traits change with increases in percent IC?** I hypothesized that there would be a decline in shredder richness and an increase in herbivore-scraper richness in response to increases in percent IC. I expected to see a shift in collector diversity from collector-filterers to collector-gatherers in response to increases in percent IC while no change in the Predator trait diversity is expected.

It is well documented that increases in percent IC associated with urbanization alters the natural hydrologic flow regime. In this study, I examined the usefulness of the Indicators of Hydrologic Alterations (IHA) metrics in evaluating the alterations of the hydrologic flow regime due to urbanization by focusing on the following question and hypothesis:

**Q2.4 Which Indicators of Hydrologic Alterations have greater impacts on EPT taxa and trait richness and diversity?** I hypothesized that the IHA metrics more closely associated with the flashiness of a storm event would have greater impacts on taxa and trait richness and diversity.

## 2.4 METHODOLOGY

### 2.4.1 STUDY SITES

This study utilized data collected by the Charlotte-Mecklenburg Storm Water Services (CMSWS) from 15 streams in Mecklenburg County, North Carolina, whose watersheds span a gradient of rural, suburban, and urban land use over a 26-year period (1994–2020). The streams were assigned to one of the following percent Impervious Cover (percent IC) groups: Low (<10% IC), Mid (10 to 25% IC), and High (>25% IC). These impervious cover groups are the same three impervious groups used by Schueler (1994), Exum et al. (2005), and Shuster et al. (2005). The three percent IC groups represent a broad range of percent IC coverage in Mecklenburg County and enabled the selection of at least 3 watersheds per group (Figure 2.1; Table 2.3). The placement of each stream into a percent IC group was based on the percent IC of each watershed upstream of the study site using the 2016 USDA GIS Land Use/Land Cover (LULC) dataset for Mecklenburg County, North Carolina. GIS LULC data were used to determine the percent IC for each of the 15 watersheds for 1992, 2001, 2006, 2008 through 2014, 2016, and 2019, the years for which GIS LULC data are available for Mecklenburg County. The 1992, 2001, and 2006 percent IC were calculated using National Land Cover Datasets (NLCD) and the 2008 through 2019 percent IC were calculated using the USDA LULC datasets (1992–2014 LULC was calculated by Ms. Jing Deng, CAGIS, UNC-Charlotte; 2016 and 2019 LULC was calculated by James Scanlon, CMSWS GIS).

The watersheds for each study site ranged from 3.5 mi$^2$ (Gar Creek, MC50) to 38.6 mi$^2$ (McAlpine Creek, MC38; Table 2.3). In 2019, percent IC ranged from 5.6 to 80.6 percent IC. Several watersheds of the study sites underwent extensive development between 1994 and 2014. For example, the percent IC in the Reedy Creek watershed above MY13 increased from 4.7% to

16.2%, McKee Creek increased from 2.3% to 16.0% above MY7B, and McDowell Creek increased from 4.2% to 32.6% above MC4. In contrast, the percent IC in streams that were urban in 1992, such as Little Sugar, Briar, and McMullen Creeks, did not increase greatly. For example, the percent IC in the Briar Creek watershed above MC33 increased from 34.6% to 48.5% and Little Sugar Creek increased from 59.1% to 78.2% above MC 29A1. A USGS stream gage is located at each of these stream sites from which stream discharge and gage height data were obtained (Table 2.3).

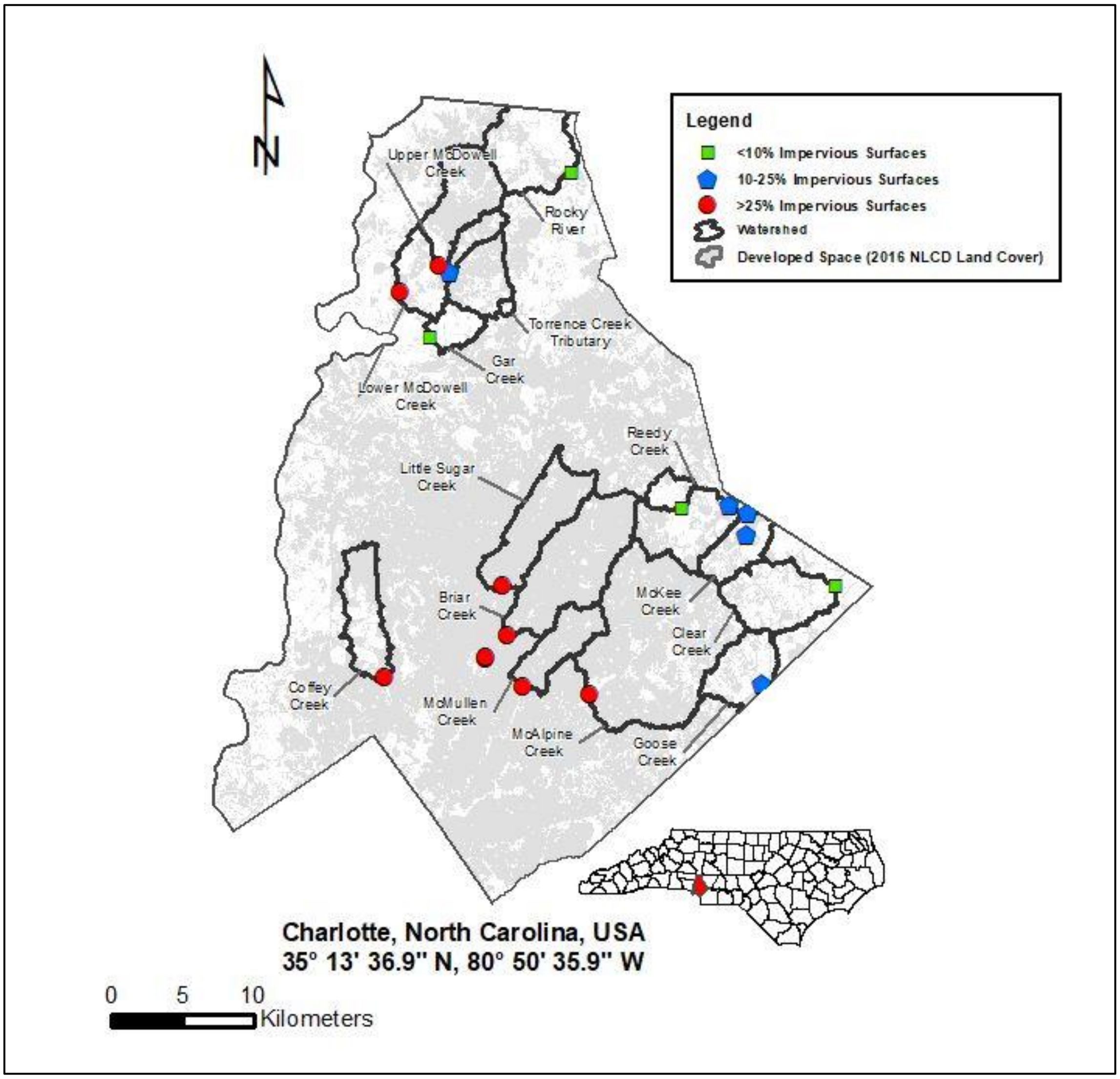

**Figure 2.1**. Map of Stream Assessment Sites in Mecklenburg County in Piedmont North Carolina. *% Impervious Cover Conditions*: Low (<10% IC) – Green; Mid (10% to 25% IC) – Orange; and High (>25% IC) – Red.

**Table 2.3**. Stream Sites in Mecklenburg County, North Carolina. 4 sites were relocated by CMCWS in 2002 to a nearby location with a USGS stream gage (Goose Creek site MY9B to MY9; McKee Creek site MY7 to MY7B; Briar Creek site MC31 to MC33; Little Sugar Creek site MC29 to MC29A1).

| Stream | Site | Drainage Area (mi$^2$) | 1992 % Impervious Cover | 2019 % Impervious Cover | USGS Stream Gage | Latitude | Longitude |
|---|---|---|---|---|---|---|---|
| **Gar Cr** | MC50 | 3.5 | 1.3 | 5.6 | 0214266080 | 35.3615 | -80.8975 |
| **Reedy Cr** | MY13A | 2.53 | 0.7 | 9.1 | 0212427947 | 35.2562 | -80.7006 |
| **W Rocky R** | MY1B | 20.7 | 2.0 | 15.3 | 0212393300 | 35.4678 | -80.7903 |
| **Clear Cr** | MY8 | 12.5 | 1.3 | 11.8 | 0212466000 | 35.2082 | -80.5798 |
| **Goose Cr** | MY9B | 3.09 | ǂ | ǂ |  | 35.1455 | -80.6358 |
| **Goose Cr** | MY9 | 8.7 | 2.2 | 17.3 | 0212467451 | 35.1304 | -80.6312 |
| **McKee Cr** | MY7 | 4.1 | 3.2 | ǂ |  | 35.2401 | -80.6492 |
| **McKee Cr** | MY7B | 5.8 | 2.3 | 25.9 | 0212430653 | 35.2539 | -80.6480 |
| **Reedy Cr** | MY13 | 12.7 | 4.7 | 19.6 | 0212430293 | 35.2586 | -80.6628 |
| **Torrence Cr** | MC3E | 7.3 | 15.4 | 42.3 | 0214265808 | 35.4036 | -80.8828 |
| **Coffey Cr** | MC25 | 8.7 | 11.6 | 46.3 | 02146348 | 35.1455 | -80.9267 |
| **McDowell Cr** | MC4 | 26.5 | 4.2 | 42.4 | 0214266000 | 35.3896 | -80.9210 |
| **McDowell** | MC2A1 | 10.2 | 4.8 | 53.6 | 02142654 | 35.4070 | -80.8909 |
| **McAlpine Cr** | MC38 | 38.6 | 19.2 | 42.5 | 02146600 | 35.1373 | -80.7681 |
| **Briar Cr** | MC31 | 21.6 | 33.5 | ǂ |  | 35.1583 | -80.8487 |
| **Briar Cr** | MC33 | 18.9 | 34.6 | 54.8 | 0214645022 | 35.1739 | -80.8330 |
| **McMullen Cr** | MC42 | 7.1 | 26.7 | 45.7 | 02146700 | 35.1409 | -80.8200 |
| **Little Sugar Cr** | MC29 | 15.9 | 52.9 | ǂ |  | 35.1600 | -80.8489 |
| **Little Sugar Cr** | MC29A1 | 12.0 | 59.1 | 80.6 | 02146409 | 35.2046 | -80.8370 |

ǂ - impervious cover data for nearby site was used for samples collected. It was assumed that by being close to the nearby site that the % IC of these sites would not be significantly different.

### 2.4.2 BENTHIC MACROINVERTEBRATES

The CMSWS has monitored stream benthic macroinvertebrates in streams throughout Charlotte and Mecklenburg County, North Carolina, since 1994. As an employee of CMSWS, I supervised the collection and identification of the benthic macroinvertebrates, as well as the annual training of CMSWS staff that conducted the collections and identifications. The benthic macroinvertebrates were collected from a 100-meter segment at each site using the Standard Qualitative Method developed by North Carolina Department of Environmental Quality (NCDEQ) Biological Assessment Unit (NCDEQ 2016) and described in the Charlotte-Mecklenburg Stormwater Services Bioassessment Standard Operating Procedures (CMSWS 2017). The Standard Qualitative Method collections consists of 2 kick net riffle samples, 3 sweep-net samples from microhabitats found within runs, glides, and pool such as root wads, soft sediment in undercut bank areas, woody debris, macrophyte beds, and overhanging vegetation, 1 leaf-pack sample, 2 rock and/or log wash samples, and visual collections. The benthic macroinvertebrates in these samples were sorted in the field and preserved in glass vials containing 95% ethanol. All benthic macroinvertebrates were identified to the lowest possible taxonomic level (Taxa list is in Appendix Table A1).

### 2.4.3 BACKGROUND ENVIRONMENTAL PARAMETERS

During the bioassessment sampling at each stream site, field measurements of conductivity, dissolved oxygen (DO), pH, and temperature were taken using a YSI Pro DSS hand-held multi-probe meter.

### 2.4.4 STREAM HABITAT ASSESSMENT

CMSWS has been evaluating stream habitat conditions since 2000 using the Mecklenburg Stream Habitat Assessment Protocols (MHAP) which were based on the USEPA Rapid Bioassessment Protocols (Barbour et al., 1999). The MHAP evaluates 10 habitat condition parameters including instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, frequency of riffles, channel flow status, bank vegetation protection, bank stability, and vegetative riparian zone width (Appendix, Table A2). From 2000 through 2014, the MHAP protocol was conducted by 3 CMSWS assessors following the *visual* approach described by Barbour et al. (1999). The scores of each assessor were averaged to produce a final score for each parameter which were added for a final MHAP score (200 maximum). In 2015, CMSWS modified the MHAP procedure (EMHAP; CMSWS 2020a; CMSWS 2020b) to reduce the high variability observed in the data attributed to the visual approach. Several of the parameters were modified to include field counts and measurements that proved to be both consistent with the visual MHAP approach and reduced the high variability observed in the visual MHAP submetric scores (personal observations; CMSWS 2020a; CMSWS 2020b). The EMHAP scores have been associated with stream habitat conditions ranging from degraded (<60) to fully supporting (≥160). In this study, the terms MHAP and EMHAP are used interchangeably as either method produced equivalent results (personal observations). This analysis gives a broad picture of the impact of urbanization on stream habitat conditions and functional trait diversity.

### 2.4.5 HYDROLOGY

A USGS stream gage is located at each of the 15 stream sites from which stream discharge and gage height data were obtained (Table 2.3). Daily flow data for the 15 streams were downloaded from the USGS website. The IHA metrics that were calculated using the IHA software developed by The Nature Conservancy (The Nature Conservancy 2009). Linear regressions were conducted by the IHA software. Eighteen IHA metrics were identified as having the highest number of significant results within the 15 sites were initially selected to be used in this study. These significant metrics were then reduced to 13 by taking the top 4 or 5 metrics in groups 2, 3, 4, and 5. Analyses focused on Group 2 metrics, annual 1- and 90-day maximums and minimum flows, and base flow index, Group 3 metrics, dates of minimum and maximum flows, Group 4 metrics, the number and duration of high-flow and low-flow pulses in each year, and high and low pulse duration, and Group 5 metrics, fall rate and rise rate (Table 2.2). Alterations in flow magnitude and frequency measured by these metrics have been shown to have the potential to modify the habitat critical for fish and benthic macroinvertebrates (Kiesling 2003; Konrad and Booth 2005; Poff et al., 2006; DeGasperi et al., 2009; Onwuka et al., 2021).

The R-B Index was calculated for each year using daily streamflow data to determine changes in flow from one day to the next ($q_i$ - $q_{i-1}$) divided by the total daily average flow using equation 2.1 (Baker et al., 2004). Larger daily changes in flow results in higher R-B Index values indicating flashy flow conditions while more stable flows will have values closer to zero. Daily flow data for the 15 streams were downloaded from the Water Quality Portal (https://www.waterqualitydata.us/) separately for each year from 1994 through 2020 using the *dataRetrieval* function in the United States Geological Survey ‘dataRetrieval’ package in R.

**Equation 2.1**

$$R - B\ Index = \frac{\sum_{i=1}^{n} |q_i - q_{i-1}|}{\sum_{i=1}^{n} q_i}$$

## 2.5 DATA ANALYSIS

### 2.5.1 STREAM HABITAT CONDITION AND PERCENT IMPERVIOUS COVER

Each stream site was assigned an impervious cover group based on the percent IC of the stream’s watershed above the study reach in 2016. These groups are Low IC (<10% IC), Mid IC (10–25% IC), and High IC (>25% IC). These percent IC groups are similar to the IC groups used in previous studies (Schueler 1994; Exum et al., 2005; Shuster et al., 2005).

The relationship between stream habitat conditions as measured by the MHAP and percent IC in the 15 study streams (grouped by Low-, Mid-, and High-percent IC) were compared. The Shapiro-Wilk Normality test indicated that both the MHAP and percent IC data sets did not have normal distributions. Therefore, I used the Kruskal-Wallis rank sum test to test the null hypothesis that the MHAP scores were the same in each percent IC group (kruskal.test function in the 'stats' package in R; R Core Team, 2020). The Dunn Test for multiple comparisons was used to evaluate pairwise comparisons between each percent IC group to identify any significant interactions (*dunnTest* function in 'FSA' package in R; Ogle et al., 2020). To examine how MHAP and percent IC change with respect to each other, a correlation analysis was conducted on MHAP and percent IC (*cor.test* function in the 'stats' package in R; R Core Team, 2020).

### 2.5.2 BENTHIC MACROINVERTEBRATES

For this study, I focused on the benthic macroinvertebrate pollution sensitive aquatic insect orders, Ephemeroptera, Plecoptera, and Trichoptera (EPT). I evaluated all EPT data for normality using the Shapiro-Wilk Normality test and found that the EPT taxa and trait richness and trait diversity data were significantly different from normal distribution. The EPT taxa diversity data were not significantly different from normal distribution. Therefore, I chose to use non-parametric statistics to evaluate differences among groups of parameters. I used non-metric multidimensional scaling (NMDS) analysis to evaluate the relationship of EPT taxa and trait distribution among streams in different percent IC groups with varying MHAP scores, percent IC, and water chemistry (*metaMDS* function in the 'vegan' package in R; Oksanen et al., 2020). EPT taxa and trait diversity were calculated as Shannon-Wiener Diversity (H′) indices (*diversity* function in the 'vegan' package in R; Oksanen et al., 2020).

I used the Kruskal-Wallis rank sum test to test each null hypothesis that the EPT taxa and trait richness and diversity data sets, respectively, were identical in each percent IC group. The Dunn Test for multiple comparisons was used to evaluate pairwise comparisons between each percent IC group to identify any significant interactions.

As a watershed is developed, the amount and severity of negative impacts predicted by the urban stream syndrome increases. To determine if there are EPT taxa and traits that can serve as indicators of habitat condition or watershed percent IC, the *multipatt* function in the 'indicspecies' package in R (version 1.7.12; De Cáceres and Legendre 2009) was utilized on the EPT taxa and trait data sets with the MHAP scores and with the percent IC descriptors for each site. The presence of ecological thresholds along the environmental gradients, percent IC and steam habitat condition, at which changes in the EPT taxa and trait assemblages occurred were determined using the **T**hreshold **I**ndicator **T**axa **AN**alysis (*titan* function in the 'TITAN2' package in R; Baker and King 2010).

### 2.5.3 IHA/MULTIPLE LINEAR REGRESSION

To investigate the relationship between EPT taxa and trait richness and diversity, and habitat (MHAP scores), percent IC, and the 13 indicators of hydrologic alterations calculated using the IHA software, I used the Kendall Rank Correlation Test (*cor.test* function in the 'stats' package in R; R Core Team, 2020). To identify which determinate variables had the greatest contribution to EPT taxa and trait richness and diversity, I used multiple linear regression. A stepwise approach was performed using the *lm* function in the 'stats' package in R (R Core Team, 2020).

The R-B Index was calculated for each site on the annual daily USGS flow data downloaded from the from the Water Quality Portal (https://www.waterqualitydata.us/) using the

*RBIcalc* function in the ‘ContDataQC’ package in R (Leppo, 2022). The relationship between R-B Index values and percent IC in the 15 study streams (grouped by Low-, Mid-, and High-percent IC) were compared. The Shapiro-Wilk Normality test indicated that the R-B Index data set did not have normal distributions. Therefore, I used the Kruskal-Wallis rank sum test to test the null hypothesis that the R-B Index values were the same in each percent IC group (kruskal.test function in the ‘stats’ package in R; R Core Team, 2020). The Dunn Test for multiple comparisons was used to evaluate pairwise comparisons between each percent IC group to identify any significant interactions (*dunnTest* function in ‘FSA’ package in R; Ogle et al., 2020). To examine the relationship between R-B Index and percent IC, a correlation analysis was conducted on R-B Index and percent IC (*cor.test* function in the ‘stats’ package in R; R Core Team, 2020).

## 2.6 RESULTS

### 2.6.1 STREAM HABITAT CONDITION

Stream habitat quality declined as percent IC increased with development (Figure 2.2). When comparing streams grouped by percent IC (Low IC <10%; Mid IC 10–25%; High IC >25%), MHAP scores were significantly different among the IC Stream groups (Kruskal-Wallis Rank Sum Test $p<0.05$; Figure 2.3). The instream habitat MHAP scores of the Low IC streams were significantly higher than those of the streams with percent IC > 10% (Dunn Test for Kruskal-Wallis multiple comparison $p<0.05$; Figure 2.3). There was no significant difference in the MHAP scores of both stream groups with percent IC >10%. MHAP was significantly negatively correlated with percent IC ($r=-0.141$, $p<0.005$).

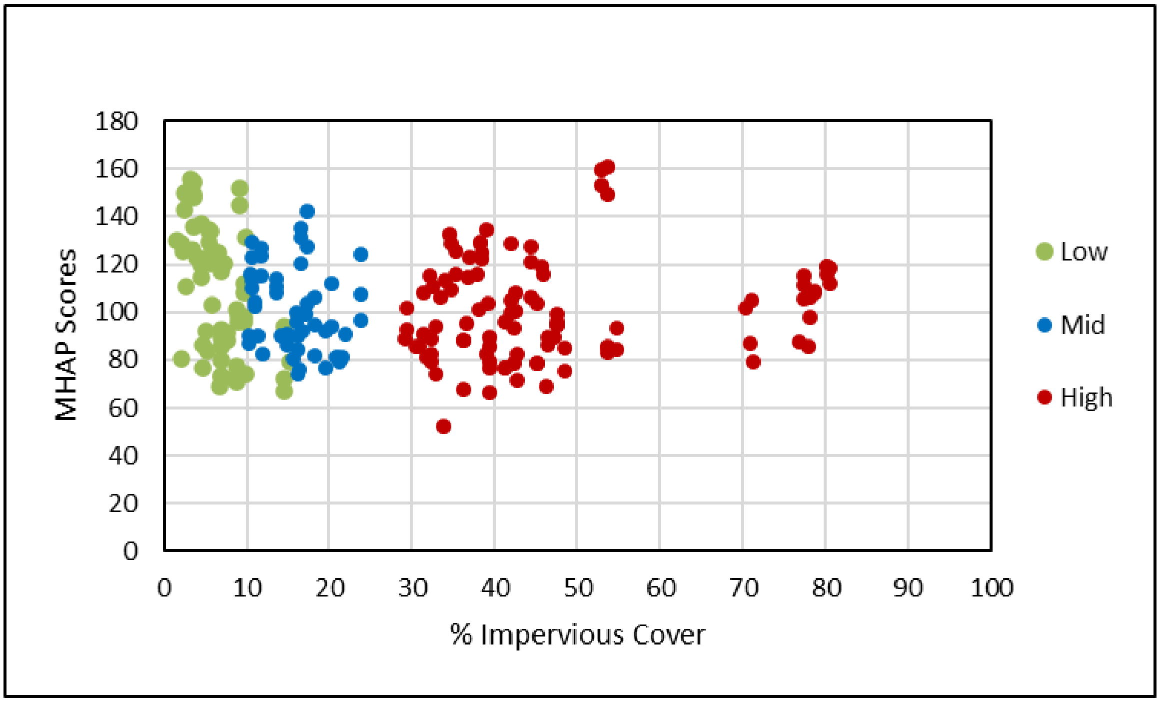

**Figure 2.2**. Changes in MHAP scores with percent impervious cover for all sites (N=15) and years 1994–2020. Sites were grouped by percent impervious cover where Low < 10%, Mid 10–25%, and High >25%. Stream habitat condition declined as percent impervious cover increased with development.

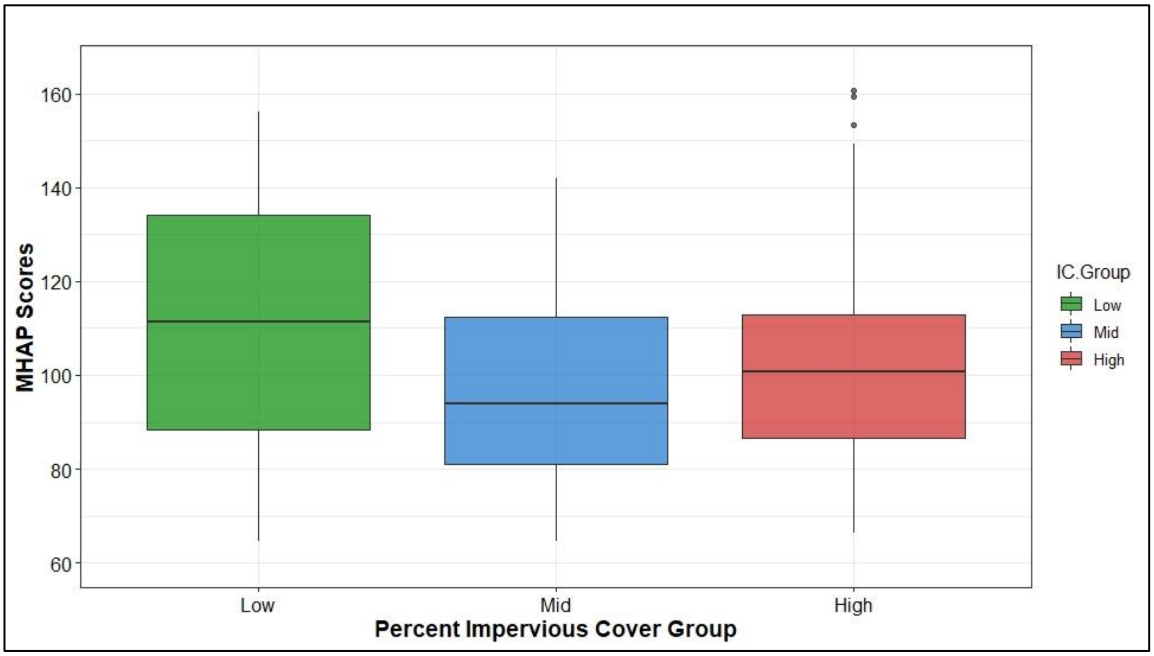

**Figure 2.3**. The MHAP scores for Low percent IC streams were significantly higher than the MHAP scores for streams with percent IC>10 (Dunn Test for Kruskal-Wallis multiple comparison $p<0.05$). (N=119 for High percent IC sites; 68 for Mid percent IC sites; 60 for Low percent IC sites).

### 2.6.2 TAXA AND TRAIT RICHNESS AND DIVERSITY

NMDS plots of EPT taxa and traits by percent IC groups, Low IC (<10% IC), Mid IC (10–25% IC), and High IC (>25% IC), showed that EPT taxa and traits assemblages in the Low percent IC and High percent IC streams were different (Figure 2.4 and 2.5). EPT taxa and trait assemblages in the Mid percent IC streams overlap both the Low percent IC and High percent IC streams. EPT taxa assemblages were impacted by MHAP and percent IC. Conductivity and temperature also influenced the taxa assemblages. EPT taxa NMDS1 axis can be explained by percent IC and temperature while NMDS2 axis can be explained by MHAP and conductivity. EPT trait assemblages were also driven inversely by MHAP and percent IC. Conductivity, DO, and temperature also influenced the trait assemblages. EPT trait NMDS1 axis can be partially explained by percent IC and DO. EPT trait NMDS2 can be explained by percent IC, MHAP temperature, and conductivity.

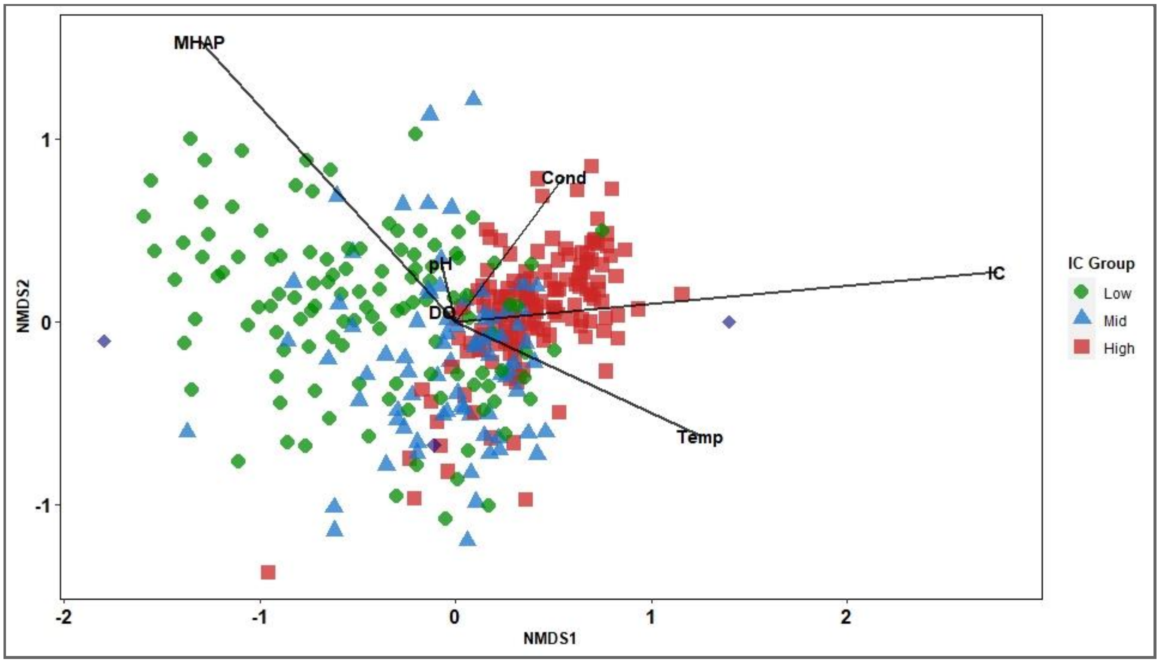

**Figure 2.4**. NMDS plot shows that EPT taxa assemblages are different in the low (green), mid (blue), and high (red) percent impervious cover (IC) streams (Stress = 0.22; goodness of fit r2=0.313 α<0.001).

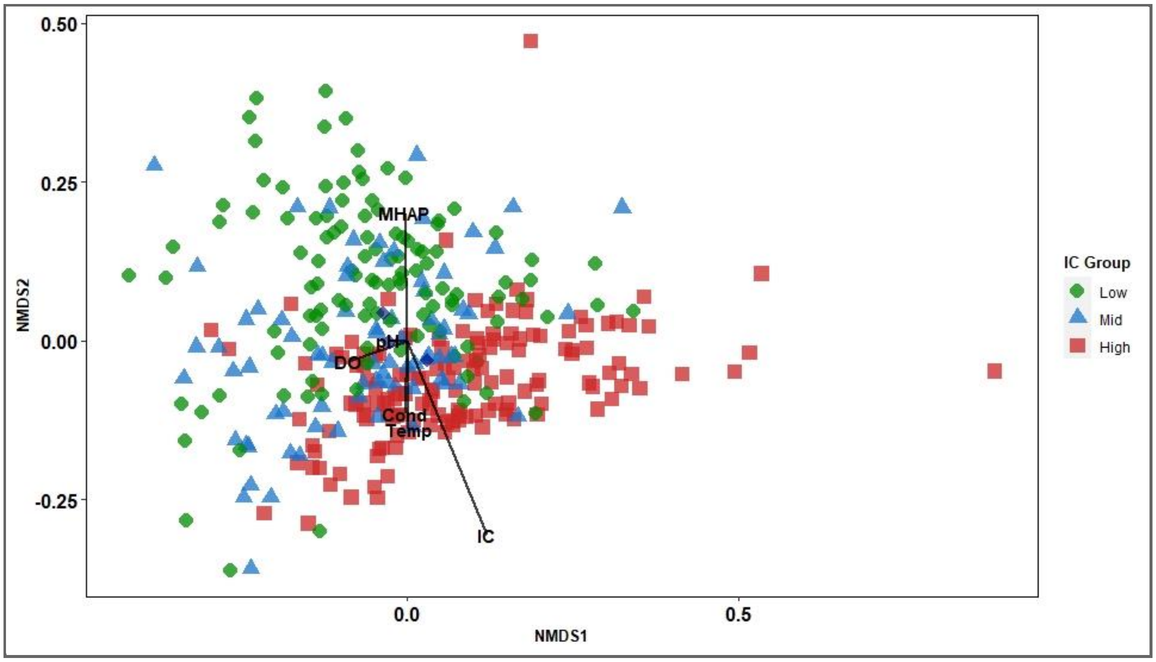

**Figure 2.5**. NMDS plot shows that EPT trait assemblages in High IC (red) streams are different from the EPT trait assemblages found in the Low IC (green) streams (Stress = 0.17; goodness of fit $r^2$=0.170 α<0.001).

Both EPT taxa richness and diversity declined with increasing percent impervious cover (percent IC) at percent IC as low as 5% (Figures 2.6 and 2.7). EPT trait richness and diversity also declined with increasing percent IC, but at a lesser degree than EPT taxa richness and diversity, with trait richness ranging from 53 to 35 traits (Figure 2.6). EPT trait diversity did not change with increasing percent IC (Figure 2.7).

Kruskal-Wallis Rank Sum Tests on EPT taxa and trait richness and diversity by percent IC Stream Groups showed that EPT taxa and trait richness and diversity were significantly different among the percent IC stream groups (Figures 2.8–2.11; EPT taxa richness p<0.001; taxa diversity p<0.001; trait richness p<0.001; trait diversity p<0.001). EPT taxa richness and diversity and trait richness were significantly higher in the Low IC streams (Dunn Test for multiple comparison p<0.001). EPT trait diversity in the Low IC streams were significantly higher than streams with >10% IC (Dunn Test p<0.01) while there was no significant difference in trait diversity between the Mid and High IC streams.

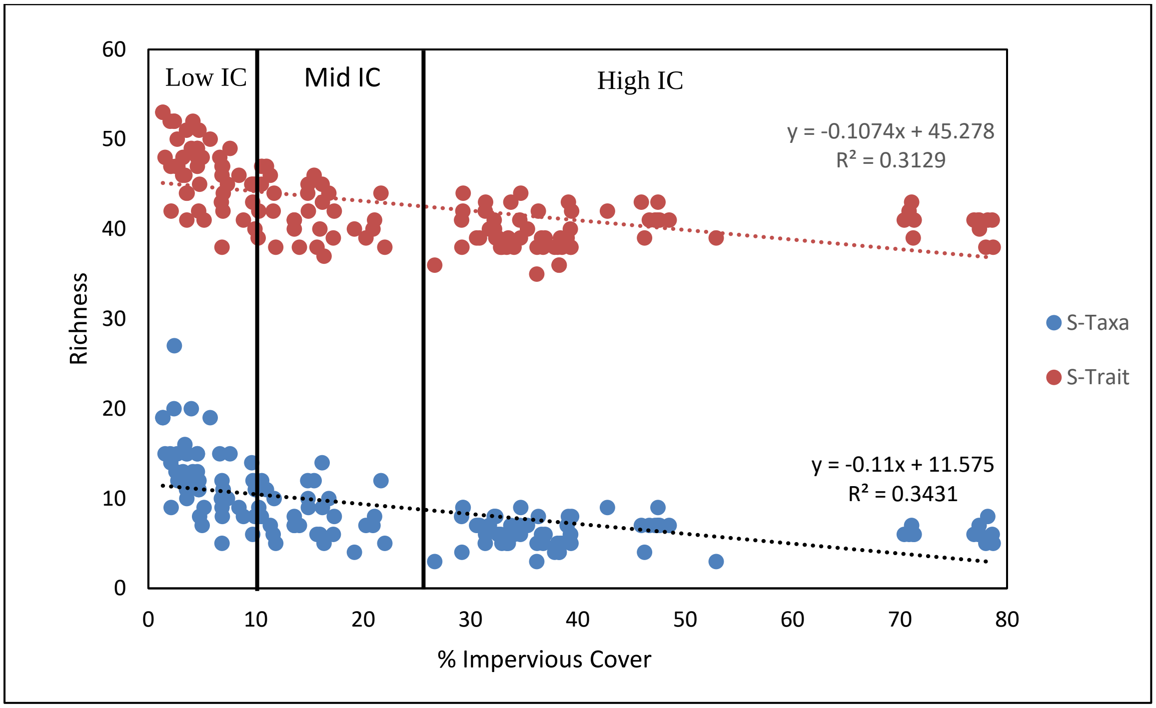

**Figure 2.6**. EPT taxa and trait richness declined as early as 5% impervious cover. Taxa richness declined greater than trait richness ranging from 27 to 3 taxa while trait richness ranged from 53 to 35 traits.

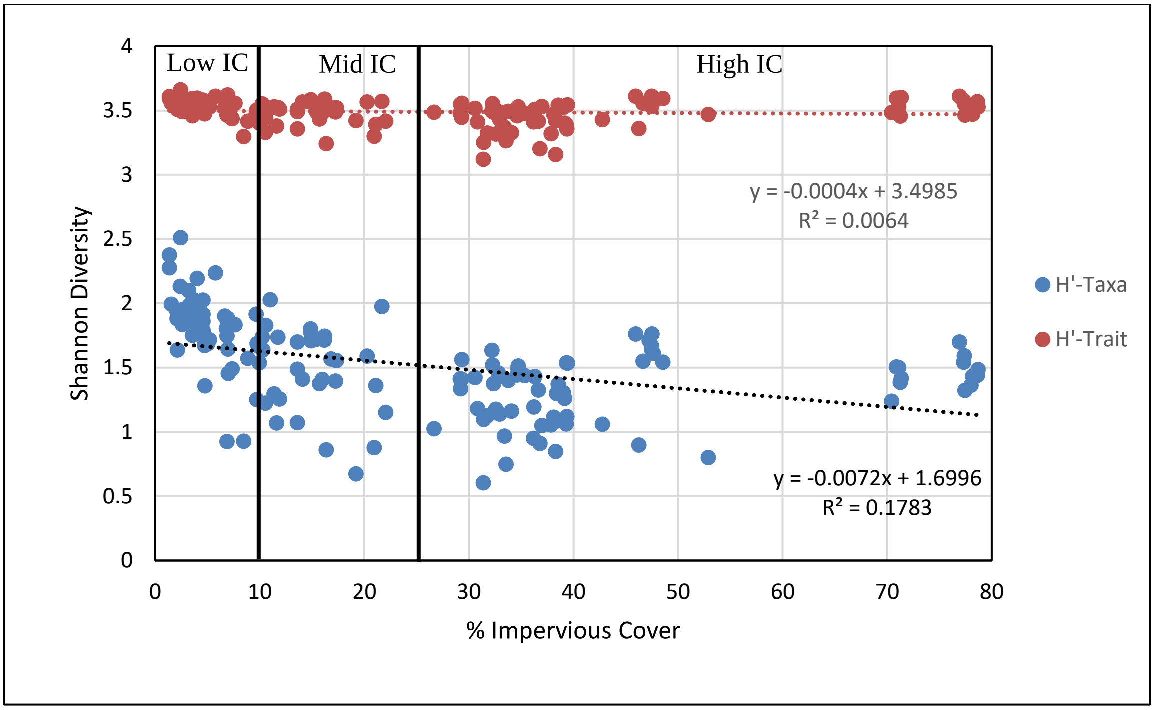

**Figure 2.7**. EPT taxa diversity declined as early as 5% impervious cover. Taxa diversity declined greater than trait diversity ranging from 2.5 to 0.6 taxa while trait diversity changed very little.

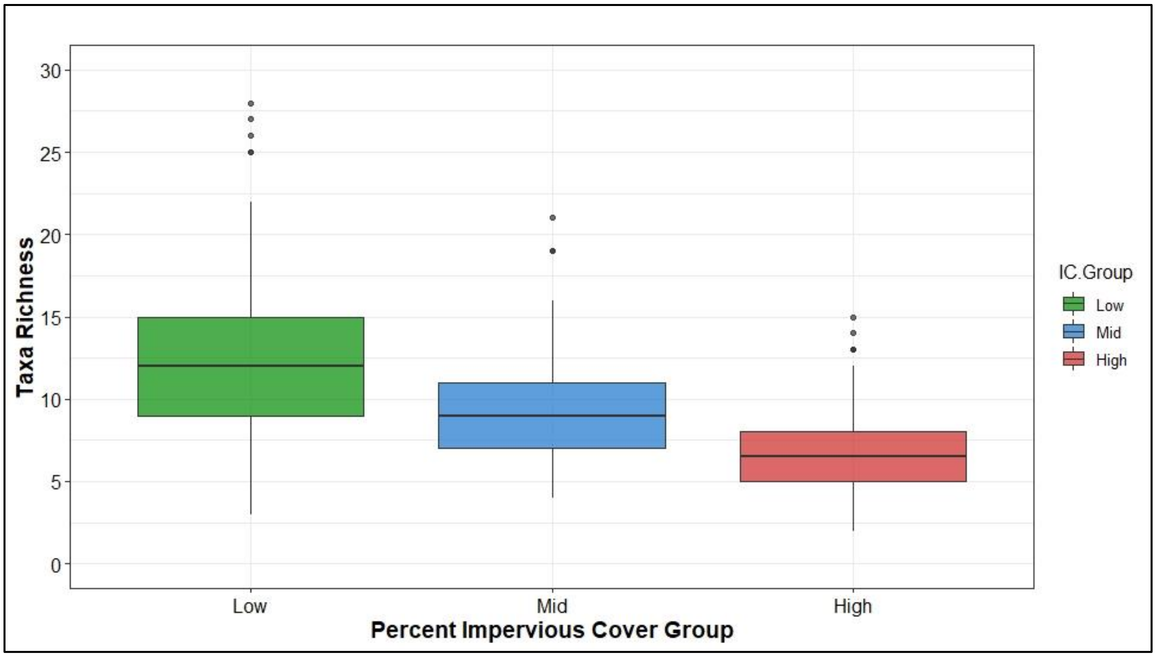

**Figure 2.8**. Box plots of EPT taxa richness in each percent IC stream group. EPT taxa richness in the Low IC (<10% IC) streams was significantly higher than in streams with IC > 10% (Dunn Test for multiple comparison $p<0.001$). Taxa richness was significantly different between the Mid and High IC streams.

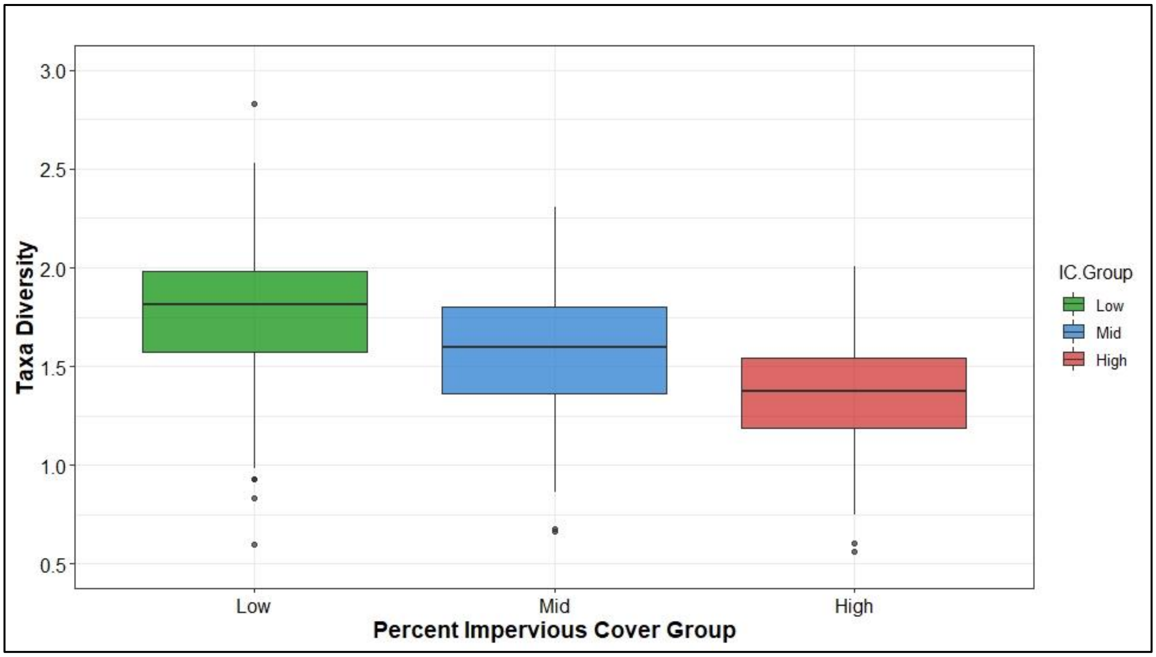

**Figure 2.9**. Box plots of EPT taxa diversity in each percent IC stream group. EPT taxa diversity in the Low IC (<10% IC) streams was significantly higher than in streams with IC > 10% (Dunn Test for multiple comparison p<0.001). Taxa diversity was significantly different between the Mid and High IC streams.

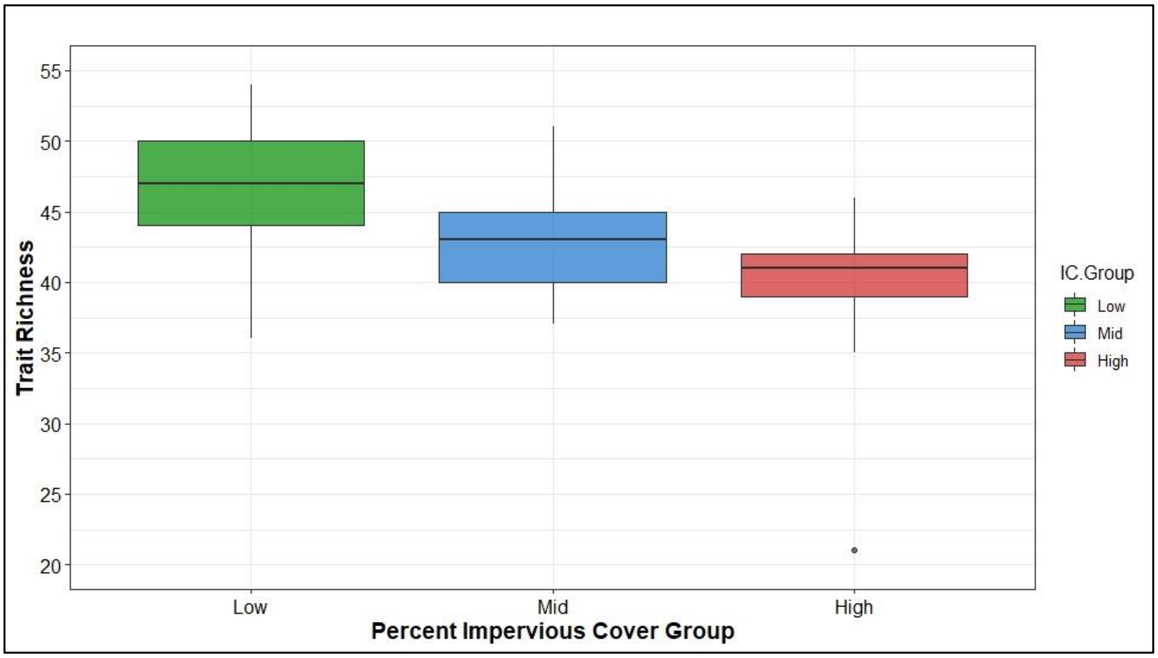

**Figure 2.10**. Box plots of EPT trait richness in each percent IC stream group. EPT trait richness in the Low IC (<10% IC) streams was significantly higher than in streams with IC > 10% (Dunn Test for multiple comparison p<0.001). Trait richness was significantly different between the Mid and High IC streams.

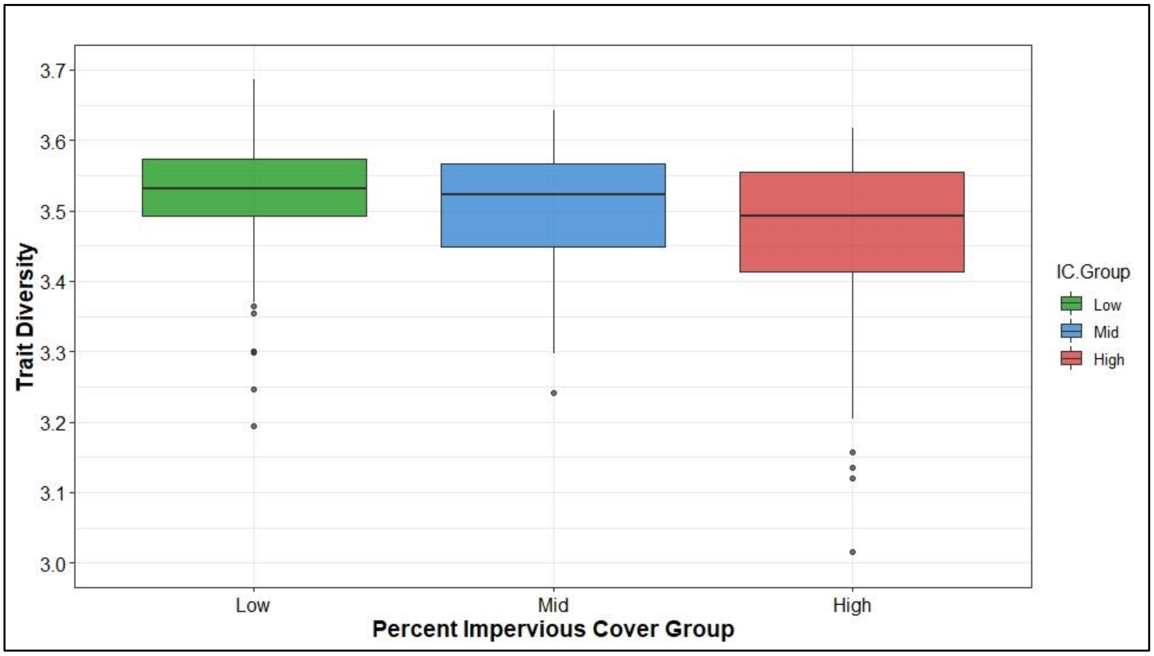

**Figure 2.11**. Box plots of EPT trait diversity in each percent IC stream group. EPT trait diversity in the Low IC (<10% IC) streams was significantly higher than in streams with IC > 10% (Dunn Test for multiple comparison p<0.01). Trait diversity was not significantly different between the Mid and High IC streams.

### 2.6.3 INDICATOR SPECIES

MHAP categories were not good indicators of EPT taxa. Out of 101 EPT taxa, only 2 taxa, *Paracloeodes fleeki* McCafferty and Lenat 2004 (supporting) and *Neophylax atlanta* Ross 1947 (partially supporting), were identified as indicator taxa (Appendix Table A3). Both taxa were rarely found (9 total *Paracloeodes fleeki* organisms at 7 sites and 24 total *Neophylax atlanta* organisms at 5 sites), making these taxa indicator species doubtful. There were no indicator traits identified for MHAP categories.

Three EPT taxa, *Hydropsyche betteni* Ross 1938, *Hydroptila* Dalman 1819., and *Baetis flavistriga* McDunnough 1921, were identified as indicator taxa for High percent IC (Figure 2.12; Appendix Table A4) streams while 40 EPT taxa were found to be indicators of Low percent IC streams and 5 EPT taxa, including *Baetis* Leach 1815, *Acentrella nadineae*

McCafferty, Waltz, and Webb 2009, *Triaenodes ignitus* (Walker 1852), *Oecetis persimilis* (Banks 1907), and *Mystacides sepulchralis* (Walker 1852), were found to be indicators of Mid percent IC streams. The top 4 Low percent IC indicator EPT taxa are shown in Figure 2.13. One EPT taxon was found to be an indicator of both High and Mid percent IC streams and three EPT taxa were found to be indicators of both Mid and Low percent IC streams (Figure 2.14; 1 taxon from each combination group shown).

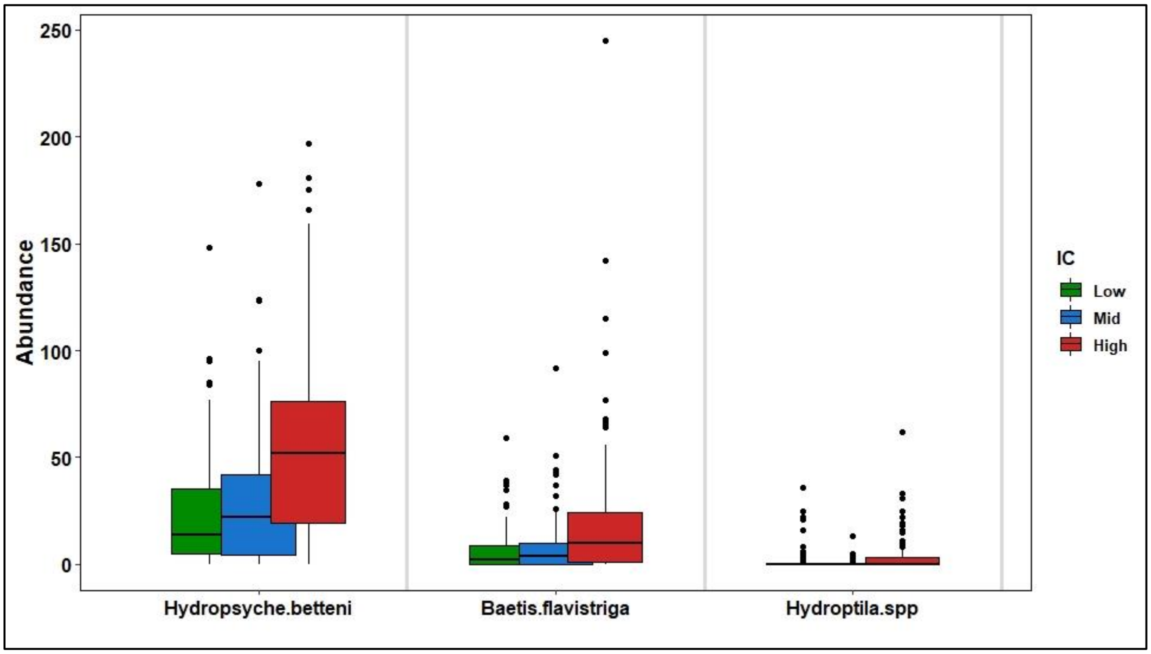

**Figure 2.12**. Three EPT taxa were identified by the Indicator Species Analysis as indicators of High percent IC urban streams. These EPT taxa are commonly found in urban streams in the Piedmont region of North Carolina.

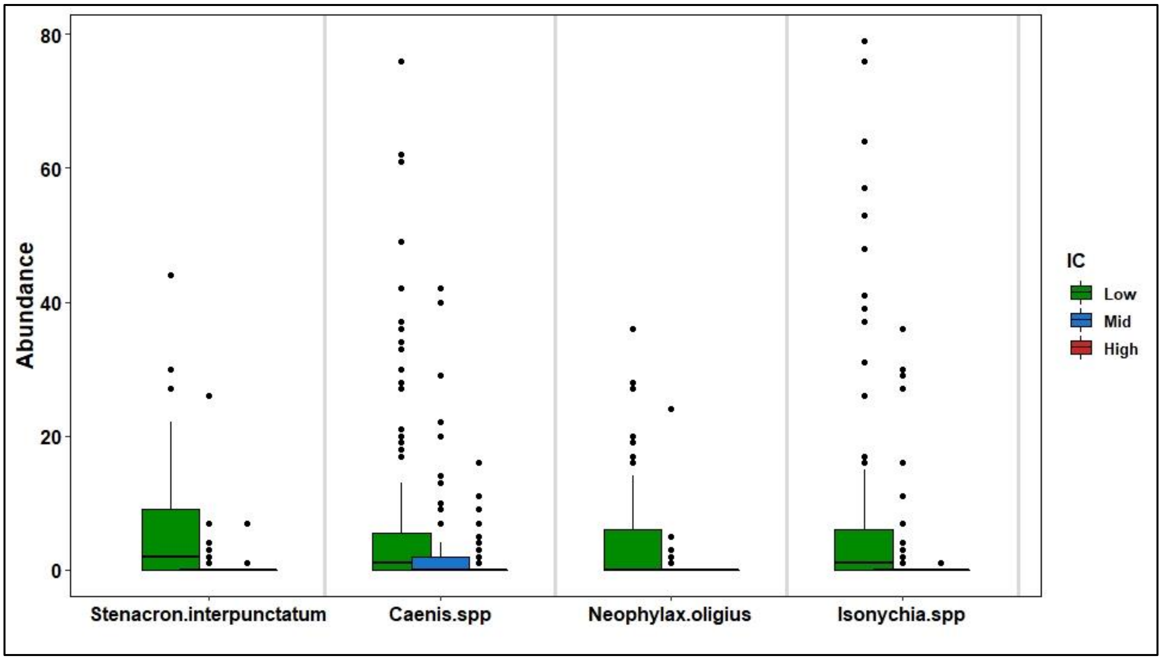

**Figure 2.13**. Top 4 of 40 taxa identified by the Indicator Species Analysis as indicator EPT taxa found in Piedmont streams with less than 10% IC.

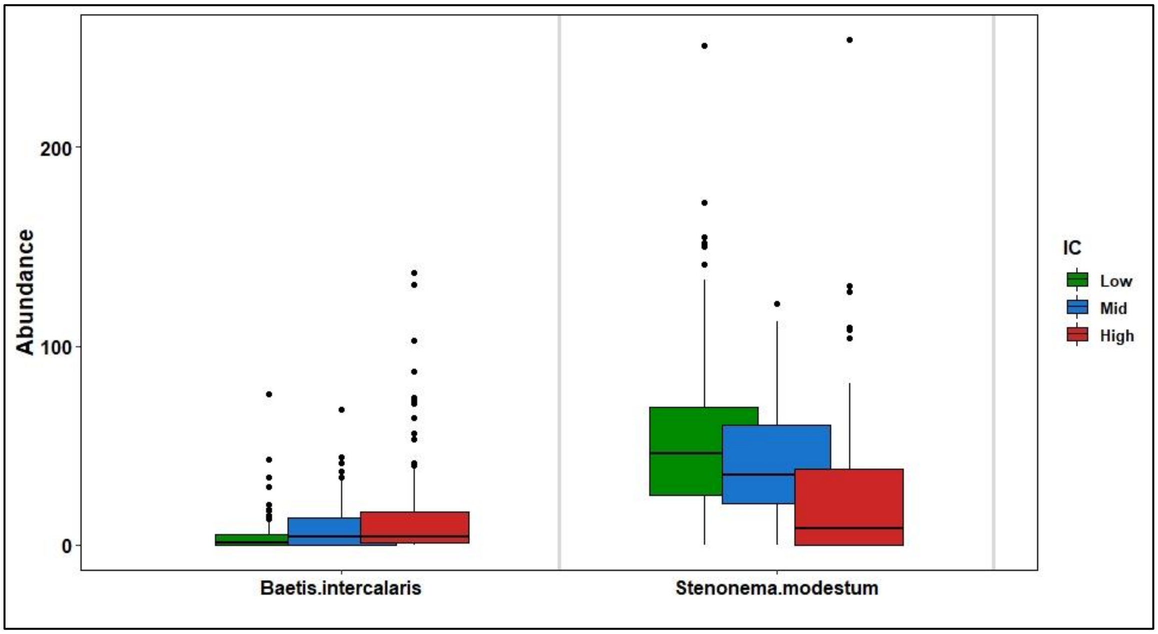

**Figure 2.14**. EPT taxa that the Indicator Species Analysis identified as indicators of multiple percent IC groups. *Baetis intercalaris* McDunnough 1921 was found to be an indicator of both High and Mid IC streams and *Stenonema modestum* (Banks 1910) was an indicator of both Low and Mid percent IC streams.

Two traits, adult ability to exit present (EPR) and abundant occurrence in drift (ADRF), were identified as indicators of High percent IC streams (Figure 2.15; Appendix Table A1) while 38 traits were found to be indicators of Low percent IC. The top 4 Low percent IC indicator traits are shown in Figure 2.16. One trait, climbing habit (CLB), was found to be an indicator in Mid percent IC streams. Six traits, including preference for erosional rheophily (ERO), nonswimmer (NSW), strong flying ability (SFLY), collector-filter (CF), high female dispersal (HDID), and some attachment (SATT), were found to be indicators of both High and Low percent IC streams (Figure 2.17). Two traits, herbivore-scraper (HB) and preference for depositional rheophily (DEP), were found to be associated with both Mid and Low percent IC streams. Collector-filterers (CF), collector-gatherers (CG), and herbivore-scrapers (HB) were the most abundant of the functional feeding groups (Figure2.18).

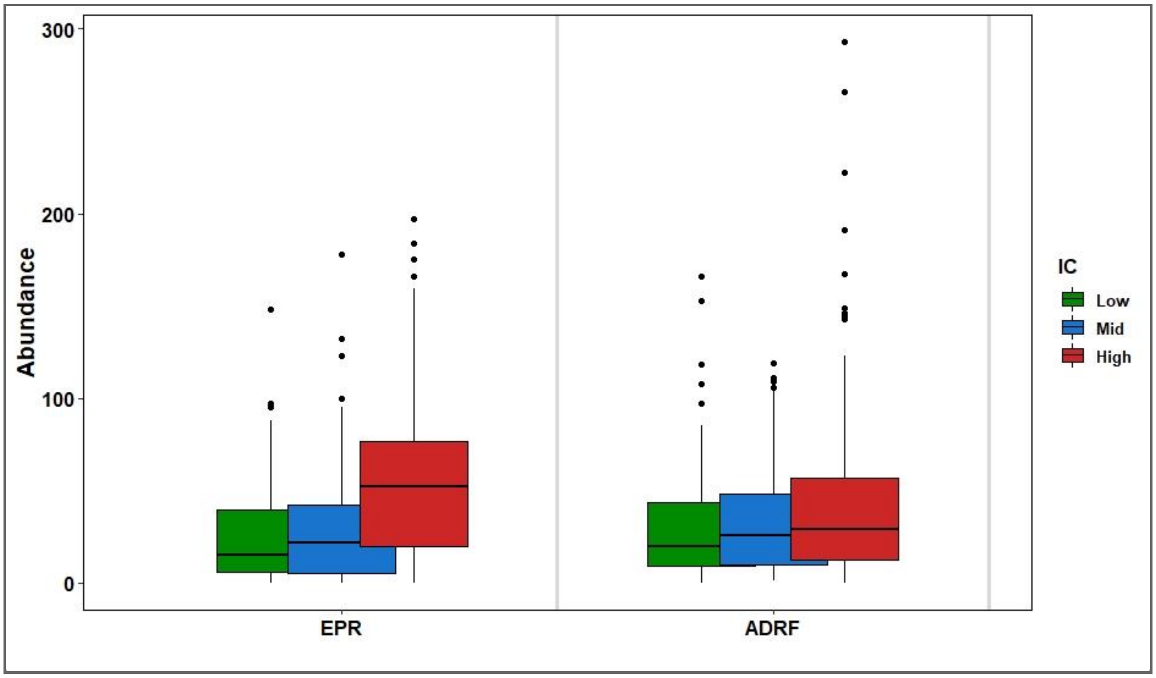

**Figure 2.15**. The Indicator Species Analysis identified 2 traits to be indicators of High percent IC urban streams. (EPR – Adult ability to exit present; ADRF – abundant occurrence in drift)

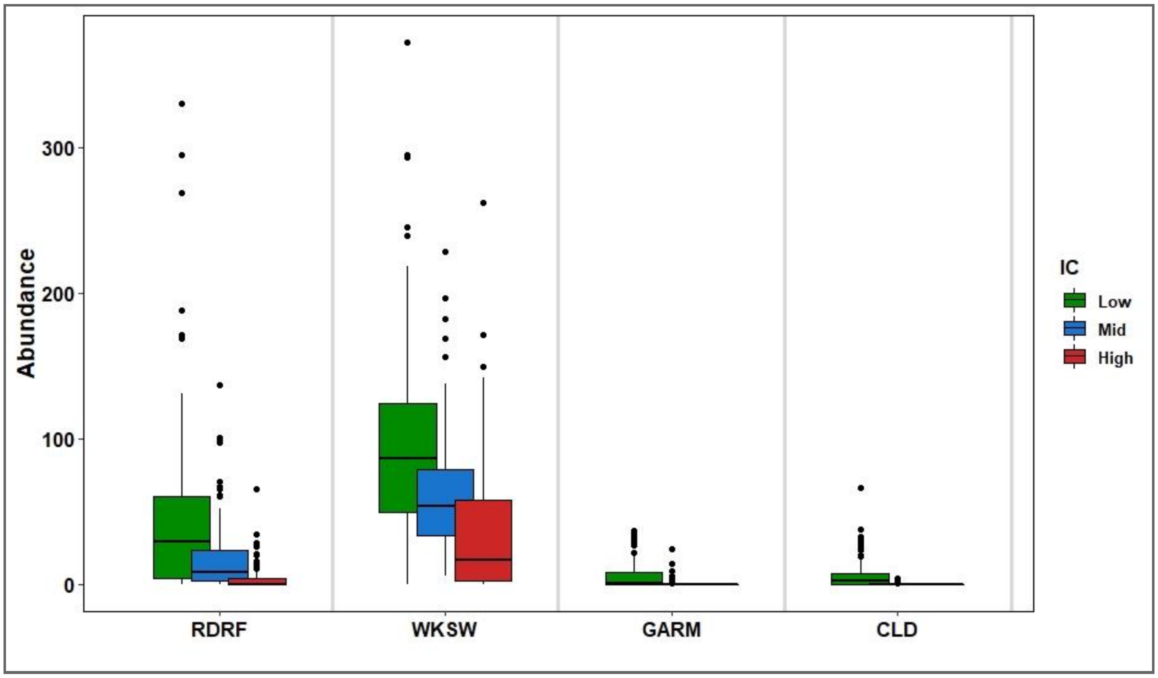

**Figure 2.16**. Top 4 of 38 traits identified by the Indicator Species Analysis to be indicators of Low percent IC streams. (RDRF – rare occurrence in drift; WKSK – weak swimming ability; GARM – good armoring; CLD – cold stenothermal preference).

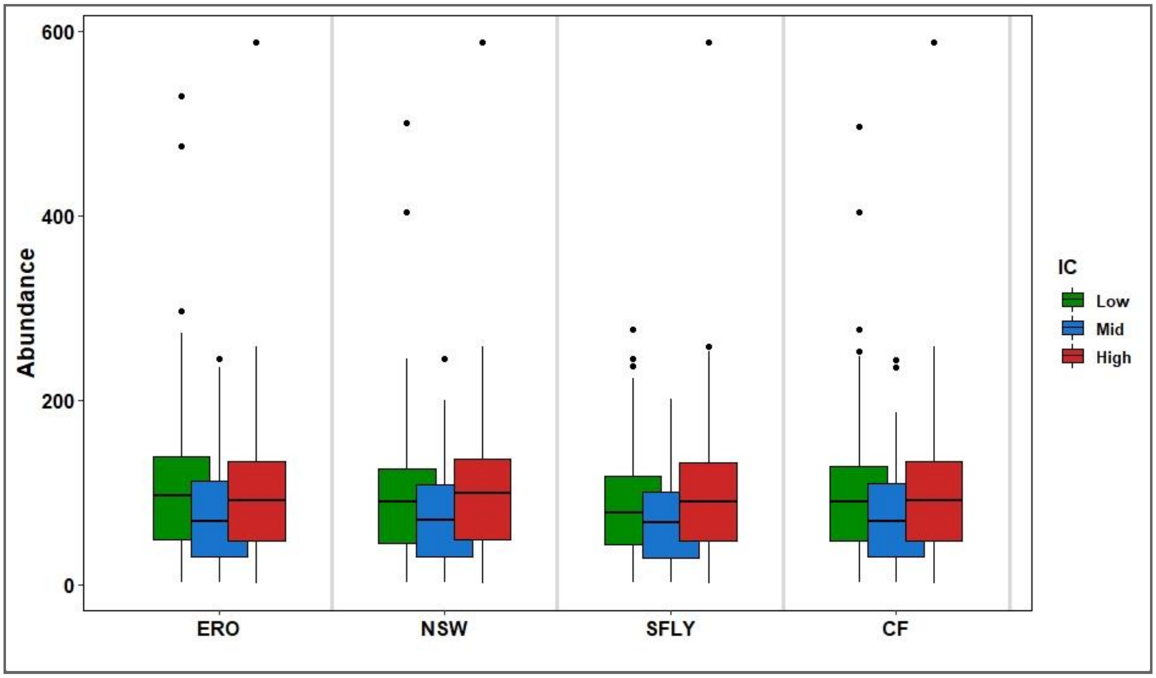

**Figure 2.17**. Top 4 of 6 traits identified by the  Indicator Species Analysis to be indicators for both High and Low percent IC streams. (ERO – preference for running water riffles; NSW – no swimming ability; SFLY – strong flying ability; CF – collector-filterer)

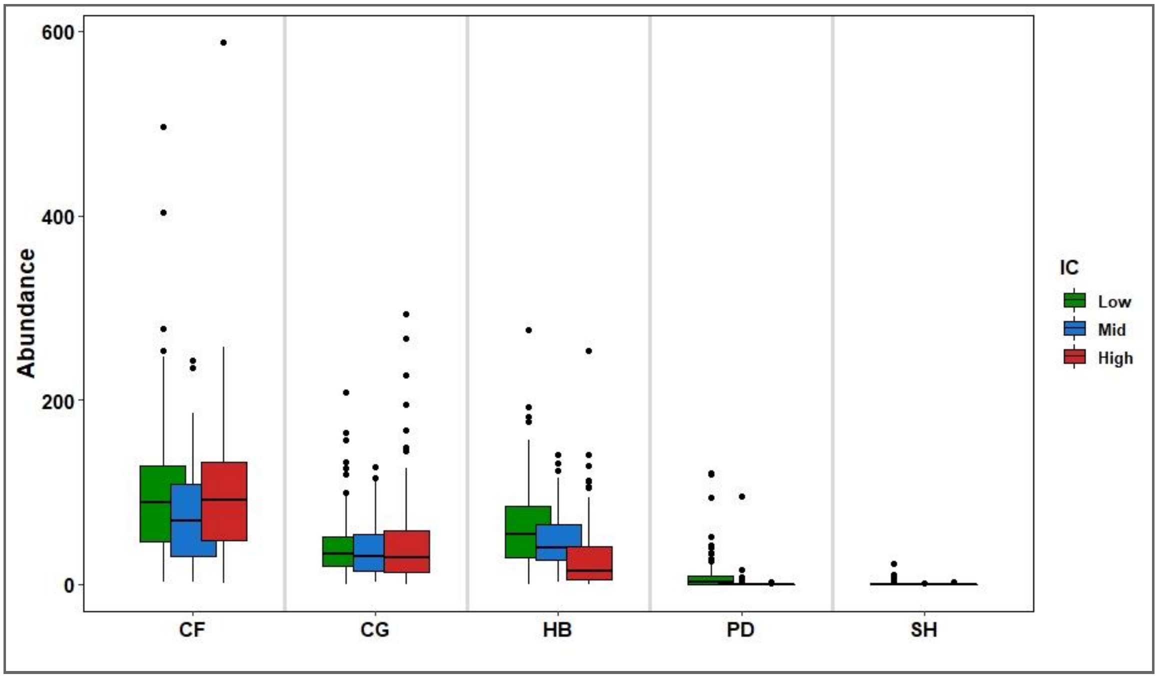

**Figure 2.18**. Collector-filterers (CF), collector-gathers (CG), and herbivore-scrapers (HB) were most abundant of the functional feeding groups found in urban streams in Piedmont North Carolina.

### 2.6.4 THRESHOLD INDICATOR TAXA ANALYSIS (TITAN)

Threshold Indicator Taxa Analysis (TITAN2; Baker and King 2010; King and Baker 2010; King and Baker 2011; King et al., 2011) was conducted on the EPT taxa and trait assemblages found in the 15 streams in this study using percent IC and stream habitat condition (MHAP scores) as gradients. Nineteen EPT taxa, including Ephemeroptera taxa *Teloganopsis deficiens* (Morgan 1911), *Eurylophella temporalis* (McDunnough 1924), and *Leucrocuta aphrodite* (McDunnough 1926), Plecoptera taxa *Perlesta placida* (Hagen 1861), *Neoperla* Needham 1905, and *Isoperla holochlora* (Klapálek 1923), and Trichoptera taxa *Pycnopsyche guttifera* (Walker 1852), *Polycentropus* Curtis 1835, and *Neophylax oligius* Ross 1938, declined when IC was as low as 5% IC (Figure 2.19). All these taxa were found to be indicators of streams in watersheds with <10% IC. Nine EPT taxa were found to increase in abundance above 30% IC, including Ephemeroptera taxa *Baetis flavistriga* McDunnough 1921, *Baetis intercalaris*

McDunnough 1921, *Paracloeodes minutus* (Daggy 1945), and *Tricorythodes* Ulmer 1920, and Trichoptera taxa *Hydropsyche betteni* Ross 1938, *Hydroptila* Dalman, 1819, and *Leucotrichia pictipes* (Banks 1911). The *Baetis* Leach 1815 taxa were also found to be indicators of streams in watersheds with >25% IC.

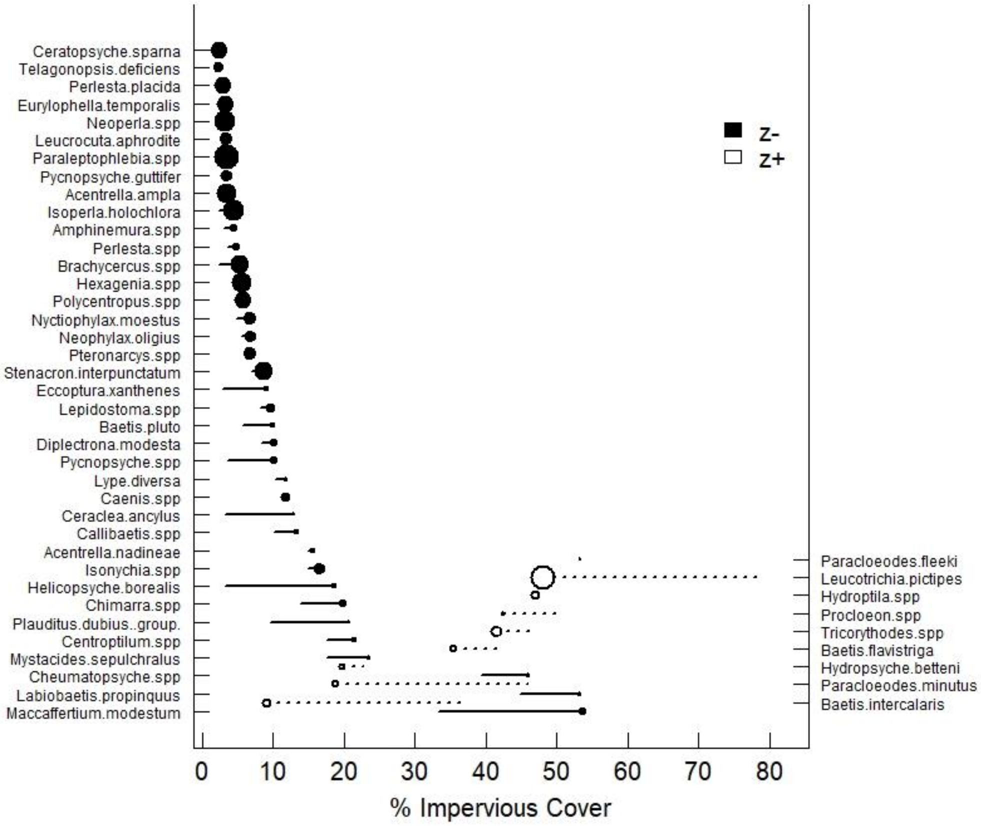

**Figure 2.19**. Threshold Indicator Taxa Analysis for Taxa Richness by percent IC. Taxa richness declined at IC as low as 5% IC with significant declines in taxa occurring from 5% through 20% IC. Tolerant taxa increase in abundance beginning around 5% IC.

Like taxa, about 20 traits declined in abundance at percent IC as low as 5% (Figure 2.20). These traits are generally found in taxa that are intolerant of pollution or unstable habitat and include Life History traits semivoltine (SV), well synchronized emergence (WSY), slow seasonal development (SSE), and adult ability to exit absent (SAB); Morphology traits respiration by gills (GIL), not streamlined body (NOSTR), and large body size (LG); Mobility traits low female dispersal (LDIS), weak flyer (WFLY), and high crawling rate (HCW); and Ecology traits burrower habit (BRW) and feeding groups predator (PD) and shredder (SH). These traits were also found to be indicators of streams in watersheds with <10% IC. Eight traits increased in abundance above 30% IC. These traits are generally found in taxa that are tolerant of pollution or unstable habitats and include Life History traits multivoltism (MV) and adult ability to exit (EPR); Mobility traits strong swimming ability (STSW) and abundant occurrence in drift (ADRF); Morphology trait small size at maturity (SM); and Ecology traits, feeding group collector-gather and swimmer habit (SWM). These traits were also found to be indicators of streams in watersheds with >30% IC.

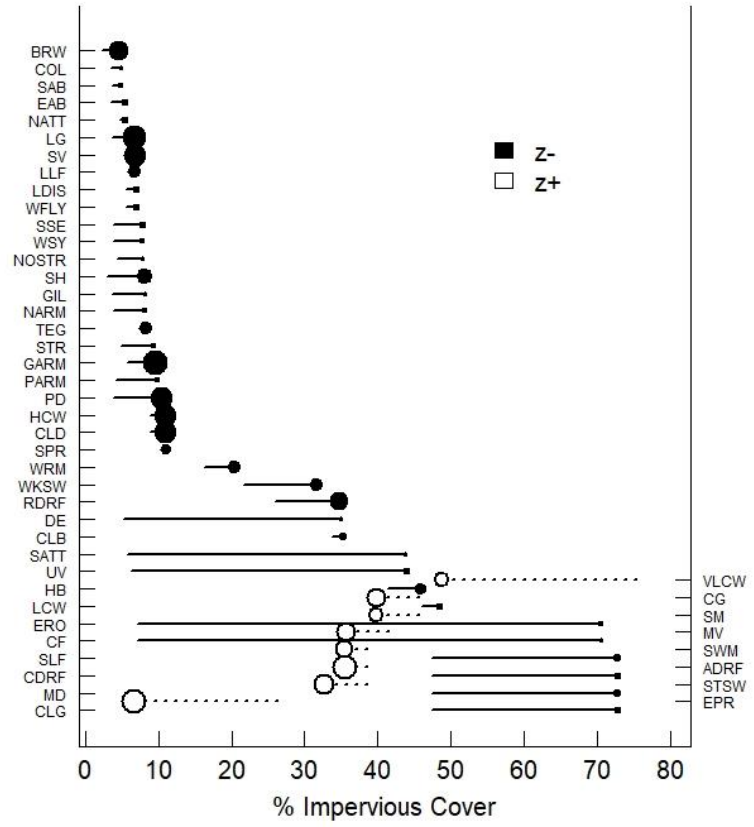

**Figure 2.20**. Threshold Indicator Taxa Analysis for Trait Richness by percent IC. Trait richness declined at IC as low as 5% IC with significant declines in taxa occurring from 5% through 10% IC. Tolerant taxa increase in abundance beginning around 30% IC

EPT taxa abundance and richness declined as stream habitat conditions declined (as measured using the MHAP scores; Figure 2.21). EPT taxa in streams with MHAP scores above 120 (partially supporting to supporting) have tolerance ratings <4.0 (Appendix Table A1; NCDEQ 2016; MCSWS 2017) while EPT taxa in streams with scores below 110 (impaired to degraded) have tolerance rating >4.5. Like EPT taxa, traits decline dramatically when MHAP scores decline to 120 (Figure 2.22). Another MHAP threshold where more traits decline is at MHAP score of 80.

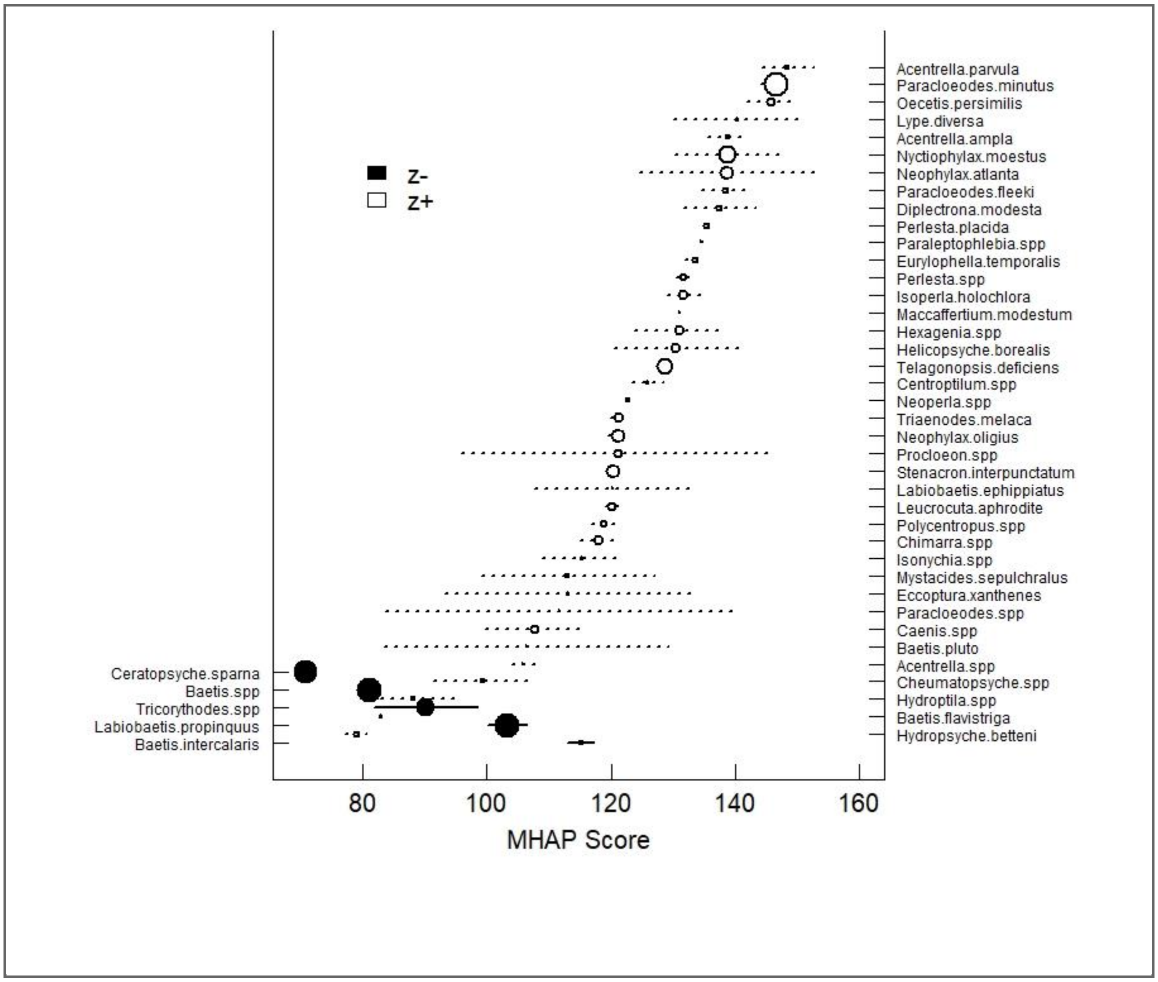

**Figure 2.21**. Threshold Indicator Taxa Analysis for Taxa Richness by MHAP score. Taxa richness began declining as MHAP score below 140 with significant declines in taxa occurring between 120 and 140. Tolerant taxa increased in abundance as MHAP declined below 110.

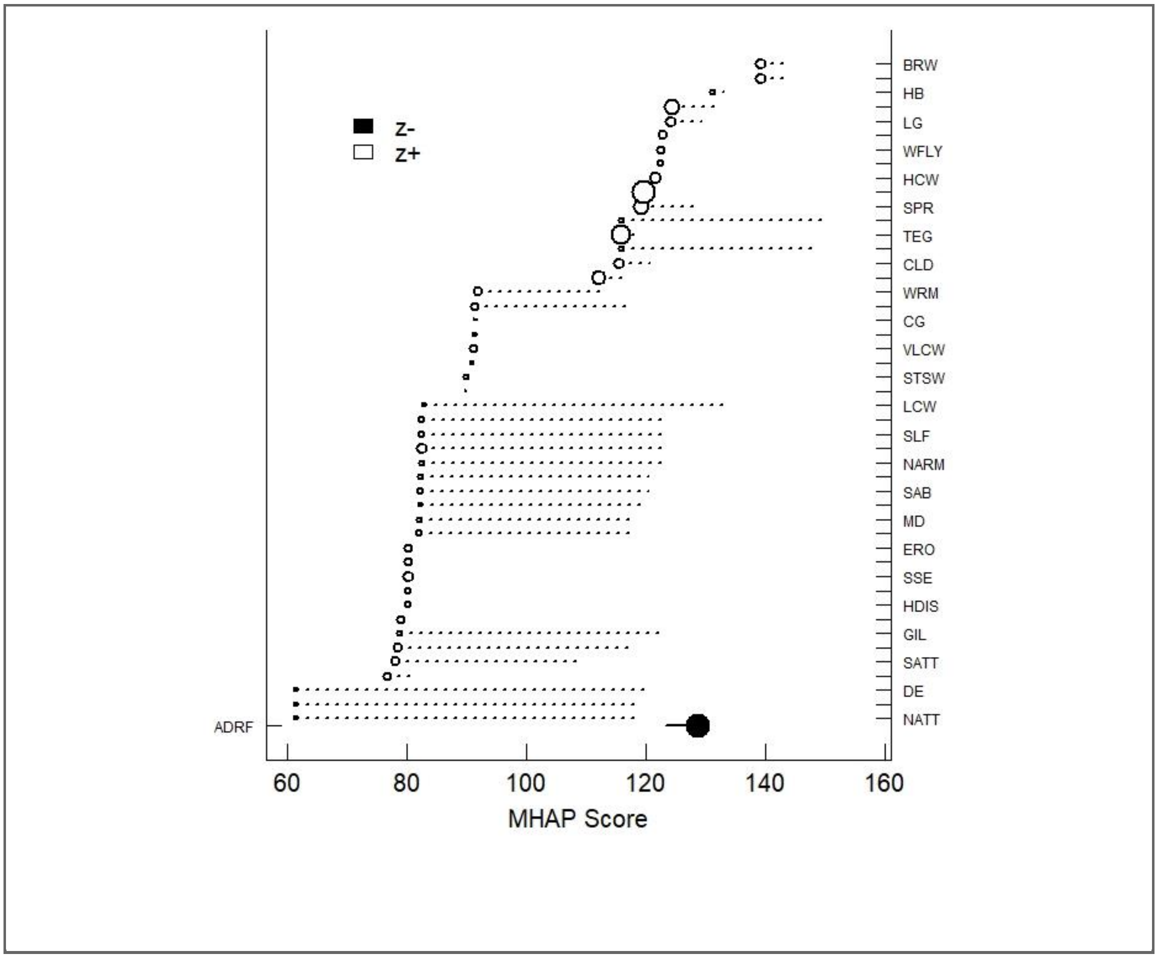

**Figure 2.22**. Threshold Indicator Taxa Analysis for Trait Richness by MHAP scores. Trait richness began declining as MHAP score dropped below 140 with significant declines in taxa occurring between 120 and 140. Another significant decline in traits occurred between MHAP scores of 80 to 90.

### 2.6.5 INDICATORS OF HYDROLOGIC ALTERATION

Correlation analyses between EPT taxa and trait richness and diversity, and percent IC, MHAP, and the 13 IHA metrics showed that EPT taxa and trait richness and taxa diversity were negatively correlated with all metrics except for MHAP, High and Low Pulse Duration, and Fall Rate (Table 2.4). They were also significantly correlated with all metrics except for Base Flow and Date of Maximum and Minimum Flow. Trait diversity was minimally correlated with these metrics (tau ranged between -0.15 to 0.12) and was significantly correlated with MHAP (positive) and with Low Pulse Count (negative).

**Table 2.4**. Correlations analyses between taxa and trait richness and diversity and percent IC, MHAP, IHA Group 2 metric (1 Day Min, 1 Day Max, 90 Day Min, 90 Day Max, Base Flow), IHA Group 3 metrics (Date Min, Date Max), IHA Group 4 metrics (High Pulse Count, High Pulse Duration, Low Pulse Count, Low Pulse Duration), and IHA Group 5 metrics (Fall Rate, Rise Rate).

| | **Kendall's Rank Correlation tau** | | | | | |
|---|---|---|---|---|---|---|
| **Metric** | **Taxa Richness** | **Taxa Diversity (H′)** | **Trait Richness** | **Trait Diversity (H′)** | **%IC** | **MHAP** |
| percent IC | -0.448*** | -0.362*** | -0.452*** | -0.0545 | 1 | -0.114* |
| MHAP | 0.192*** | 0.193*** | 0.215*** | 0.118* | -0.114* | 1 |
| IHA Group 2: 1 Day Min | -0.144** | -0.0842 | -0.168*** | 0.0547 | 0.335*** | -0.316*** |
| IHA Group 2: 1 Day Max | -0.116** | -0.0607 | -0.1348** | 0.0611 | 0.303*** | -0.112* |
| IHA Group 2: 90 Day Min | -0.240*** | -0.178*** | -0.240*** | 0.00246 | 0.506*** | -0.222*** |
| IHA Group 2: 90 Day Max | -0.102* | -0.0572 | -0.124* | 0.0783 | 0.357*** | -0.197*** |
| IHA Group 2: Base Flow | -0.0513 | -0.0475 | -0.0634 | 0.0345 | 0.115* | -0.272*** |
| IHA Group 3: Date Min | -0.0104 | -0.0212 | -0.0637 | -0.0408 | 0.0351 | -0.0433 |
| IHA Group 3: Date Max | -0.0430 | -0.0322 | -0.0421 | -0.0477 | -0.0464 | -0.0361 |
| IHA Group 4: High Pulse Count | -0.403*** | -0.297*** | -0.394*** | -0.0414 | 0.619*** | -0.0514 |
| IHA Group 4: High Pulse Duration | 0.137* | 0.0749 | 0.0853 | 0.027 | -0.117* | -0.0216 |
| IHA Group 4: Low Pulse Count | -0.286*** | -0.311*** | -0.286*** | -0.214*** | 0.248*** | -0.0174 |

| | | | | | | |
|---|---|---|---|---|---|---|
| IHA Group 4: Low Pulse Duration | 0.131* | 0.0915 | 0.145** | -0.0578 | -0.246*** | -0.00914 |
| IHA Group 5: Rise Rate | -0.245*** | -0.1825*** | -0.242*** | 0.00796 | 0.496*** | -0.181*** |
| IHA Group 5: Fall Rate | 0.152** | 0.175*** | 0.186*** | 0.0244 | -0.396*** | 0.229 *** |

Correlation coefficients significant p-values - * $\alpha < 0.05$, ** $\alpha < 0.01$, and *** < 0.001.

Multiple linear regression analyses developed models that relate taxa richness, trait richness, taxa diversity and trait diversity to indicators of hydrologic alteration (IHA), steam habitat conditions (MHAP scores) and percent impervious cover (percent IC) (Table 2.5). The regression model for taxa richness includes percent IC, MHAP, and IHA metrics Low Pulse Number, 1 Day Minimum, High Pulse Number, and 90 Day Maximum. The model for taxa diversity includes IC, MHAP, and IHA metrics 1 Day Minimum, Low Pulse Number, High Pulse Number, 90DayMax, and Fall Rate. The model for trait richness includes percent MHAP, and IHA metrics Low Pulse Number, High Pulse Number, Date Minimum, and Rise Rate. The model for trait diversity includes MHAP and IHA metrics Low Pulse Number, 1 Day Minimum, 1 Day Maximum, 90 Day Minimum, 90 Day Maximum, and Fall Rate. All the models include MHAP and Low Pulse Number while 3 models also included 1 Day Minimum and High Pulse Number reflecting the importance of increasing flashiness of storm runoff on negatively impacting stream habitat stability.

**Table 2.5**. Multiple linear regression models for Taxa and Trait Richness and Diversity (H′). The initial set of parameters tested included: IHA Metrics (1 Day Minimum, 90 Day Minimum, 1 Day Maximum, 90 Day Maximum, Base Flow, Date Minimum, Date Maximum, Low Pulse Number, Low Pulse Length, High Pulse Number, High Pulse Length, Rise Rate, Fall Rate), percent IC, and MHAP Scores.

| | **Goodness of fit** | | **Regression model** |
|---|---|---|---|
| | **Adj. R2** | **p** | |
| **Taxa Richness** | 0.4808 | 2.2e-16 | 7.91175 - 0.05083 (IC) + 0.06242 (MHAP) - 0.10308 (LowPulseNum) - 0.10169 (HighPulseNum) + 0.01560 (90DayMax) + 0.44162 (1DayMin) |
| **Taxa Diversity (H′)** | 0.4028 | < 2.2e-16 | 1.427671– 0.011595 (LowPulseNum) + 0.005181 (MHAP) - 0.007623 (HighPulseNum) + 0.068488 (1DayMin) – 0.002830 (IC) + 0.002919 (90DayMax) + 0.084386 (FallRate) |
| **Trait Richness** | 0.4701 | 2.2e-16 | 47.24618 - 0.19176 (HighPulseNum) + 0.04865 (MHAP) - 0.11379 (LowPulseNum) - 0.00996 (DateMin) + 0.15846 (RiseRate) |
| **Trait Diversity (H′)** | 0.1639 | 6.744e-07 | 3.45 - 0.002895 (LowPulseNum) + 0.0008219 (MHAP) + 1.427 (1DayMin) + 0.04642 (FallRate) + 0.001257 (90DayMax) – 0.00003975 (1DayMax) + 0.003641 (90DayMin) |

The Richards-Baker Flashiness Index (R-B Index) was significantly higher in streams in watersheds with percent IC greater than 25% (Dunn Test for multiple comparisons p<0.001) while the R-B Index was not significantly different in streams in watersheds with percent IC less than 25% (Figure 2.23). The R-B Index was significantly positively correlated with percent IC (Kendall's rank correlation p<0.001; Table 2.6). However, the R-B Index was positively correlated with MHAP but not significant ( p=0.046). The R-B Index was significantly negatively correlated with taxa and trait richness and taxa diversity (p<0.001) but was not significantly correlated with trait diversity.

**Table 2.6**. Correlations Analyses between Richards-Baker Flashiness Index and percent IC, MHAP scores, and Taxa and Trait Richness and Diversity.

|  | **Kendall's rank correlation tau** |  |  |  |  |  |
|---|---|---|---|---|---|---|
| **Metric** | **% IC** | **MHAP** | **Taxa Richness** | **Taxa Diversity (H′)** | **Trait Richness** | **Trait Diversity (H′)** |
| **R-B Index** | 0.380*** | 0.0833 | -0.307*** | -0.201*** | -0.287*** | -0.012 |

***- Correlation coefficients significant p-values * α < 0.05, ** α < 0.01, and *** < 0.001.**

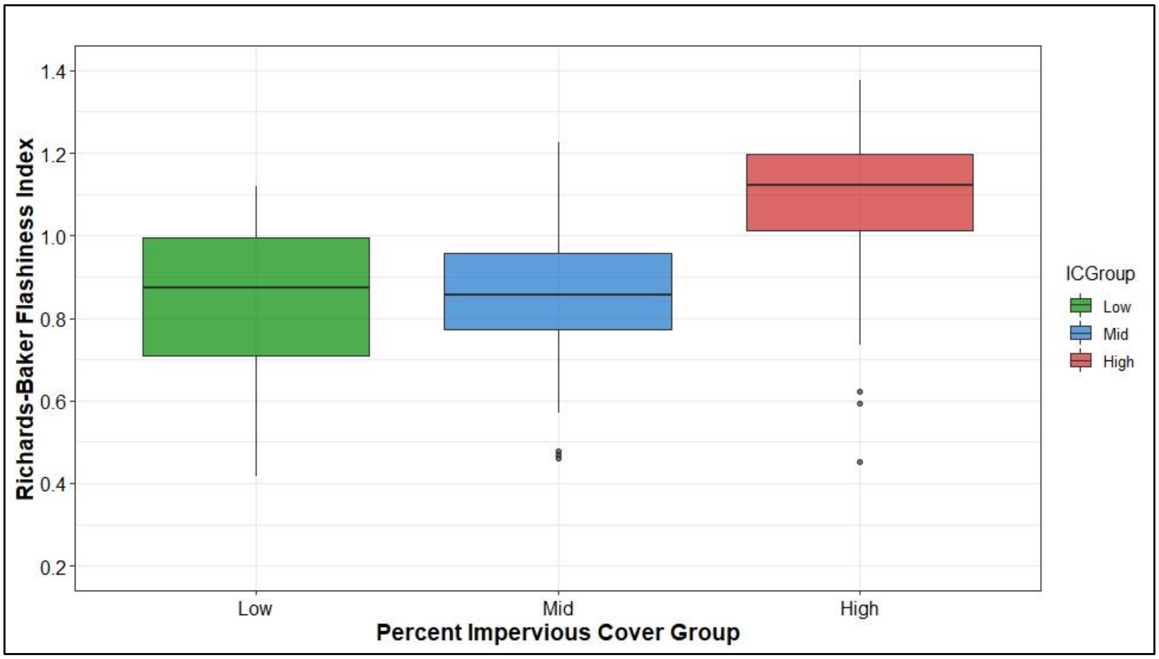

**Figure 2.23**. Box plots of Richards-Baker Flashiness Index (R-B Index) scores in each percent IC stream group. The R-B Index in the High IC (>25% IC) streams was significantly higher than in streams with IC > 25% (Dunn Test for multiple comparisons p<0.001). The R-B Index was not significantly different in the Mid and Low percent IC streams.

## 2.7 DISCUSSION

My first objective was to examine the relationship between increases in percent impervious cover associated with urban land development with benthic macroinvertebrate EPT taxa and trait richness and diversity and with stream habitat conditions by evaluating 15 streams in the Piedmont, North Carolina, spanning a gradient of low to high percent IC at the watershed scale over a 26-year period. My second objective was to determine which indicators of hydrologic alteration (IHA) were most influential for controlling aquatic insect EPT taxa and trait richness and diversity by evaluating the correlations between the IHA metrics and taxa and trait richness and diversity. A better understanding of how urbanization impacts macroinvertebrate taxa diversity and trait diversity is essential for the management and restoration of river ecosystems.

### 2.7.1 EPT TAXA RICHNESS AND DIVERSITY

The NMDS analyses showed that the EPT taxa and trait assemblages found in the Low IC stream were distinctly different from taxa and trait assemblages found in streams with higher levels of percent IC. The major drivers of the differences in the taxa and trait assemblages in the different IC group streams were percent IC and MHAP. Conductivity and temperature also influence the taxa and trait assemblages. The EPT taxa richness and diversity significantly declined with increasing impervious cover. Impervious cover as low as 5% in a watershed has been shown to have negative effects on benthic macroinvertebrate taxa diversity and biomass (Schueler 1994; Paul and Meyer 2001; Stepenuck et al., 2002; Center for Watershed Protection 2003; Morse et al., 2003; Ourso and Frenzel 2003; Cuffney et al., 2010).

EPT taxa richness and diversity declined with increasing impervious cover associated with urban development; however, taxa richness and diversity do not tell the whole story about what happens to the benthic macroinvertebrate community during the urbanization process. While taxa richness may decline, the decline is not linear as taxa that are less sensitive to environmental changes and more tolerant of the urban stream environment (flashy storm events, less stable substrates, poor habitat diversity, higher water temperatures, increased stormwater pollutants) may become more abundant (Walsh et al., 2005). Tolerant taxa may replace the sensitive taxa that are lost from the benthic macroinvertebrate assemblage, thus reducing the overall decline in taxa richness (Boulton 2003; Gresens et al., 2007; Marques et al., 2019; Maloney et al., 2021). The TITAN analysis (Figure 2.4xg) revealed that several of the more sensitive EPT taxa that have pollution tolerance values (TV) less than 4.0 (NCDEQ 2016), including all Plecopteran taxa, declined in abundance at percent IC as low as 5% and were lost to the benthic macroinvertebrate assemblage when the percent IC rose above 10%. These taxa were among the EPT taxa found to be indicators of streams with percent IC <10%. At the same, the abundance of several of the more tolerant EPT taxa (TV > 4.5) began increasing when the percent IC rose above 30%. These taxa were also among the EPT taxa that were found to be indicators of streams in watersheds with percent IC >25%. These results indicate that the benthic macroinvertebrate assemblage changes as percent IC increases with development as the more sensitive taxa declined in abundance while the abundance of tolerant taxa increased. Some sensitive taxa were lost while tolerant taxa new to the benthic macroinvertebrate assemblage thrived. These tolerant taxa may have been present but rare when the percent IC was <10% making their detection rate low. They also could have come in from nearby urban streams. Therefore, just focusing on the taxa richness changes in a stream in a developing watershed does not reflect the changes taking place in the composition in the taxa assemblages.

One of the more significant negative impacts to stream ecosystems due to urbanization is the alteration of the natural hydrologic regime (Brown et al., 2009; Cuffney et al., 2010; Konrad and Booth, 2005; Konrad et al., 2002; Roy et al., 2005; Walsh et al., 2005; Walsh et al., 2007). The multiple linear regression model showed that 3 IHA metrics, Low Pulse Number, High Pulse Number, and 1 Day Minimum, significantly impacted both taxa richness and diversity.

The frequency and duration of high pulses, number of flow reversals, pulse counts, rise and fall rates, and 1-day minimum and maximum, are general indicators of stormwater flashiness (Baker et al., 2004; Onwuka et al., 2021). Stormwater flashiness has been shown to increase with % IC (Baker et al., 2004; DeGasperi et al., 2009; Schwendel et al., 2010). In this study, I found that the Richards-Baker Flashiness index (R-B Index) was significantly higher in streams with watersheds greater than 25% IC. I also found that the R-B Index was significantly positively correlated with % IC, but not significantly correlated with MHAP scores. However, as percent IC was significantly negatively correlated with MHAP scores, I expect that the increased frequency of stormwater flashiness indirectly and negatively impacts stream habitats available to benthic macroinvertebrates and other aquatic organisms. Streambed material was disturbed more frequently in flashy urban streams (Konrad et al., 2005; Schwendel et al., 2010; Anim et al., 2018; Russell et al., 2020). Anim et al. (2018) found that shear stress was significantly higher in urban streams than in forested streams. Shear stress was predicted to be higher during the same bankfull event in a stormwater impacted tributary than in an adjacent forested tributary resulting in lower MHAP scores and taxa richness in the stormwater impacted tributary (Chapter 4, page 159). Merigoux and Doledec (2004) found that taxa richness declined with increased shear stress. The higher shear stress associated with flashy stormwater runoff (Baker et al., 2004; Konrad et al., 2005; Schwendel et al., 2010; Russell et al., 2020) contributed to the less stable and more homogenous habitats found in the urban streams with watersheds having greater than 25% IC. Bond and Downes (2000, 2003) observed that benthic macroinvertebrates were initially impacted by movement of fine sediments. They also observed that flows at or above critical flow velocity can move larger particles and negatively impact habitat and benthic macroinvertebrate assemblages. I found that the R-B Index was significantly negatively correlated with taxa and trait richness and taxa diversity but was not significantly correlated with trait diversity. DeGasperi et al. (2009) also found that the R-B Index was significantly negatively correlated with benthic macroinvertebrate B-IBI scores and was significantly positively correlated with total impervious area. They concluded that the R-B Index was the most sensitive of the 8 indicators of hydrologic alteration (six of which were IHA metrics) that they tested to detect trends in urbanization. These results support my hypothesis that IHA indicators associated with storm runoff flashiness have the greatest impact on EPT taxa and trait richness and diversity.

Stream habitat condition has been shown to decline with increases in % IC (Center for Watershed Protection 2003; Morse et al., 2003; Walsh et al., 2005; Vietz et al., 2016). In this study I found that stream habitat condition, as measured by MHAP, was significantly negatively correlated with percent IC, and declined as percent IC increased. The MHAP scores were significantly higher in streams with less than 10% IC. There were no significant MHAP score differences in streams in watersheds with percent IC greater than 10%. The TITAN analysis revealed that the same group of taxa and traits that declined with increases % IC also declined as stream habitat conditions deteriorated with development. Sensitive EPT taxa abundance began declining as MHAP scores dropped below 140. The TITAN analysis shows a distinct threshold near a MHAP score of 120. MHAP scores between 120 and140 are in the lower range of Partially Support Habitat conditions and correspond to the transition range between Partially

Supporting and Impaired habitat (Barbour et al., 1999). In this study, streams in watersheds with >10% had MHAP scores 120 or less.

### 2.7.2 EPT TRAIT RICHNESS AND DIVERSITY

EPT trait richness and diversity also declined with increasing percent IC, but not to the same degree as EPT taxa richness and diversity supporting my hypothesis that watershed development would cause EPT taxa richness and diversity to decline more than EPT trait richness and diversity. Beche et al. (2006) also found the decline in trait richness and diversity due to a disturbance was significantly less when compared to the response of taxa richness to the same disturbance. This may be due to the redundancy of traits throughout the benthic macroinvertebrate assemblage (Walker 1992; Rosenfeld 2002; Heatherly et al., 2007; Bêche and Statzner 2009; Flynn et al., 2009; Peru and Doledec 2010; Lamothe et al., 2018). Like taxa richness, the TITAN analysis revealed that there are several traits that decline at IC as low as 5%, most of which were commonly found in the sensitive taxa that decline when percent IC increases above 5%. Several of these traits were found to be indicators of streams in watersheds with < 10% IC. These are traits that also favor streams with diverse habitats including abundant riffles (Berger et al., 2018; Castro et al., 2018; Monk et al., 2018; Ntloko et al., 2021). There were several traits that increased in abundance when the percent IC rose above 30%, most of which are associated with the more tolerant taxa that increased in abundance above 30% IC. Several traits were found to be indicators of streams in watersheds with > 25% IC. These results support my hypothesis that individual traits responses to urbanization vary by individual trait. Like in this study, Berger et al. (2018) and Castro et al. (2018) found traits associated with sensitive taxa and with undisturbed sites to include univoltism, larger body size, and climbing habit. Monk et al. (2018) found traits positively correlated with flow velocity to include climber and swimmer habits, preference for cold-cool eurythermal water, and ability to survive desiccation while traits negatively correlated to flow velocity include sprawler and burrower habits, preference for warm eurythermal water, and inability to survive desiccation.

The multiple linear regression model showed that 3 IHA metrics, Low Pulse Number, High Pulse Number, and Rise Rate, significantly impacted trait richness and 6 IHA metrics, including Low Pulse Number, 1-Day Minimum, 1-Day Maximum, and Fall Rate, significantly impacted trait diversity. These IHA metrics are associated with stormwater flashiness (Baker et al., 2004; DeGasperi et al., 2009; Onwuka et al., 2021) which has been shown to negatively impact stream habitat conditions (Konrad et al., 2005; Schwendel et al., 2010; Russell et al., 2020). Both stream habitat (MHAP) and percent IC were part of the model for EPT trait richness while stream habitat (MHAP) was part of the model for EPT trait diversity indicating that habitat condition, which is impacted by stormwater flashiness, is also an important factor affecting trait composition of benthic macroinvertebrate assemblages (Menezes et al., 2010; Schwendel et al., 2010; Castro et al., 2017; Castro et al., 2018 ).

Functional feeding group traits, collector-filter (CF) and shredder (SH), were found to be indicators of streams in watersheds with <10% IC and declined in abundance at percent IC as low as 5%. Functional feeding group collector-gather was found to be an indicator of streams in watersheds with >30% IC. Merigoux and Doledec (2004) also found an inverse relationship between the proportion of collector-filterers and collector-gathers verse shear stress which is correlated with urban stream flashiness. While most herbivore-scrapers declined with increasing % IC, three herbivore-scraper taxa, *Paracloeodes* Day 1955, *Hydroptila* Dalman, 1819, and *Leucotrichia pictipes* (Banks 1911), were abundant in streams with > 30% IC. These results support my hypothesis that EPT shredder and collector-filterer richness declined and EPT collector-gatherer richness increased in response to increases in percent IC but partially supported my hypothesis that herbivore-scrapers would increase with % IC. I also found no change in the EPT Predator diversity.

### 2.7.3 LOSS OF SENSITIVE TAXA IMPACTS ECOSYSTEM FUNCTION.

The loss of both taxa and traits from the benthic macroinvertebrate assemblages due to the various stressors associated with the impacts of development can significantly impact the ecosystem function of a stream ecosystem (Gravel et al., 2016; Baumgartner et al., 2017). These stressors include a decline in habitat quality with increased stormwater flashiness, shifting of the primary energy source from allochthonous to autochthonous with the removal or thinning of streamside vegetation, increased stream summertime baseflow temperatures (Dewson et al., 2007), and increased harmful pollutant loads, such as hydrocarbons, heavy metals, fertilizers, and pesticides, accompanying the increased stormwater runoff (Cuffney et al., 2010). Changes in biodiversity can affect ecosystem function through changes in trophic interactions such as top-down or bottom-up influenced changes in community food webs (Rosemond et al., 1993; Power and Dietrich 2002; Baxter et al., 2004; Thébault and Loreau 2006). Woodward (2009) also observed that biodiversity-ecosystem function relationships are sensitive to top-down or bottom-up cascades.

The aquatic food web can be disrupted with the loss of taxa and their accompanying traits. For example, in this study, an entire aquatic insect order, Plecoptera (stoneflies), was lost from streams in watersheds with greater than 10% IC. All but 3 of the Plecoptera taxa found in the streams in this study were predators. The loss of a significant portion of the predators in the benthic macroinvertebrate community can potentially disrupt the established predator-prey interaction within the benthic macroinvertebrate community, allowing several prey taxa whose abundance were held in check by predation to increase in abundance (Woodward 2009; Gravel et al., 2016). The food webs can also be impacted as taxa from different trophic levels may be lost due to the impacts of urbanization. This can affect the overall processing of allochthonous and autochthonous organic matter through the ecosystem (Gravel et al., 2016; Baumgartner 2017: Kovalenko 2019). Ultimately, the loss of taxa from the benthic macroinvertebrate community will impact the terrestrial environment as the biomass of emerging insects may decline, impacting also the predators, detritivores, and other biota that depend on those insectivores (Baxter et al., 2005; Nakano et al., 1999; Nakano and Murakami, 2001; McKie et al., 2018).

### 2.7.4 IMPACT OF INTERMITTENT DROUGHTS BENTHIC MACROINVERTEBRATES

Not all declines in benthic macroinvertebrate assemblages in urban streams are the result of the urbanization process. For example, a drought can reduce the base flow stream levels, facilitate increases in water temperatures, and degrade or eliminate specific habitats and taxa dependent on those habitats (Boulton 2003). During the study period, there were several significant droughts in the Piedmont of North Carolina (Appendix Figure A1) which impacted the benthic macroinvertebrate assemblages. In smaller rural watersheds such as Gar Creek (MC50; drainage area 3.5 mi$^2$), ambient water levels dropped notably, and summertime temperatures rose higher than pre-drought conditions (personal observations). Both EPT taxa and trait richness dropped in the 2 to 4 years after 2 exceptional droughts in 2002 and 2008, while no change was observed in both EPT taxa and trait diversity (Appendix Figures A2 and A3). EPT median pollution tolerance ratings (NCDEQ 2016) increased from 3.6 before the 2002 drought to 4.85 and increased from 5.1 before the 2008 drought to 5.7 indicating that the more sensitive EPT taxa declined during both droughts. The water levels in Gar Creek have not returned to pre-drought levels (personal observations). This change in water level resulted in headwater taxa such as *Neophylax atlanta* (Trichoptera) becoming part of the benthic macroinvertebrate assemblage (CMSWS unpublished data; personal communication Eric Fleek, NCDEQ). While not specifically studying changes in flow regimes due to drought conditions, Schneider and Petrin (2017) observed changes in benthic macroinvertebrate assemblages due to changes in flow regimes in regulated river systems. They also observed that while taxa in benthic macroinvertebrate assemblage changed, the new taxa had similar feeding habitats resulting in little or no changes in trait diversity.

In larger rural watersheds such as Clear Creek (MY8; drainage area 12.5 mi$^2$), the ambient water levels dropped, but not as dramatically as in the smaller watersheds, and summertime temperatures rose higher than pre-drought conditions (personal observations). However, both EPT taxa and trait richness dropped in the 2 to 4 years after the 2002 and 2008 droughts while no change was observed in both taxa and trait diversity (Appendix Figures A4 and A5). EPT median pollution tolerance ratings (NCDEQ 2016) increased from 3.55 before the 2002 drought to 5.75 and increased from 5.7 before the 2008 drought to 5.75 indicating that the more sensitive EPT taxa declined during the first drought, but not during the second drought. In contrast, in large urban watersheds such as Little Sugar Creek (MC29; drainage area 15.9 mi$^2$), the ambient water levels did not drop very much, but the summertime temperatures rose higher than pre-drought conditions (personal observations). EPT taxa and trait richness and diversity did not change after the 2002 and 2008 droughts (Appendix Figures A6 and A7). EPT median pollution tolerance rating (NCDEQ 2016) was 6.5 before the 2002 drought and remained 6.5 after the 2008 drought. This may be due to prior adaptations of benthic macroinvertebrate assemblages in the urban stream to higher ambient summer water temperatures and lower ambient water levels.

Chessman (2015) found several traits, including high rheophily, low thermophily, slower maturation, and absence of atmospheric respiration, were negatively impacted by drought conditions that reduced stream water levels and flow velocity, and increased water temperatures. Nelson et al. (2009) warned that, while urbanization caused significant declines in a small number of fish taxa in Chesapeake Bay watersheds, the combined effects of both urbanization and climate change on fish populations could be much larger and could result in loss of fish taxa richness and diversity. Hung et al. (2020) predicted that the combined effects of land-use and climate change could increase the impact of urbanization's alteration of the hydrological processes and could negatively impact the success of stream restoration projects in improving storm runoff and water quality. Suttles et al. (2018) also predicted that the combined effects of land use change and climate change would be greater than when considered separately. They also identified that land use change could have a greater impact than climate in the urban watersheds that experience significant forest loss. Watershed managers should consider the severity of climate change in planning stream restoration projects.

## 2.8 IMPLICATIONS FOR IMPROVING RESTORATION

The alteration of the natural hydrologic regime is one of the most significant impacts to the stream in a developing watershed resulting in degradation of the physical and biological components of the stream ecosystem (Center for Watershed Protection 2003; Walsh et al., 2005; Anim et al., 2018; Erba et al., 2020; Russell et al., 2020; Anim and Banahene 2021; Maloney et al., 2021; Zerega et al., 2021; Hawley 2022). My results show the benefit of use of long-term data sets in evaluating watersheds for impacts to environmental changes including urban development. Using the 26-year benthic macroinvertebrate data set collected by the Charlotte-Mecklenburg Storm Water Services, I demonstrated that the alteration of the natural hydrologic regime is a root cause of the declines observed in EPT taxa and trait richness and diversity. Maloney et al. (2021) drew similar conclusions in their study of streams in the Chesapeake Bay watershed where they found that macroinvertebrate assemblages were 3.8 to 4.7 times more likely to become degraded in flow-altered streams.

Watershed managers respond to the degraded urban stream by restoring the stream using various stream restoration techniques. A general assumption for stream restoration projects has been that restoring channel geomorphology to resemble an undisturbed stream would result in the recovery of the benthic macroinvertebrate community (Sudduth et al., 2011). Numerous studies have shown that stream restorations that just address geomorphological stream channel characteristics without taking into consideration ecosystem function and the ecological requirements of the benthic macroinvertebrate community fail to stimulate the recovery of the benthic macroinvertebrates (Shields et al., 2003; Palmer et al., 2005; Suren and McMurtrie 2005; Sudduth et al., 2011; Hawley 2018). Violin et al. (2011) found no significant differences in stream function and benthic macroinvertebrate communities in urban-restored and urban-unrestored streams while at the same time they found forested undisturbed streams had significantly greater diversity and function compared to urban streams.

Studies of disturbances in streams have shown that it can take up to 10 or more years for recovery to return to pre-disturbance conditions (Dunham et al., 2007; Robinson and Uehlinger 2008). It is important to note that urban streams are subject to a wide range of disturbances including land-use histories, high volume and intensity stormwater runoff, nonpoint and point source pollution, alteration of riparian-zone canopy cover, and drought. The stream restoration process is yet another disturbance to the stream biological community. Most stream restoration studies investigate benthic macroinvertebrate taxa richness found in the project segment before and within 3 to 5 years after project construction to evaluate restoration success. This relatively short monitoring period is not long enough to show impact of restoration on stream ecosystems and provides very little information regarding the success of projects restoring the macroinvertebrate functional traits of the stream (Shields et al., 2003; Bernhardt et al., 2005; Palmer et al., 2005; Sudduth 2005; Bernhardt and Palmer 2007; Palmer et al., 2007; Sudduth et al., 2011).

A significant obstacle to restoring a degraded urban stream ecosystem is the infrastructure (utility lines, roads, and buildings) adjacent to the stream which limits the type of restoration that can be implemented. Infrastructure constraints also limit the ability to address the increased volume of stormwater runoff that reaches the stream with each storm event. Another limitation to improving the benthic macroinvertebrate community is the distance that the restoration is to the closest source of a good benthic macroinvertebrate population. Tonkin et al. (2014) found that the distance from the source of a good population to colonize the restored stream and dispersal factors were significant barriers to improving the restored stream's benthic macroinvertebrate community. Sundermann et al. (2011) found that good recolonizing populations located 0–5 km from the restored stream were effective in impacting the restored stream.

Most stream restorations consist of restoring small segments of a watershed and focus on geomorphologic stabilization with restoration of the riffle-pool complex (Doll et al., 2003; Shields et al., 2003; Bernhardt et al., 2005; Palmer et al., 2005; Bernhardt and Palmer 2007; Bernhardt et al., 2007; Palmer et al., 2007; Palmer et al., 2010; Sudduth et al., 2011; Hawley 2018). Restoring short stream segments fails to address the increased volume of stormwater that originates upstream of the project, which caused the stream degradation that led to the need for restoration.  Unfortunately, little attention has been given to restoring the hydrologic regime (Russell et al., 2020; Anim and Banahene 2021; Zerega et al., 2021; Hawley 2022). Zerega et al. (2021) reported that the restorations that included designs to increase habitat diversity without addressing the restoration of the hydrologic regime were not successful in improving the aquatic biota. They and Hawley (2022) recommended adding measures designed to restore the natural hydrological patterns to the stream. Another improvement to stream restoration design to improve the benthic macroinvertebrate community would be to expand the habitat improvement designs that currently focus primarily on the larval aquatic insect stage to include habitats required by the adult aquatic insects (Merten et al., 2014; Jordt and Taylor 2021).

Geomorphological stability of restored streams is easily measured. However, the degree to which ecological uplift is obtained by restoration is rarely assessed. This may be due to a lack of a good definition of ecological uplift. Once a definition is in place, future research can be directed on how to measure ecological uplift. Since most urban stream restorations take place greater than 5 km from a good population source for recolonization, another means of reintroducing benthic macroinvertebrates to the restored stream is needed. Reintroductions of benthic macroinvertebrates from streams with a high-quality community have been explored by several researchers (Dumeier et al., 2018; Jourdan et al., 2019; Dumeier et al., 2020; Clinton et al., 2022). Research is needed to identify successful reintroduction techniques.

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## 2.10 APPENDIX

**Table A1**. EPT taxa collected from the 15 study sites.

| **BugClass** | **BugOrder** | **Family** | **Genus Species** | **Tolerance** |
|---|---|---|---|---|
| Insecta | Ephemeroptera | Baetidae | *Acentrella alachua* | 3 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella ampla* | 3.6 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella nadineae* | 1.9 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella parvula* | 4.8 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella* spp. | 2.5 |
| Insecta | Ephemeroptera | Baetidae | Acentrella turb*ida* | 2 |
| Insecta | Ephemeroptera | Baetidae | *Baetis flavistriga* | 6.8 |
| Insecta | Ephemeroptera | Baetidae | *Baetis intercalaris* | 5 |
| Insecta | Ephemeroptera | Baetidae | *Baetis pluto* | 3.4 |
| Insecta | Ephemeroptera | Baetidae | *Baetis* spp. | 5 |
| Insecta | Ephemeroptera | Baetidae | *Baetis tricaudatus* | 1.5 |
| Insecta | Ephemeroptera | Baetidae | *Callibaetis* spp. | 9.2 |
| Insecta | Ephemeroptera | Baetidae | *Centroptilum* spp. | 3.8 |
| Insecta | Ephemeroptera | Baetidae | *Heterocloeon amplum* | 3.4 |
| Insecta | Ephemeroptera | Baetidae | *Heterocloeon* spp. | 3.7 |
| Insecta | Ephemeroptera | Baetidae | *Labiobaetis ephippiatus* | 3.5 |
| Insecta | Ephemeroptera | Baetidae | *Labiobaetis propinquus* | 5.8 |

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|---|---|---|---|---|
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes fleeki* | 8 |
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes minutus* | 8 |
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes* spp. | 8 |
| Insecta | Ephemeroptera | Baetidae | *Plauditus cestus* | 4.6 |
| Insecta | Ephemeroptera | Baetidae | *Plauditus dubius* | 2.2 |
| Insecta | Ephemeroptera | Baetidae | *Plauditus punctiventris* | 4 |
| Insecta | Ephemeroptera | Baetidae | *Procloeon* spp. | 1.9 |
| Insecta | Ephemeroptera | Baetidae | *Pseudocentroptiloides usa* | 4.8 |
| Insecta | Ephemeroptera | Baetidae | *Pseudocloeon* spp. | 4 |
| Insecta | Ephemeroptera | Caenidae | *Brachycercus* spp. | 2.1 |
| Insecta | Ephemeroptera | Caenidae | *Caenis* spp. | 6.8 |
| Insecta | Ephemeroptera | Ephemerellidae | *Dannella simplex* | 3.4 |
| Insecta | Ephemeroptera | Ephemerellidae | *Ephemerella catawba* | 0 |
| Insecta | Ephemeroptera | Ephemerellidae | *Eurylophella temporalis* | 4.8 |
| Insecta | Ephemeroptera | Ephemerellidae | *Telagonopsis deficiens* | 2.6 |
| Insecta | Ephemeroptera | Ephemeridae | *Hexagenia* spp. | 4.4 |
| Insecta | Ephemeroptera | Heptageniidae | *Heptagenia marginalis* | 2.2 |
| Insecta | Ephemeroptera | Heptageniidae | *Heptagenia* spp. | 1.9 |
| Insecta | Ephemeroptera | Heptageniidae | *Leucrocuta aphrodite* | 2.9 |

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|---|---|---|---|---|
| Insecta | Ephemeroptera | Heptageniidae | *Stenonema modestum* | 5.7 |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron interpunctatum* | 6.4 |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron pallidum* | 2.8 |
| Insecta | Ephemeroptera | Heptageniidae | *Stenonema femoratum* | 6.9 |
| Insecta | Ephemeroptera | Isonychiidae | *Isonychia* spp. | 3.6 |
| Insecta | Ephemeroptera | Leptohyphidae | *Tricorythodes* spp. | 5 |
| Insecta | Ephemeroptera | Leptophlebiidae | *Habrophlebiodes* spp. | 1 |
| Insecta | Ephemeroptera | Leptophlebiidae | *Paraleptophlebia* spp. | 1.2 |
| Insecta | Plecoptera | Capniidae | *Allocapnia* spp. | 3.3 |
| Insecta | Plecoptera | Leuctridae | *Leuctra* spp. | 1.5 |
| Insecta | Plecoptera | Nemouridae | *Amphinemura* spp. | 3.8 |
| Insecta | Plecoptera | Perlidae | *Acroneuria abnormis* | 2.1 |
| Insecta | Plecoptera | Perlidae | *Acroneuria mela* | 0.9 |
| Insecta | Plecoptera | Perlidae | *Agnetina annulipes* | 0 |
| Insecta | Plecoptera | Perlidae | *Agnetina capitata* | 0 |
| Insecta | Plecoptera | Perlidae | *Agnetina flavescens* | 0 |
| Insecta | Plecoptera | Perlidae | *Eccoptura xanthenes* | 4.7 |
| Insecta | Plecoptera | Perlidae | *Neoperla* spp. | 2.1 |
| Insecta | Plecoptera | Perlidae | *Perlesta placida* | 2.9 |

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|---|---|---|---|---|
| Insecta | Plecoptera | Perlidae | *Perlesta* spp. | 2.9 |
| Insecta | Plecoptera | Perlodidae | *Diploperla duplicata* | 2.8 |
| Insecta | Plecoptera | Perlodidae | *Isoperla holochlora* | 0.7 |
| Insecta | Plecoptera | Pteronarcyidae | *Pteronarcys* spp. | 1.8 |
| Insecta | Trichoptera | Helicopsychidae | *Helicopsyche borealis* | 0 |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche sparna* | 2.5 |
| Insecta | Trichoptera | Hydropsychidae | *Cheumatopsyche* spp. | 6.6 |
| Insecta | Trichoptera | Hydropsychidae | *Diplectrona modesta* | 2.3 |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche betteni* | 7.9 |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche rossi* | 4.8 |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche venularis* | 5.1 |
| Insecta | Trichoptera | Hydroptilidae | *Agraylea* spp. | 1 |
| Insecta | Trichoptera | Hydroptilidae | *Hydroptila* spp. | 6.5 |
| Insecta | Trichoptera | Hydroptilidae | *Leucotrichia pictipes* | 4.6 |
| Insecta | Trichoptera | Lepidostomatidae | *Lepidostoma spp.* | 1 |
| Insecta | Trichoptera | Leptoceridae | *Ceraclea ancylus* | 2.8 |
| Insecta | Trichoptera | Leptoceridae | *Ceraclea flava* | 0 |
| Insecta | Trichoptera | Leptoceridae | *Ceraclea* spp. | 2.2 |
| Insecta | Trichoptera | Leptoceridae | *Mystacides sepulchralus* | 2.6 |

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|---|---|---|---|---|
| Insecta | Trichoptera | Leptoceridae | *Nectopsyche candida* | 6.5 |
| Insecta | Trichoptera | Leptoceridae | *Nectopsyche exquisita* | 4.3 |
| Insecta | Trichoptera | Leptoceridae | *Nectopsyche pavida* | 3.9 |
| Insecta | Trichoptera | Leptoceridae | *Nectopsyche* spp. | 2.9 |
| Insecta | Trichoptera | Leptoceridae | *Oecetis cinerascens* | 4.7 |
| Insecta | Trichoptera | Leptoceridae | *Oecetis inconspicua* | 1.9 |
| Insecta | Trichoptera | Leptoceridae | *Oecetis persimilis* | 4.6 |
| Insecta | Trichoptera | Leptoceridae | *Oecetis* spp. | 5.1 |
| Insecta | Trichoptera | Leptoceridae | *Oecetis* spp. 1 (sand case) | 4.3 |
| Insecta | Trichoptera | Leptoceridae | *Setodes* spp. | 0 |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes ignitus* | 4.8 |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes marginatus* | 4.5 |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes melaca* | 4.1 |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes tardus* | 4.5 |
| Insecta | Trichoptera | Limnephilidae | *Hydatophylax argus* | 2.4 |
| Insecta | Trichoptera | Limnephilidae | *Ironoquia punctatissima* | 6.7 |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche guttifer* | 2.2 |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche* spp. | 2.5 |
| Insecta | Trichoptera | Philopotamidae | *Chimarra* spp. | 3.3 |

| | | | | |
|---|---|---|---|---|
| Insecta | Trichoptera | Polycentropodidae | *Nyctiophylax moestus* | 3.8 |
| Insecta | Trichoptera | Polycentropodidae | *Polycentropus* spp. | 3.1 |
| Insecta | Trichoptera | Psychomyiidae | *Lype diversa* | 3.9 |
| Insecta | Trichoptera | Rhycophilidae | *Rhyacophila carolina* | 0.4 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax atlanta* | 1.6 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax consimilis* | 0.3 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax oligius* | 2.4 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax ornatus* | 1.3 |

**Table A2**. Mecklenburg County Stream Habitat Assessment Protocols (MHAP) parameters, stream conditions measured, and measurements.

| MHAP Stream Parameter | Stream Condition | Measurements |
|---|---|---|
| 1. Instream Cover | Diversity of microhabitats | Count/measure microhabitats observed |
| 2. Epifaunal Substrate | Riffle condition | Measure riffle length and width and estimate composition of major substrate categories |
| 3. Embeddedness | Degree to which substrate surrounded/covered with sediment | Conduct an abbreviated pebble count at each transect |
| 4. Channel Alteration | Man made changes to the stream channel | Count various types of anthropogenic channel disturbances observed |
| 5. Sediment Deposition | Deposition of sediment and formation of sand bars | Estimate percentage of exposed sediment within study reach |
| 6. Frequency of Riffles | Estimation of the frequency of riffles in sample reach | Measure length and frequency of riffles within the study reach |
| 7. Channel Flow Status | Measurement of channel flow status | Measurement of the active channel and wetted width at each transect |
| 8. Bank Vegetative Protection (Left/Right Bank) | Estimation of amount of stream bank vegetation cover | Estimation of percentage of bank cover every 20 meters |
| 9. Bank Stability (Left/Right Bank) | Estimation of amount of unstable stream bank present | Estimation of percentage of bank erosion every 20 meters |
| 10. Vegetated Buffer Zone Width (Left/Right Bank) | Estimation of riparian buffer width and condition | Use aerial photos to determine buffer width (up to 300 feet) |

**Table A3**. Indicator Taxa Associated with MHAP

| **Supporting** | **Stat** | **P value** |
|---|---|---|
| *Paracloeodes.fleeki* | 0.937 | 0.0297* |
| **Partially Supporting** | | |
| *Neophylax.atlanta* | 0.171 | 0.0467 * |

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

**Table A4**. Taxa Associated %IC (LowIC<10; MidIC 10-25; HighIC>25%)

| **High %IC (IC>25%)** | **stat** | **P value** |
|---|---|---|
| *Hydropsyche.betteni* | 0.330 | 0.0001 *** |
| *Baetis.flavistriga* | 0.245 | 0.0001 *** |
| *Hydroptila*.spp | 0.183 | 0.0023 *** |
| | | |
| **Mid %IC (10 - 25%)** | **stat** | **P value** |
| *Triaenodes.ignitus* | 0.246 | 0.0001 *** |
| *Baetis*.spp | 0.210 | 0.0002 *** |
| *Oecetis.persimilis* | 0.191 | 0.0005 *** |
| *Acentrella.nadineae* | 0.155 | 0.0106 * |
| *Mystacides.sepulchralis* | 0.154 | 0.0001 *** |
| | | |
| **Low %IC (<10%)** | **stat** | **P value** |
| *Stenacron.interpunctatum* | 0.424 | 0.0001 *** |
| *Caenis*.spp | 0.361 | 0.0001 *** |
| *Leucrocuta.aphrodite* | 0.336 | 0.0001 *** |

| | | |
|---|---|---|
| *Neophylax.oligius* | 0.326 | 0.0001 *** |
| *Isonychia*.spp | 0.310 | 0.0001 *** |
| *Nyctiophylax.moestus* | 0.282 | 0.0001 *** |
| *Lype.diversa* | 0.145 | 0.0170 * |
| *Allocapnia*.spp | 0.139 | 0.0389 * |
| *Chimarra*.spp | 0.260 | 0.0001 *** |
| *Neoperla*.spp | 0.237 | 0.0002 *** |
| *Pycnopsyche.guttifer* | 0.232 | 0.0001 *** |
| *Eccoptura.xanthenes* | 0.199 | 0.0004 *** |
| *Acentrella*.spp | 0.129 | 0.0396 * |
| *Perlesta*.spp | 0.187 | 0.0007 *** |
| *Cheumatopsyche*.spp | 0.187 | 0.0022 ** |
| *Pycnopsyche*.spp | 0.184 | 0.0015 ** |
| *Telagonopsis.deficiens* | 0.183 | 0.0002 *** |
| *Paraleptophlebia*.spp | 0.178 | 0.0025 ** |
| *Diplectrona.modesta* | 0.174 | 0.0012 ** |
| *Triaenodes.tardus* | 0.169 | 0.0040 ** |
| *Brachycercus*.spp | 0.166 | 0.0055 ** |
| *Hexagenia*.spp | 0.156 | 0.0106 * |
| *Pteronarcys*.spp | 0.148 | 0.0100 ** |
| *Lepidostoma*.spp | 0.144 | 0.0085 ** |
| *Polycentropus*.spp | 0.140 | 0.0262 * |
| *Acroneuria.abnormis* | 0.140 | 0.0238 * |
| *Eurylophella.temporalis* | 0.139 | 0.0098 ** |

| | | |
|---|---|---|
| *Leuctra*.spp | 0.139 | 0.0100 ** |
| *Neophylax.atlanta* | 0.138 | 0.0158 * |
| *Hydropsyche.sparna* | 0.134 | 0.0311 * |
| *Isoperla.holochlora* | 0.134 | 0.0118 * |
| *Acentrella.ampla* | 0.132 | 0.0121 * |
| *Baetis.tricaudatus* | 0.131 | 0.0213 * |
| *Rhyacophila.carolina* | 0.127 | 0.0252 * |
| *Habrophlebiodes*.spp | 0.122 | 0.0482 * |
| *Heptagenia.marginalis* | 0.119 | 0.0436 * |
| *Triaenodes.melaca* | 0.126 | 0.0406 * |
| *Plauditus.dubius* | 0.126 | 0.0469 * |
| *Amphinemura*.spp | 0.123 | 0.0311 * |
| *Dannella.simplex* | 0.114 | 0.0379 * |
| | | |
| **High %IC + Mid %IC** | **stat** | **P value** |
| *Baetis.intercalaris* | 0.184 | 0.0032 ** |
| | | |
| **Low %IC + Mid %IC** | **stat** | **P value** |
| *Stenonema.modestum* | 0.300 | 0.0001 *** |
| *Centroptilum*.spp | 0.154 | 0.0048 ** |
| *Helicopsyche.borealis* | 0.159 | 0.0057 ** |

**Table A5.** Traits Associated %IC (LowIC<10; MidIC 10-25; High>25%)

| **High %IC (IC>25%)** | **stat** | **P value** |
|---|---|---|
| EPR | 0.324 | 0.0001 *** |
| ADRF | 0.134 | 0.0410 * |
| | | |
| **Mid %IC (10-25%)** | **stat** | **P value** |
| CLB | 0.25 | 0.0001 *** |
| | | |
| **Low %IC (<10%)** | **stat** | **P value** |
| RDRF | 0.443 | 0.0001 *** |
| WKSW | 0.439 | 0.0001 *** |
| GARM | 0.401 | 0.0001 *** |
| CLD | 0.399 | 0.0001 *** |
| EAB | 0.373 | 0.0001 *** |
| WFLY | 0.358 | 0.0001 *** |
| WRM | 0.356 | 0.0001 *** |
| LDIS | 0.354 | 0.0001 *** |
| HCW | 0.352 | 0.0001 *** |
| NATT | 0.321 | 0.0001 *** |
| UV | 0.289 | 0.0001 *** |
| TEG | 0.287 | 0.0001 *** |
| SH | 0.273 | 0.0001 *** |
| SPL | 0.261 | 0.0002 *** |
| CLG | 0.256 | 0.0001 *** |
| DEP | 0.255 | 0.0001 *** |

|  |  |  |
|---|---|---|
| STR | 0.253 | 0.0001 *** |
| PD | 0.248 | 0.0001 *** |
| SAB | 0.246 | 0.0002 *** |
| MD | 0.245 | 0.0001 *** |
| SSE | 0.241 | 0.0002 *** |
| DE | 0.228 | 0.0005 *** |
| SLF | 0.225 | 0.0004 *** |
| LCW | 0.223 | 0.0002 *** |
| SPR | 0.220 | 0.0001 *** |
| NARM | 0.213 | 0.0005 *** |
| GIL | 0.212 | 0.0006 *** |
| LG | 0.206 | 0.0002 *** |
| PARM | 0.206 | 0.0005 *** |
| COL | 0.203 | 0.0012 ** |
| PSY | 0.198 | 0.0015 ** |
| CDRF | 0.169 | 0.0069 ** |
| FSE | 0.166 | 0.0104 * |
| SV | 0.155 | 0.0046 ** |
| LLF | 0.149 | 0.0101 * |
| WSY | 0.189 | 0.0014 *** |
| NOSTR | 0.220 | 0.0008 *** |
| BRW | 0.155 | 0.0082 * |
| VSLF | 0.189 | 0.0026 ** |
|  |  |  |

| **High %IC + Low %IC** | **stat** | **P value** |
|---|---|---|
| ERO | 0.201 | 0.0012 ** |
| NSW | 0.198 | 0.0013 ** |
| SATT | 0.197 | 0.0015 ** |
| CF | 0.188 | 0.0028 ** |
| HDIS | 0.186 | 0.0034 ** |
| SFLY | 0.174 | 0.0063 ** |
| | | |
| **Low %IC + Mid %IC** | **stat** | **P value** |
| HB | 0.324 | 0.0001 *** |
| DEP | | 0.0337 * |

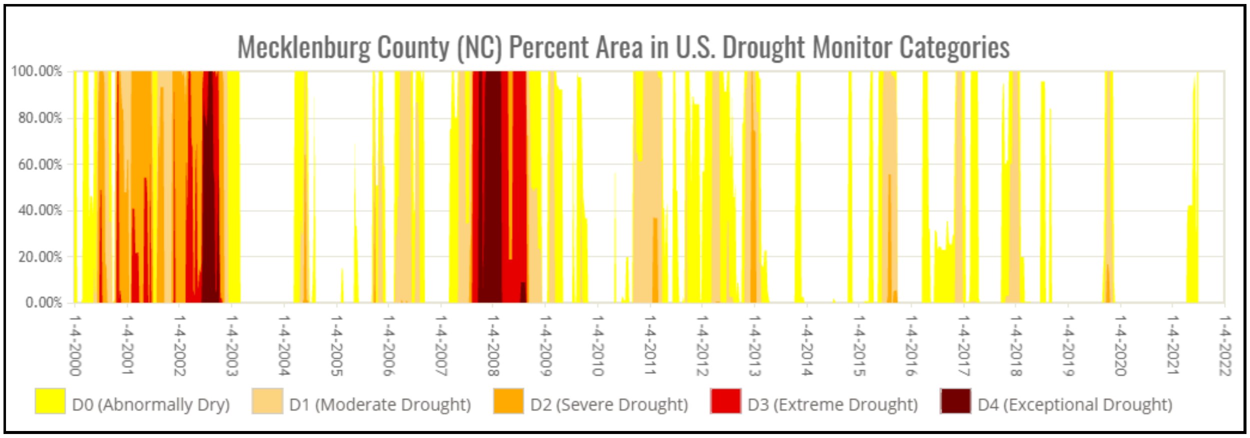


**Figure A1**. Drought History in Mecklenburg County, NC from 2000 through 2021. (https://droughtmonitor.unl.edu/DmData/TimeSeries.aspx)

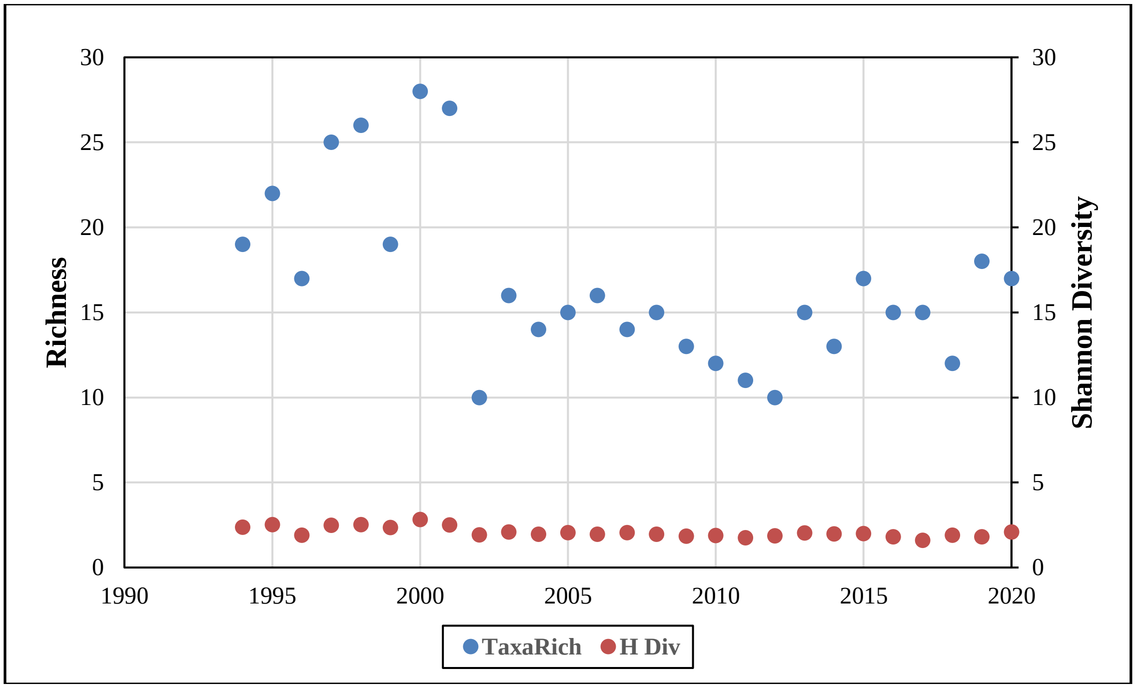


**Figure A2**. Taxa richness and diversity in Gar Creek (site MC50). Taxa richness declined significantly after the 2003 and 2008 droughts. Taxa diversity changed very little after each drought.

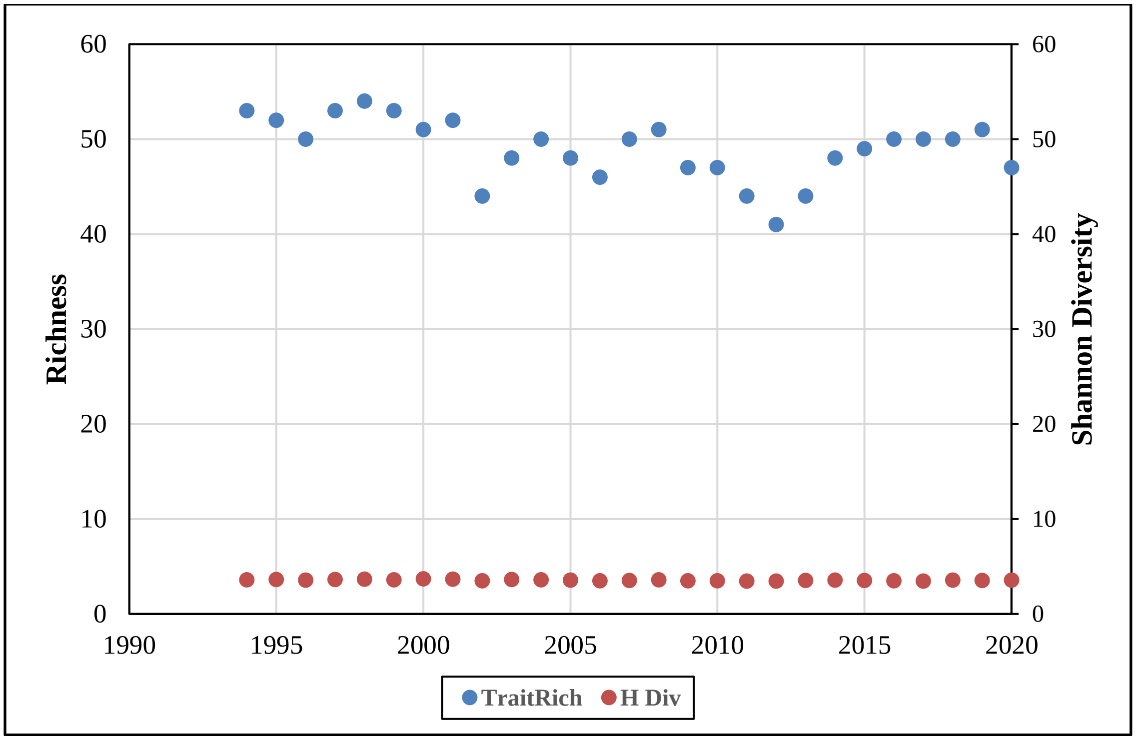


**Figure A3**. Trait richness and diversity in Gar Creek (site MC50). Trait richness declined significantly after the 2003 and 2008 droughts. Taxa diversity changed very little after each drought.

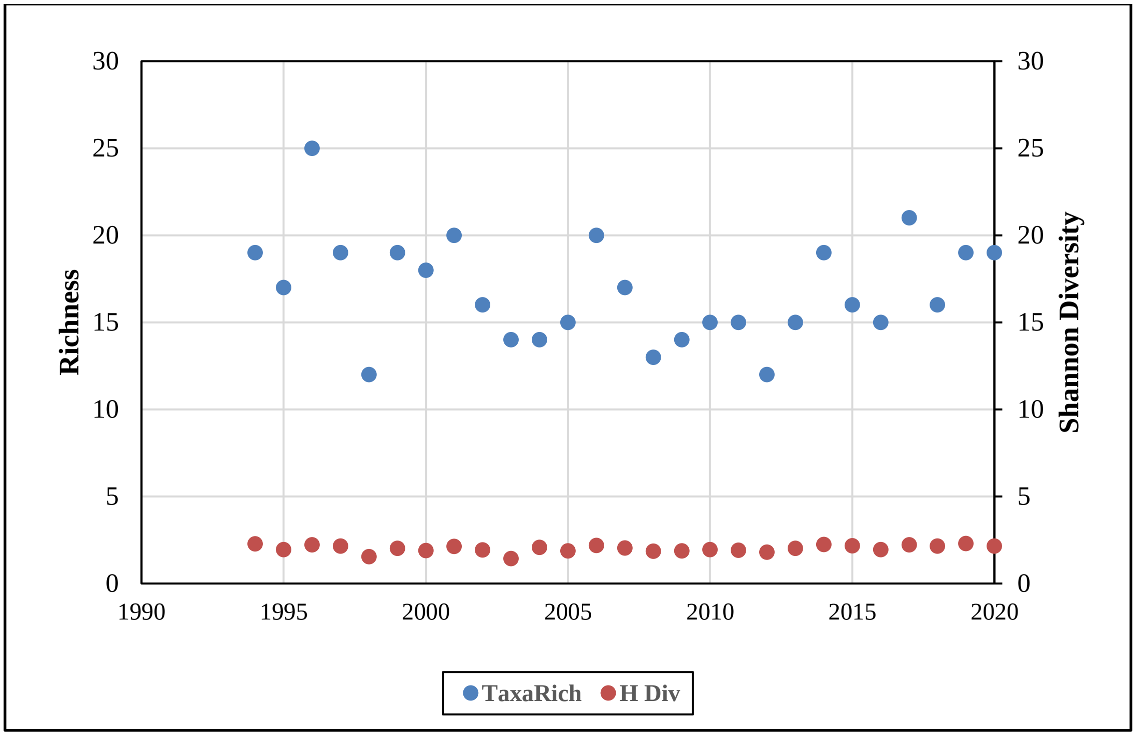


**Figure A4**. Taxa richness and diversity in Clear Creek (site MY8). Taxa richness declined after the 2003 and 2008 droughts. Taxa diversity changed very little after each drought.

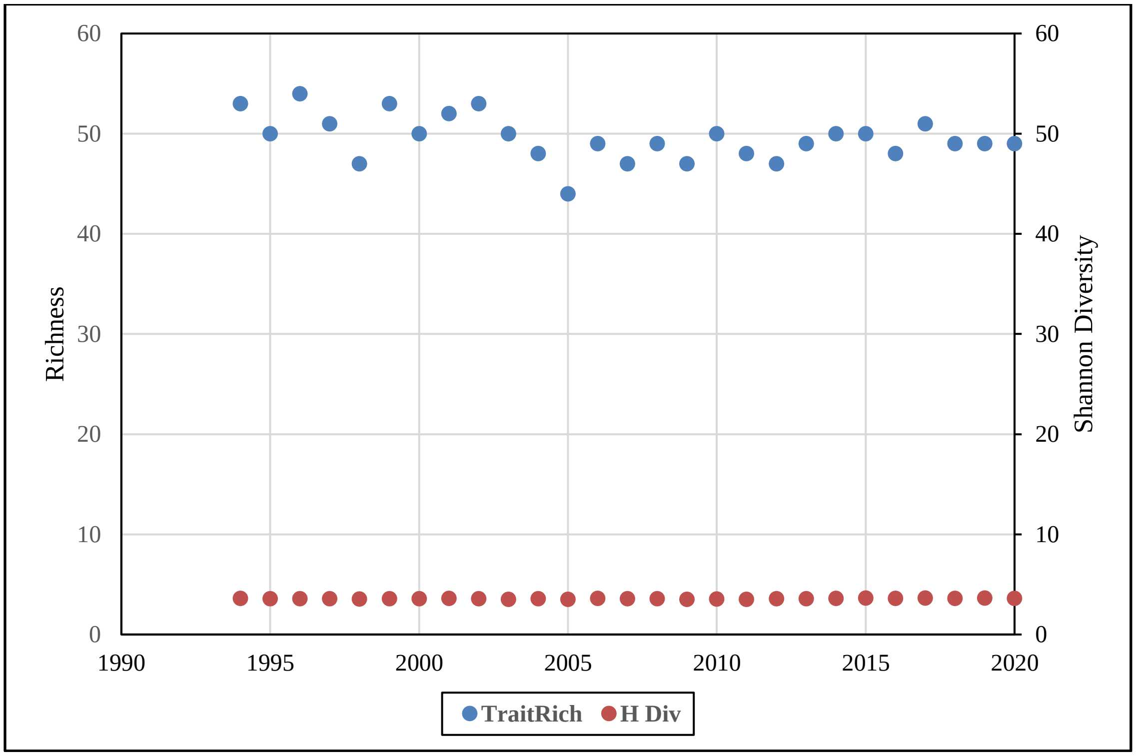


**Figure A5**. Trait richness and diversity in Clear Creek (site MY8). Trait richness declined slightly after the 2003 and 2008 droughts. Trait diversity changed very little after each drought.

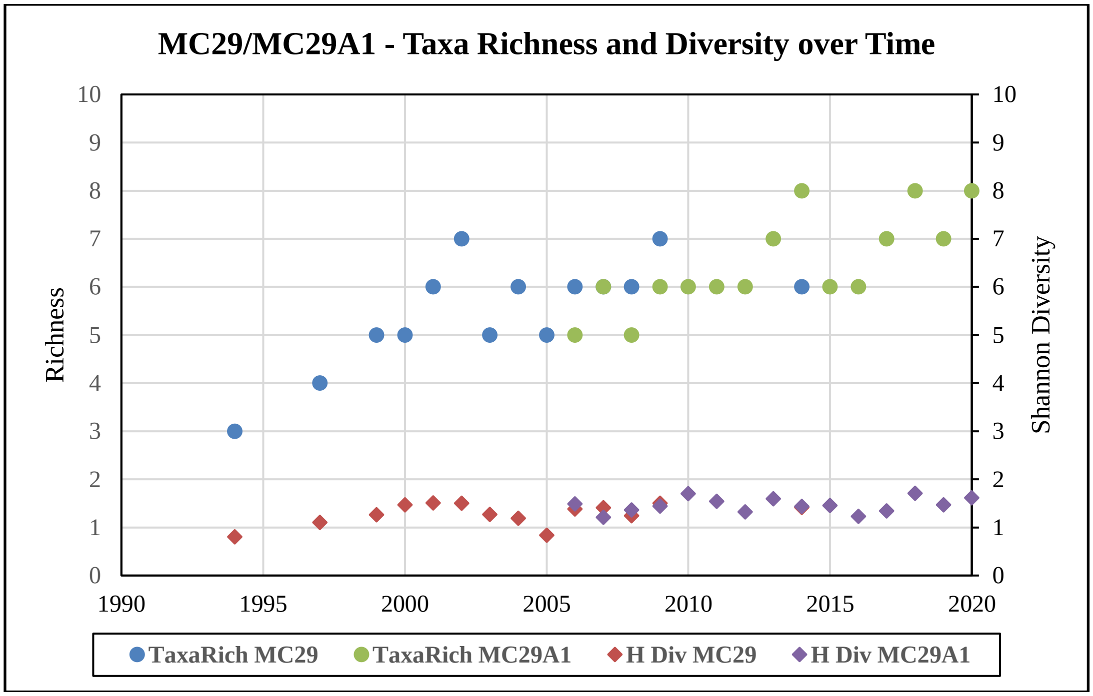


**Figure A6**. Taxa richness and diversity in Little Sugar Creek (sites MC29 and MC29A1). The 2003 and 2008 droughts did not impact taxa richness and diversity.

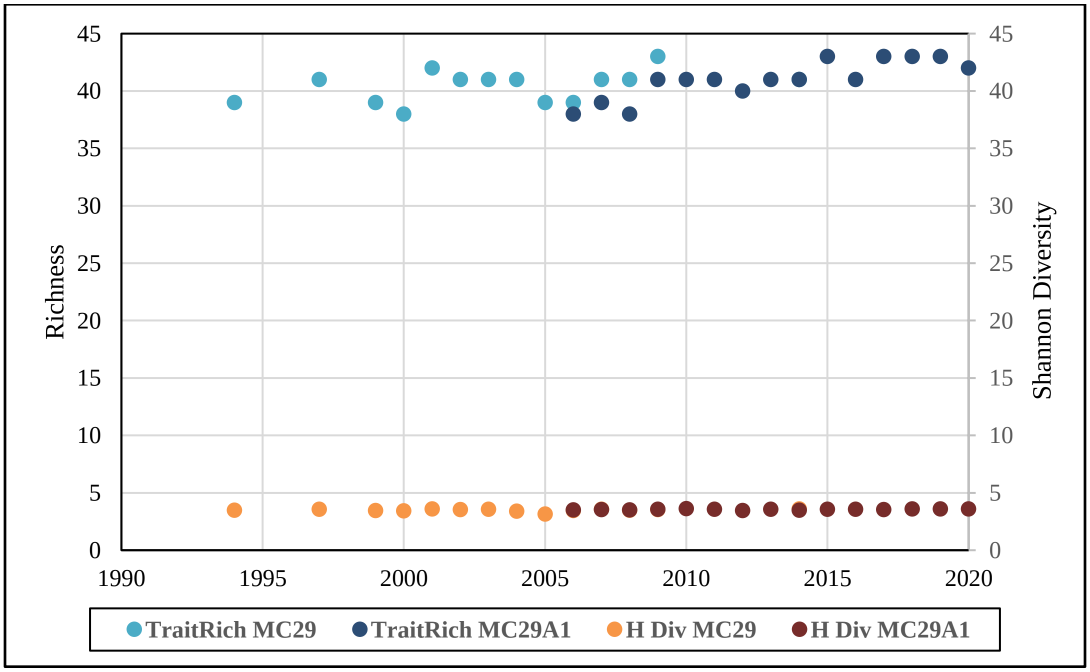


**Figure A7**. Trait richness and diversity in Little Sugar Creek (sites MC29 and MC29A1). The 2003 and 2008 droughts did not impact trait richness and diversity.

# CHAPTER 3: EVALUATION OF THE RELATIONSHIP BETWEEN STREAM HABITAT QUALITY AND TAXA AND TRAIT RICHNESS AND DIVERSITY IN PIEDMONT STREAMS IN NORTH CAROLINA

## 3.1 ABSTRACT

As impervious cover increases with development, stream channels are degraded by increased stormwater runoff volume and intensity. The Urban Stream Syndrome describes a predictable collection of negative impacts of urbanization that alter natural hydrology and subsequently impacts channel geomorphology, stream habitat diversity and stability, and stream biota. To understand better the impact of urbanization on biodiversity and ecosystem function, I proposed to examine the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity at the watershed scale. I investigated: 1) How taxa and trait richness and diversity respond to decreases in stream habitat condition. I hypothesized that the decrease in total taxa richness and diversity would be greater than the decrease in total trait richness and diversity with increasing habitat diversity. My second objective was to quantify the relationship between taxa and trait richness and diversity and microhabitats at the reach level in 10 streams with high habitat quality. I investigated: 2) How taxa and traits are distributed within and between the microhabitats found in streams. I hypothesized that similar traits are found among the aquatic insect taxa residing in similar microhabitats within the same stream.

I sampled 30 streams near Mecklenburg County spanning a gradient of good to poor habitat quality and percent impervious cover ranging from 4.8–97.8% to answer the watershed level questions. Ten of these streams, with Good to Excellent NCBI scores, good habitat conditions, and impervious cover ranging from 4.8 to 10.9%, were selected for the reach level study. I sampled benthic macroinvertebrates in the 20 sites in Mecklenburg County streams

during the summer of 2015 using the qualitative assessment methods developed by the NC Department of Environmental Quality. In the 10 streams with high quality habitat, I collected benthic macroinvertebrates from 7 microhabitats (riffles, root wads, leaf packs, woody debris, undercut banks, backwater, and sandy areas) using a quantitative approach to correlate taxa and their traits with each microhabitat. At each stream site, I quantified the stream habitat quality using the Mecklenburg Habitat Assessment Protocols (EMHAP). The habitat quality of the rural streams was rated as Partially Supporting to Supporting, receiving habitat scores ranging from 137 to 168. In comparison, impaired urban streams in Charlotte, NC received an average MHAP score of 86.4.

Streams with fully supporting habitat conditions were more like each other than to partially supporting and impaired streams when described by taxa abundance; however, fully supporting and partially supporting streams were more similar when described by trait abundance. As EMHAP scores declined, habitat diversity had greater variability in the partially supporting and impaired sites. I found that both taxa richness and diversity declined at a greater rate than trait richness and diversity along an in-stream habitat diversity gradient. Trait richness and diversity changed very little with changes in habitat condition. The impaired streams generally had lower habitat diversity than both the partially supporting and supporting streams. The supporting streams' habitats were more heterogeneous, sustaining a more complex benthic macroinvertebrate community.

Taxa richness was positively correlated with pools, runs, backwater, leaf packs, and riffles, while trait richness was positively correlated with runs, small wood, and riffles. Taxa diversity was positively correlated with pools and leaf packs while trait diversity was positively correlated with runs, leaf packs, small wood, and riffles.

Riffles, leaf packs, large wood, and runs were important microhabitats for taxa richness while undercut banks were important for taxa diversity, and habitat diversity and small wood were important for trait richness. The taxa and traits found in riffles and leaf packs were more similar than taxa and traits found in undercut banks and root wads. This may be due to the differences observed in the stream flow velocities within the microhabitats. I found the highest taxa richness and diversity in the riffles. Functional redundancy was higher in urban streams with lower habitat diversity which indicated that trait composition was not changing with habitat condition and reflected the resiliency of the community trait composition. The benthic macroinvertebrates in the urban streams are living in the similar types of habitats that favor similar traits.

Indicator-species analysis revealed that there were specific taxa and traits that were associated with specific microhabitats or combinations of microhabitats. While most traits were associated with riffles, several traits were significantly associated with other microhabitats such as root wads (CLB) and leaf packs (PLA).

## 3.2 INTRODUCTION

Stream habitat quality and aquatic insect community diversity are negatively impacted by urbanization. Walsh et al. (2005) described a predictable collection of negative impacts of urbanization that alter natural hydrology and subsequently impacts channel geomorphology and stream biota. Collectively, this group of predictable impacts to stream channel geomorphology and stream biota has been labeled as the Urban Stream Syndrome (Walsh et al. 2005). As Percent Impervious Cover (% IC) increases with development, stream channels are degraded by increased stormwater runoff volume and intensity (Schueler 1994; Bledsoe and Watson 2001; Paul and Meyer 2001; Center for Watershed Protection 2003; Coleman et al. 2011; Vietz et al. 2016). Rabeni and Minshall (1977) found that particle size, stream flow, and silt deposition impacted the habitats occupied by aquatic insects. Habitat critical for fish and aquatic insects is impaired by urbanization (Paul and Meyer 2001; Roy et al. 2003; Coleman et al. 2011).

The response of watershed managers to urban stream degradation is to return the stream to a more natural condition through stream restoration techniques such as the natural channel design method (Rosgen 1998; Doll et al. 2003). A general assumption for stream restoration projects has been that restoring channel geomorphology to resemble an undisturbed stream would result in the recovery of the aquatic insect community (Sudduth et al. 2011). Unfortunately, most natural channel design approaches do not result in an uplift of the aquatic insect community diversity and function (Suren and McMurtrie 2005; Bernhardt and Palmer 2007; Palmer et al. 2010; Stranko et al. 2012). Violin et al. (2011) found no significant difference in aquatic insect communities in urban restored and urban unrestored streams while at the same time they found significant differences between the urban streams and a forested undisturbed stream. Sudduth et al. (2011) found no differences in stream metabolism in urban-unrestored, urban-restored, and forest streams, but did find that urban-restored streams had higher summertime nitrate uptake kinetics than the urban-unrestored and forested streams due to higher instream temperatures due to canopy removal during restoration. Several hypotheses have been suggested to explain why macroinvertebrate communities do not improve, including (1) urban infrastructure constraints limiting restoration options (Bernhardt and Palmer 2007), (2) restoration scale being too small to have an impact (Sudduth and Meyer 2006; Bernhardt and Palmer 2007; Palmer et al. 2010; Violin et al. 2011; Hering et al. 2015), and (3) relatively little attention being given to managing upstream influences from the watershed, including increases in stormwater volume and intensity (Walsh et al. 2005; Poff et al. 2006b; Richardson et al. 2011; Walsh et al. 2012; Walsh et al. 2016).

A healthy stream ecosystem has been described as having a high diversity of taxa (Hynes 1970; Cummins 1974). A diversity of habitats with heterogeneous substrates is important for a stream to support a high diversity of aquatic insect species and their associated functional traits (Cummins and Lauff 1969; Rabeni and Minshall 1977; Erman and Erman 1984; Wohl et al. 1995; Beisel et al. 2000; Lamouroux et al. 2004; Milesi et al. 2016; Verdonschot et al. 2016) since each species possesses traits that reflect adaptations to the specific microhabitats where they are found (Lamouroux et al. 2004). Species traits have been used to characterize the functional composition of aquatic insect communities (Poff et al. 2006a) including Life History (rate of development), Mobility (ability to fly, crawling rate), Morphology (size, shape, armoring), and Ecology (habitat preference, thermal preference, feeding preference).

Biodiversity has been shown to be correlated to changes in ecosystem function in both terrestrial (Naeem et al. 1994; Tilman 1997; Naeem 1998; Cardinale et al. 2006a; Cardinale et al. 2006b; Cadotte et al. 2011; Cardinale 2012) and aquatic (Giller et al. 2004; Lamouroux et al. 2004; Pool et al. 2016) communities. Functional diversity is the diversity of organismal traits that impact ecosystem functioning (Tilman 2001; Schleuter et al. 2010). Tilman (2001) further defined functional diversity as the component of biodiversity which drives ecosystem dynamics such as productivity, nutrient cycling, stability, and community composition. The habitat template theory states that species found within a specific habitat or even smaller microhabitats are those species that have been filtered from a larger regional species pool based on the functional traits that enable those species to survive and reproduce in that habitat (Southwood 1977; Poff and Ward 1990; Townsend and Hildrew 1994). Lamouroux et al. (2004) supported the habitat template theory proposition that habitats with similar characteristics should support species with similar functional traits. This suggests that similar microhabitats should be able to support different species with similar functional traits. Functional redundancy, an important component of ecosystem resilience, occurs when more than one species shares one or more functional traits (Lamothe et al. 2018). The species redundancy hypothesis proposes that within a functional group that contains many species, it is the species redundancy that provides ecosystem stability (Walker 1992). Kang et al. (2015) found that functional redundancy, instead of species diversity or species redundancy, is correlated with ecosystem stability.

### 3.2.1 STUDY OBJECTIVES AND RESEARCH QUESTIONS

Urban streams are presented with predictable problems that are largely due to the alteration of the natural hydrologic regime (Walsh et al. 2005). Numerous researchers have shown that stream restorations that just address geomorphological stream channel characteristics, without considering stream functional traits and the ecological requirements of the aquatic insects, fail to stimulate the recovery of the aquatic insect community (Shields et al. 2003; Palmer et al. 2005; Suren and McMurtrie 2005; Sudduth et al. 2011). To inform restoration design and implementation more fully, I examined the relationship between stream habitat quality and aquatic insect community diversity and function with 2 overall objectives.

My first objective was to examine the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity by evaluating 30 streams in the Piedmont, North Carolina, spanning a gradient of good to poor habitat quality at the watershed scale.

**Q3.1. How do taxa and trait richness and diversity respond to decreases in stream habitat condition?** I hypothesized that the decrease in total taxa richness and diversity would be greater than the decrease in total trait richness and diversity with decreasing habitat diversity. I also expected that the diversity of traits is positively correlated with habitat diversity.

My second objective was to quantify the relationship between taxa and trait richness and diversity and microhabitats at the reach level in 10 streams with high habitat quality. While several taxa traits may be found commonly throughout the various stream microhabitats, adaptations of species to specific microhabitats may result in a sensitivity of the community to loss of taxa traits with loss of microhabitats. The redundancy of functional traits among microhabitats will make stream ecosystems more resilient to change when a few species with redundant functions are lost due to some disturbance.

**Q3.2. How are taxa and traits distributed within and between the microhabitats found in streams?** I hypothesized that similar traits are found among the aquatic insect taxa residing in similar microhabitats within the same stream. Furthermore, I hypothesized that the diversity of traits is positively correlated with the diversity of microhabitats.

## 3.3 METHODS

### 3.3.1 STUDY SITES

Thirty streams near Mecklenburg County, North Carolina, spanning a gradient of good to poor habitat quality and percent impervious cover ranging from 4.8–78.5% were identified (Figure 3.1) to answer the watershed level questions. Ten of these streams, with Good to Excellent NCBI scores, good habitat conditions, and impervious cover ranging from 4.8% to 10.9%, were selected for the reach level study (Eric Fleek, NCDEQ Bioassessment Team – personal communication). The annual precipitation in Mecklenburg County in 2015 was 125.7 cm, which was 19.9 cm above normal. However, this rainfall total was not very different from the average rainfall of 112.4 inches for the previous 3 years. The annual average temperature in Mecklenburg County in 2015 was 17.0$^{\circ\mathrm{C}}$ which was approximately normal (15.5°$^{\mathrm{C}}$) (https://www.weather.gov/gsp/cltcli). Watershed size, percent impervious cover, EMHAP scores and location are summarized in Table 3.1. General environmental data for each site is presented in Appendix Table A6.

Stream habitat conditions were assessed at all 30 sites using EMHAP which were based on the USEPA Rapid Bioassessment Protocols (Barbour et al. 1999). EMHAP evaluates 10 habitat condition parameters including instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, frequency of riffles, channel flow status, bank vegetation protection, bank stability, and vegetative riparian zone width (Appendix Table A2; CMSWS 2020a; CMSWS 2020b). EMHAP scores have been associated with stream habitat conditions ranging from degraded (<60) to fully supporting (≥160). EMHAP scores ranged from 58.3 - 169.7 across all sites and 137.3 - 169.7 for the 10 reach scale sites (CMSWS 2015 annual monitoring data). This analysis gives a broad picture of the impact of urbanization on stream habitat conditions and functional trait diversity.

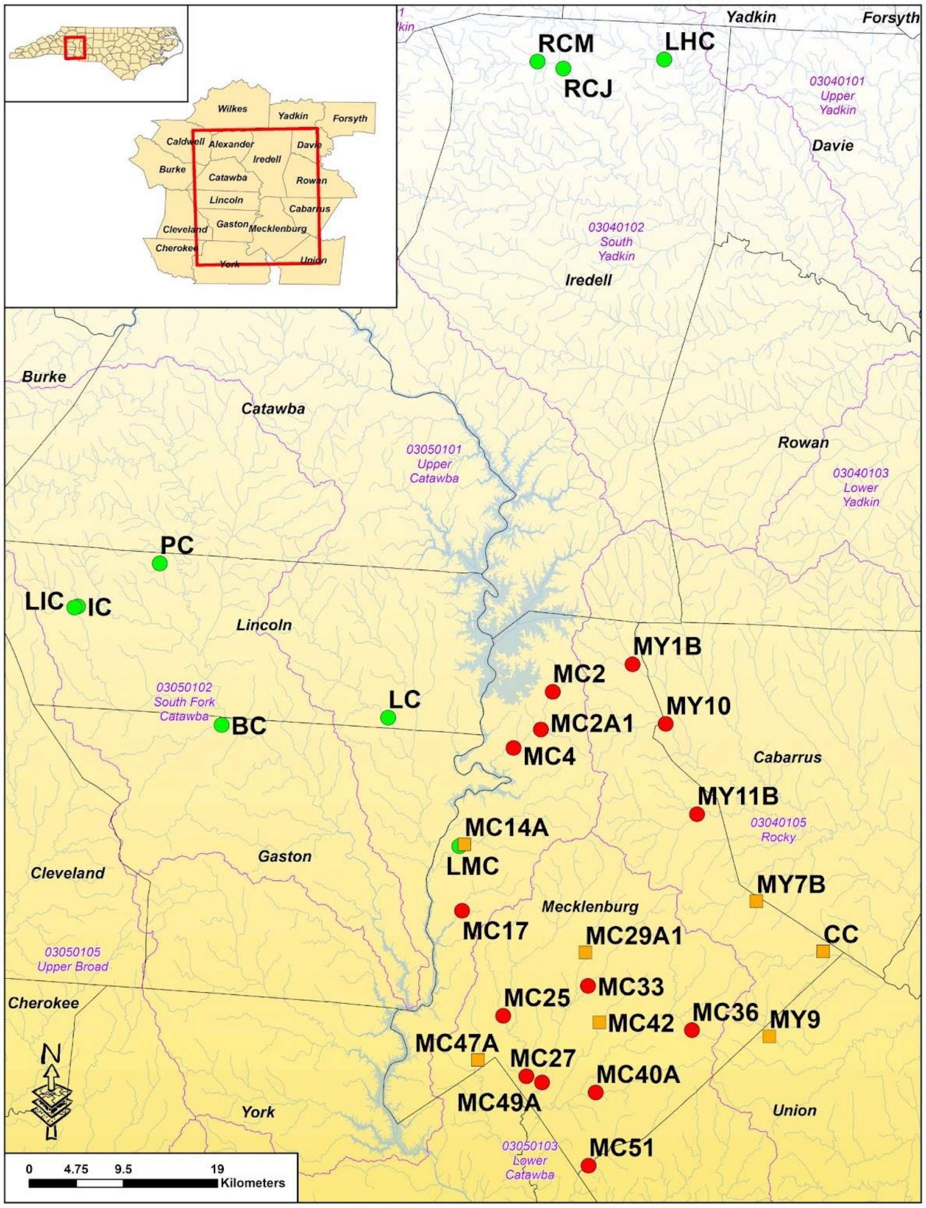


**Figure 3.1**. Map of Rural and Urban Stream Study Sites in Mecklenburg, Lincoln, and Iredell Counties in Piedmont North Carolina. *Stream Habitat Conditions*: Supporting (green); Partially Supporting (Orange); Impaired (Red).

**Table 3.1.** Stream Sites in Piedmont, North Carolina**.** The first 10 sites listed were used to collect reach scale data.

| Stream | Site | Drainage Area (mi$^2$) | 2014 % Impervious Cover | 2015 EMHAP | Latitude | Longitude |
|---|---|---|---|---|---|---|
| **L Indian Cr** | LIC | 12.0 | 8.06 | 169.7 | 35.509 | -81.413 |
| **Rocky Cr M** | RCM | 38.3 | 4.84 | 168.0 | 36.016 | -80.908 |
| **Leeper's Cr** | LC | 53.1 | 10.9 | 165.7 | 35.415 | -81.061 |
| **Rocky Cr J** | RCJ | 42.5 | 4.81 | 165.7 | 36.010 | -80.880 |
| **Long Cr** | LCM | 32.4 | 65.2 | 163.3 | 35.299 | -80.979 |
| **L Hunting Cr** | LHC | 54.5 | 9.11 | 160.3 | 36.020 | -80.767 |
| **Indian Cr** | IC | 5.67 | 6.17 | 156.0 | 35.510 | -81.409 |
| **Pott Cr** | PC | 21.1 | 6.68 | 148.0 | 35.551 | -81.318 |
| **Beaverdam** | BC | 22.8 | 10.2 | 145.0 | 35.405 | -81.246 |
| **Clear Cr** | CC | 12.8 | 24.0 | 137.3 | 35.209 | -80.573 |
| **Sugar Cr** | MC27 | 64.7 | 63.0 | 109.7 | 35.090 | -80.899 |
| **Long Cr** | MC14A | 31.9 | 35.06 | 108.3 | 35.300 | -80.973 |
| **Steele Cr** | MC47A | 7.04 | 57.3 | 104.7 | 35.105 | -80.954 |
| **Goose Cr** | MY9 | 8.68 | 10.99 | 104.3 | 35.130 | -80.631 |
| **West Rocky R** | MY1B | 33.4 | 8.85 | 101.0 | 35.468 | -80.790 |
| **McKee Cr** | MY7B | 5.8 | 15.99 | 99.7 | 35.254 | -80.648 |
| **Little Sugar Cr** | MC29A1 | 12.0 | 78.19 | 98.0 | 35.203 | -80.837 |
| **Little Sugar Cr** | MC49A | 49.4 | 78.5 | 96.0 | 35.085 | -80.882 |
| **Sixmile Cr** | MC51 | 20.5 | 33.91 | 95.0 | 35.010 | -80.828 |
| **McDowell Cr** | MC2 | 6.8 | 48.02 | 88.7 | 35.442 | -80.878 |
| **Briar Cr** | MC33 | 18.9 | 48.53 | 85.0 | 35.254 | -80.648 |
| **McMullen Cr** | MC42 | 7.06 | 39.02 | 82.7 | 35.141 | -80.820 |
| **McDowell Cr** | MC2A1 | 10.2 | 42.78 | 82.3 | 35.407 | -80.891 |
| **McDowell Cr** | MC4 | 26.5 | 32.26 | 79.0 | 35.389 | -80.921 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Clarke Cr** | MY10 | 21.8 | 10.99 | 73.7 | 35.414 | -80.752 |
| **Mallard Cr** | MY11B | 34.7 | 38.95 | 71.3 | 35.332 | -80.716 |
| **Coffey Cr** | MC25 | 8.7 | 39.33 | 66.3 | 35.146 | -80.927 |
| **Irvins Cr** | MC36 | 2.72 | 53.09 | 66.3 | 35.134 | -80.717 |
| **Fourmile Cr** | MC40A | 18.0 | 33.45 | 59.3 | 35.077 | -80.822 |
| **Paw Cr** | MC17 | 10.4 | 38.81 | 58.3 | 35.240 | -80.974 |

### 3.3.2 AQUATIC INSECTS

Aquatic insects were collected from a 100-meter segment at 20 of the 30 sites by CMSWS during July through October 2015 using the Standard Qualitative Method developed by North Carolina Department of Environmental Quality (NCDEQ) Biological Assessment Unit (NCDEQ 2016) and described in the Mecklenburg County Bioassessment Standard Operating procedures (CMSWS 2017). The Standard Qualitative Method collections consists of 2 kick net riffle samples, 3 sweep-net samples from microhabitats found within runs and pool such as root wads, soft sediment in undercut bank areas, woody debris, macrophyte beds, and overhanging vegetation, and 1 leaf-pack sample, 2 rock and/or log wash samples, and visual collections. The aquatic insects in these samples were sorted in the field and preserved in glass vials containing 95% ethanol. All aquatic insects were identified to the lowest possible taxonomic level.

At the 10 rural sites, aquatic insects were collected quantitatively from 8 microhabitats, including riffles, root wads, undercut banks, woody debris, leaf packs, backwater, macrophyte beds, and sandy areas found in the 100-meter study reach. A *riffle* is an area in a stream where water moves quickly over hard substrates (boulders, cobble, gravel, bedrock, and woody debris) resulting in a riffling effect on the water surface. *Root wads* are tree or other plant roots exposed along the stream channel edges providing habitat for benthic macroinvertebrates and fish. An *undercut bank* area is where the wetted channel of the stream flows underneath the stream bank itself due to erosion. The stream bank forms a roof ledge over the channel itself. *Woody debris* are any stable branches, fallen trees, logs, stumps, or log jams that are present in the stream and could be used as habitat by fish or benthic macroinvertebrates. A *leaf pack* is a decomposing clump of leaves caught behind an obstruction such as a rock or piece of woody debris in the stream channel. Only seasoned leaves that have a slimy layer of bacteria on their surface are suitable habitat, for it is the bacterial growth on the leaves that attract benthic macroinvertebrate shredders. A *backwater area* is a pool adjacent to one of the banks and is located behind an obstruction such as a sand bar or woody debris or a topographical feature such as bedrock where water accumulates and does not follow the natural flow of the stream. *Macrophyte beds* are areas where aquatic vegetation grows in the stream substrate and is submerged or emergent, often floating on the surface of the stream or extending above the stream. *Sandy areas* are located along the margins of the stream where slower currents allow silt and soft sediment to accumulate.

Quantitative samples of the aquatic insects found in each microhabitat were collected during July through August 2015. Within a representative riffle within the study reach at each site, a 1- by 3-meter area was sampled using a kick net. The substrate within this area was thoroughly disturbed with each rock rubbed to dislodge aquatic insects. The substrate was also visually inspected for organisms still attached. The root wads, undercut banks, leaf packs, backwater, macrophyte beds, and sandy areas were sampled using a Surber sampler through which a 0.25 $m^2$ area can be sampled.  The substrate within the area isolated by the Surber sampler was thoroughly disturbed.  A visual inspection of the substrate was conducted to collect organisms still attached.  Three Surber samples were taken from each microhabitat for a total of

$0.75\ m^2$ per microhabitat. Each of the Surber samples for each microhabitat was taken from a different area within the study reach to eliminate the effects of patchy distributions of aquatic insects. Woody debris was visually examined using a flexible square made from string the same size as the Surber sampler that could be draped over woody debris of various diameters. Woody debris of various sizes and diameters were sampled for a total of $0.75\ m^2$.

The 3 samples for each microhabitat were composited into 1 sample per microhabitat. The large debris and leaves were removed from each sample in the field. The remaining debris and organisms were preserved in 95% ethanol and sorted in the lab. All organisms were identified to the lowest taxa possible (genus or species). The 10 rural sites were added to the overall data set (20 other sites) by converting the data from quantitative to qualitative using the same protocol as described above. For detailed analysis on the impact of microhabitat on aquatic insect diversity and function the quantitative 10 site data were analyzed independently of the 20-site data. After collection was completed, macrophyte beds were removed from the analysis since they were found in only 4 sites with 8 to 27 taxa and 14 to 75 total organisms collected.

I assigned macroinvertebrate traits using the categories described by Poff et al. (2006a) and summarized in Table 2.1. The Poff dataset contains taxa traits for all aquatic insects except for Bivalvia (clams and mussels), Crustacea (amphipods and crayfish), Gastropoda (snails), Hirudinea (leeches), Oligochaeta (worms), three families of Coleoptera (Gyrinidae, Hydrophilidae, and Ptilodactylidae; beetles), and three families of Diptera (Culicidae, Dixidae, and Tabanidae; flies). I excluded from the analysis taxa that did not have traits associated with them.

### 3.3.3 HABITAT DIVERSITY

At each of the sites, the number of microhabitats, including pools, runs, root wads, undercut banks, woody debris, leaf packs, backwater, and macrophyte beds found in the 100-meter study reach were counted. Because riffles are among the most productive habitats found in streams (Hynes 1970; Allan and Castillo 2007) the length of each riffle was also measured. An index was developed by CMSWS based on EPA stream habitat assessment protocols (Barbour et al. 1999) to incorporate length into the count of riffles number (CMSWS 2020b). This index was used in the calculation of habitat Shannon-Wiener diversity (H′) index. The number of transitions between major habitat types, such as riffles, runs, pools, and backwater areas, was calculated.

## 3.4 DATA ANALYSIS

Each site was assigned as Supporting (≥ 140), Partially Supporting (100 - 139.99), and Impaired (< 100) based on EMHAP score. These EMHAP groups were selected based on the correlation of EMHAP scores with %IC and EPA Rapid Bioassessment ratings (Barbour et al. 1999).

All data analyses were conducted in R, version 4.0.3 (R Core Team 2020). I tested all data for normality using the Shapiro-Wilks Normality test and found that both the watershed and the microhabitat scale macroinvertebrate data were significantly different from normal distribution. Therefore, I chose non-parametric statistics to evaluate differences among groups of parameters. I used non-metric multidimensional scaling (NMDS) analysis to evaluate the relationship of taxa and trait distribution among streams with varying EMHAP scores and among microhabitats (*metaMDS* function in the ‘vegan’ package; Oksanen et al. 2020).

Taxa, trait, and habitat diversity were calculated as Shannon-Wiener Diversity (H′) indices at both the watershed and local scale (*diversity* function in the ‘vegan’ package; Oksanen et al. 2020) . Functional Uniqueness and Functional Redundancy of the taxa traits found in the aquatic insect communities found in each stream were calculated following the methods described by Ricotta et al. (2016). For each microhabitat, the taxa richness, the Rao diversity (Q), the Simpson index (D) and the Functional Redundancy (FR) were calculated using the *diversity* function in the 'vegan' package in R and *dbFD* function in 'FD' package in R (Laliberté and Legendre 2010; Laliberté et al 2014).

To investigate the relationship between taxa and trait metrics and habitat at both scales I used the Kendall Rank Correlation Test (*cor.test* function in the 'stats' package in R; R Core Team 2020). To investigate how these metrics varied with habitat diversity they were plotted as a linear regression and their slopes were compared. To further determine which components of habitat diversity had the greatest contribution to these metrics I used multiple linear regression. The initial set of parameters tested included: number of Pool, Run, Backwater, Root Wad, Undercut Bank, Leaf Pack, Small Wood (3 - 8 cm), Large Wood (>8 cm), Riffle microhabitats, and Habitat Diversity (H′). A stepwise approach was used using the *lm* function in the 'stats' package in R (R Core Team 2020).

At the local scale I tested differences among these metrics (diversity, richness FR) across microhabitats using the nonparametric Kruskal-Wallis rank sum test with the Dunn Test (*dunnTest* function in 'FSA' package in R; Ogle et al. 2020).

To further investigate the impact of microhabitats on the distribution of taxa and traits within a stream, the aquatic insects in the 10 rural sites with better habitat quality were collected quantitatively. However, the aquatic insects in the urban streams were collected qualitatively. I tested for significant differences between microhabitat aquatic insect Shannon-Wiener diversity (H′) of abundance per microhabitat sampling results reported as diversity of total number of organisms per taxa and diversity of number of organisms per m$^2$ per taxa using the Student's T-Test (t.test function in the 'stats' package R; R Core Team 2020). The results indicate that there was no statistical difference between the 2 data sets. To keep the data analysis consistent with the microhabitat and reach scales, it was decided that the total abundance data would be used in the analysis of the macroinvertebrate data as the differences in the total abundance and the abundance per m$^2$ data sets were not significant.

To calculate the percent occurrence of microhabitats each trait was found in, the total number of microhabitat samples a trait was found in was divided by the total number of microhabitats sampled in the study (62). I used the Kruskal-Wallis rank sum test to test the null hypothesis that the percent occurrence of each trait was the same in each microhabitat. The Dunn Test for multiple comparisons was used to determine which traits had significantly different percent occurrences among the microhabitats.

To determine if there are specific taxa associated with specific microhabitats, indicator species analysis was conducted using the multipatt function of the indicspecies R package (multipatt function in the indicspecies package in R; De Caceres and Legendre 2009).

## 3.5 RESULTS

### 3.5.1 TAXA AND TRAIT RICHNESS AND DIVERSITY

As seen in numerous studies (Schueler 1994; Center for Watershed Protection 2003; Schueler et al. 2009 ) the taxa richness in Piedmont North Carolina streams were negatively correlated with increasing watershed % Impervious Cover (%IC) (Appendix Figure A12). Likewise, stream habitat condition, as measured using the Mecklenburg Habitat Assessment Protocols (EMHAP), declined with increasing %IC (Appendix Figure

A13). Therefore, it is not surprising to see that taxa richness increases with EMHAP scores (Figure 3.2). The list of taxa collected from all sites are summarized in Appendix Table A7.

Similar taxa and traits are found in streams with similar stream habitat quality. NMDS plots of both Taxa and Trait abundance show that the taxa and traits found in streams with better stream habitat quality (supporting) are less similar than those found in the streams with poorer habitat quality (partially supporting and impaired) (Figures 3.3 and 3.4).

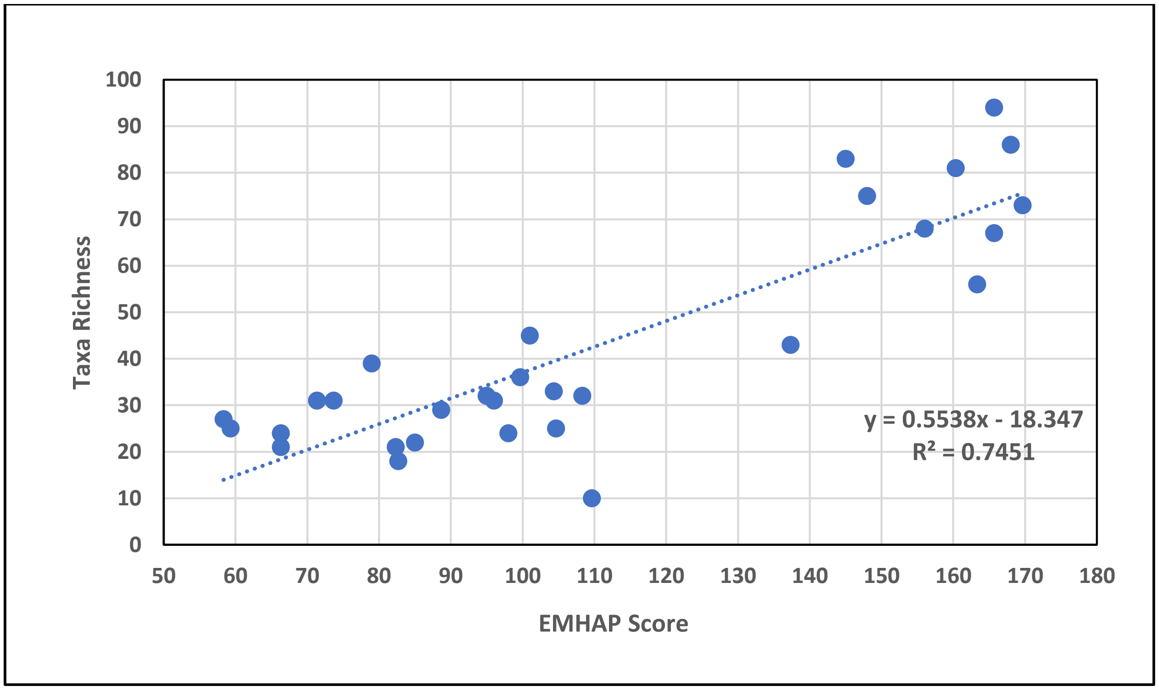


**Figure 3.2**. Taxa Richness increased with Stream habitat condition, as represented by EMHAP scores. (N=30 stream samples).

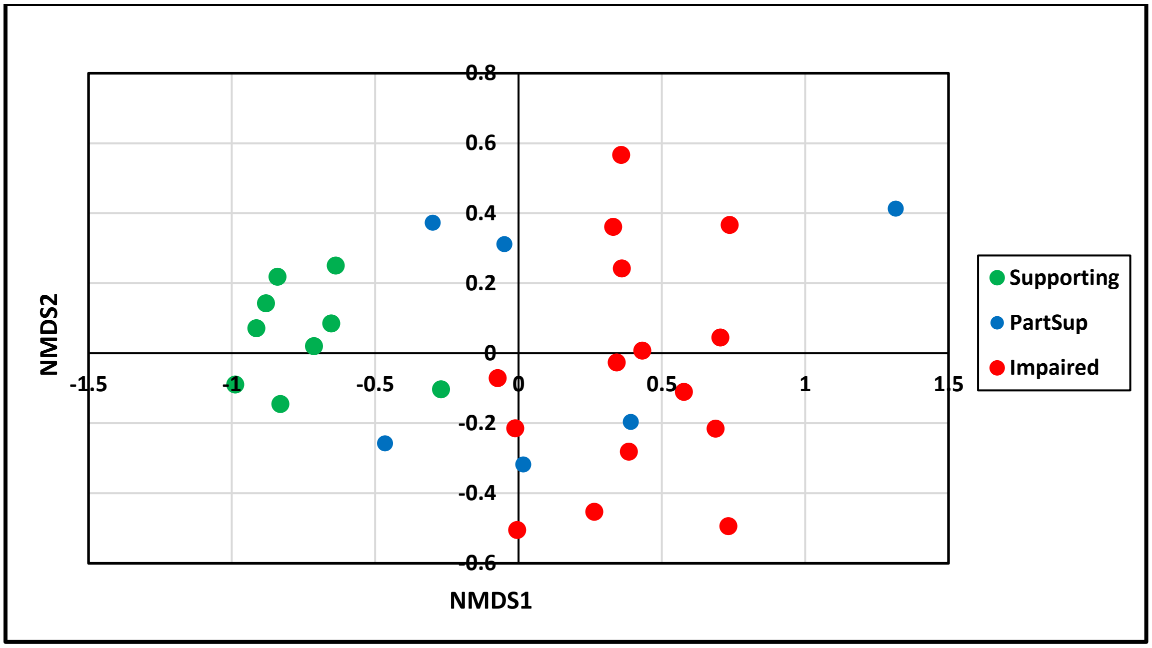


**Figure 3.3**. NMDS plot of Total Taxa Abundance found in streams spanning a gradient of stream habitat condition ranging from Good (supporting) to Poor (Impaired) EMHAP scores. Taxa found in the streams with better habitat conditions are less like the taxa found in the streams with poor habitat conditions. (N=30 stream samples).

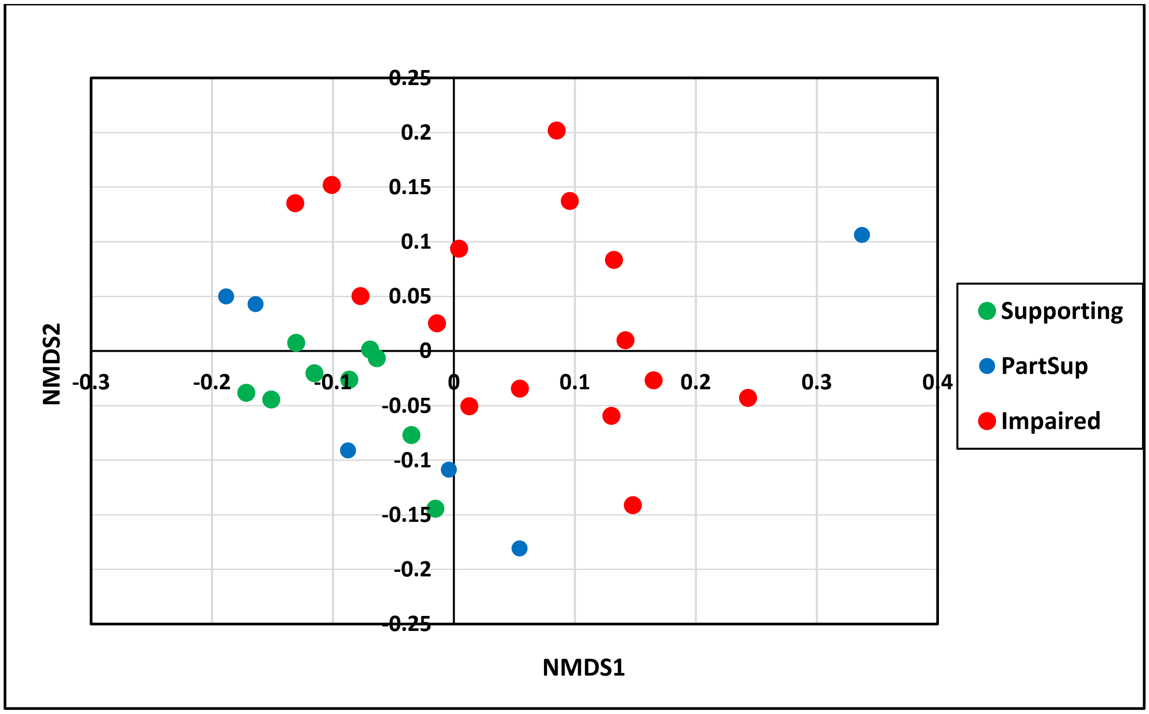


**Figure 3.4**. NMDS plot of Total Trait Abundance found in streams spanning a gradient of stream habitat condition ranging from Good (supporting) to Poor (Impaired) EMHAP scores. The traits found in the streams with better habitat conditions are less like the traits found in the streams with poor habitat conditions. (N=30 stream samples).

Taxa and trait richness are positively correlated with stream habitat condition. Impaired streams generally have lower habitat diversity (Figure 3.5). Streams with high EMHAP scores have high habitat diversity; however, non-supporting streams can also have high diversity indicating that other factors such as watershed and riparian condition can impact streams even though they have good habitat. There were significant positive correlations between taxa richness and diversity (H') and habitat Shannon-Wiener diversity (H') (Table 3.2; Kendall's rank correlation tau = 0.3828 and 0.3314 respectively; $\alpha < 0.01$ and $< 0.05$). While the correlations between trait richness and diversity and habitat Shannon-Wiener diversity (H') were not significant, the correlations were positive.

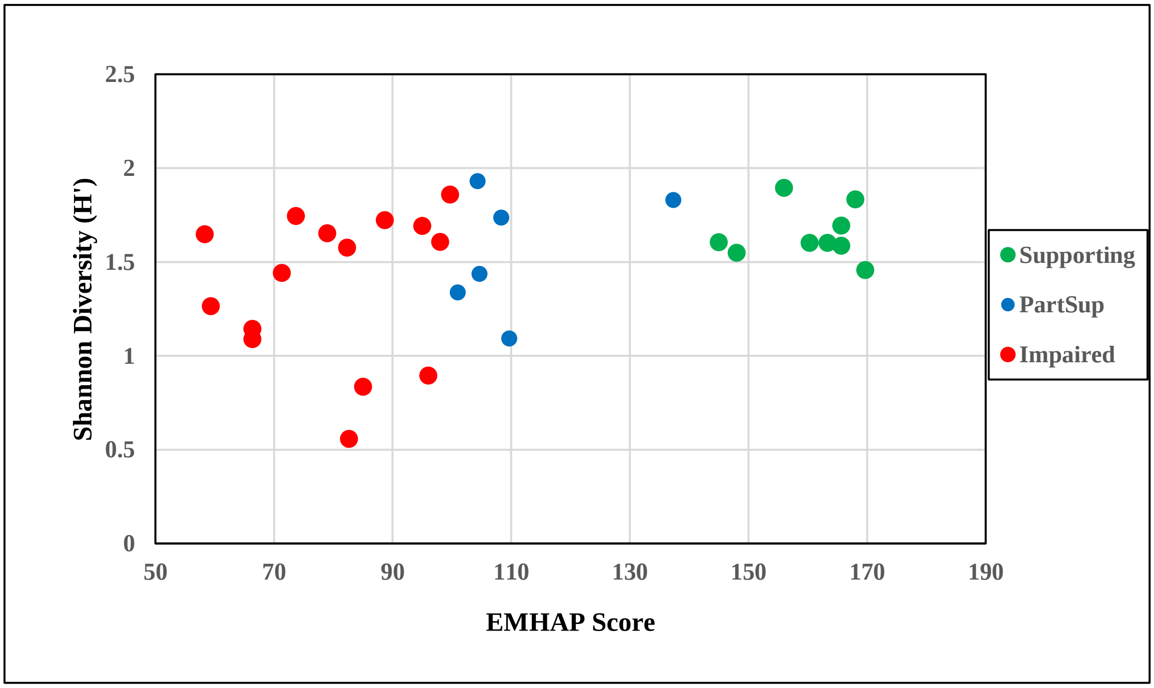


**Figure 3.5**. Habitat diversity by EMHAP scores. Habitat diversity in streams with better stream habitat conditions were less similar than the habitat diversities found in streams with partially supporting and impaired stream habitat conditions. (Supporting ≥ 140, Partially Supporting 100-139, Impaired <100).

**Table 3.2**. The Kendall Rank Correlation Test shows that Taxa Richness and Diversity (H′) are positively correlated with Habitat Shannon-Wiener Diversity H′.

| Taxa Metric | Kendall's rank correlation tau |
|---|---|
| **Taxa** | |
| Taxa Richness (S) | 0.3828399** |
| Shannon Diversity (H′) | 0.3314156* |
| **Traits** | |
| Trait Richness (S) | 0.1551329 |
| Shannon Diversity (H′) | 0.2096774 |

Correlation coefficients significant p-values - * $\alpha < 0.05$, ** $\alpha < 0.01$, and *** < 0.001.

Taxa richness and diversity decline more dramatically in response to decreases in stream habitat condition than trait richness and diversity. Taxa richness increased from 18 to 36 taxa to 56 to 94 taxa with increasing habitat diversity while trait richness did not vary as strongly (48 to 58 traits) in the same streams (Figure 3.6). Both taxa and trait diversity did not vary strongly with increasing habitat diversity (Figure 3.7) where taxa diversity increased (taxa H′=2.2-3.8) and trait diversity only increased slightly (trait H′=3.5-3.8). Functional redundancy decreases as stream habitat quality increased (Figure 3.8). However, the pattern is not as straightforward as the patterns seen with the taxa and trait richness and diversity.

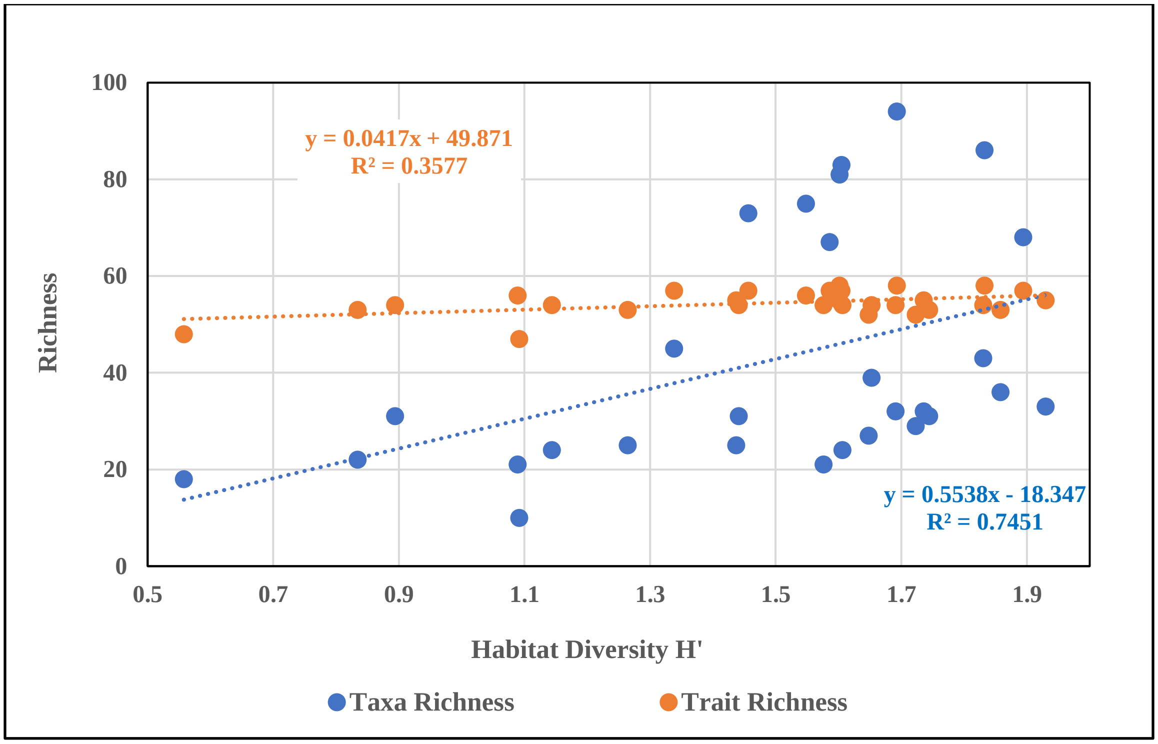


**Figure 3.6**. Taxa and trait richness by stream habitat diversity (H′). Taxa richness increased at a greater rate than trait richness with stream habitat quality. (N=30 stream samples).

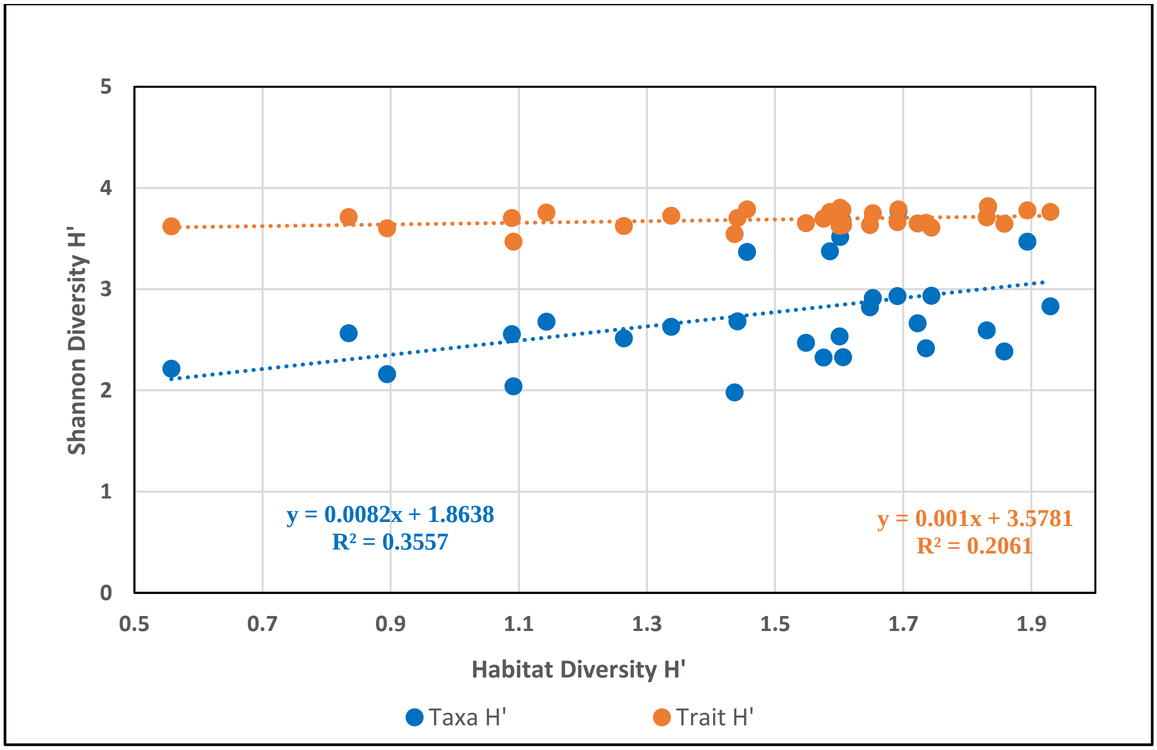


**Figure 3.7**.  Taxa and trait diversity (H′) by stream habitat diversity (H′). Taxa diversity increased at a greater rate than trait diversity with stream habitat quality. (N=30 stream sites).

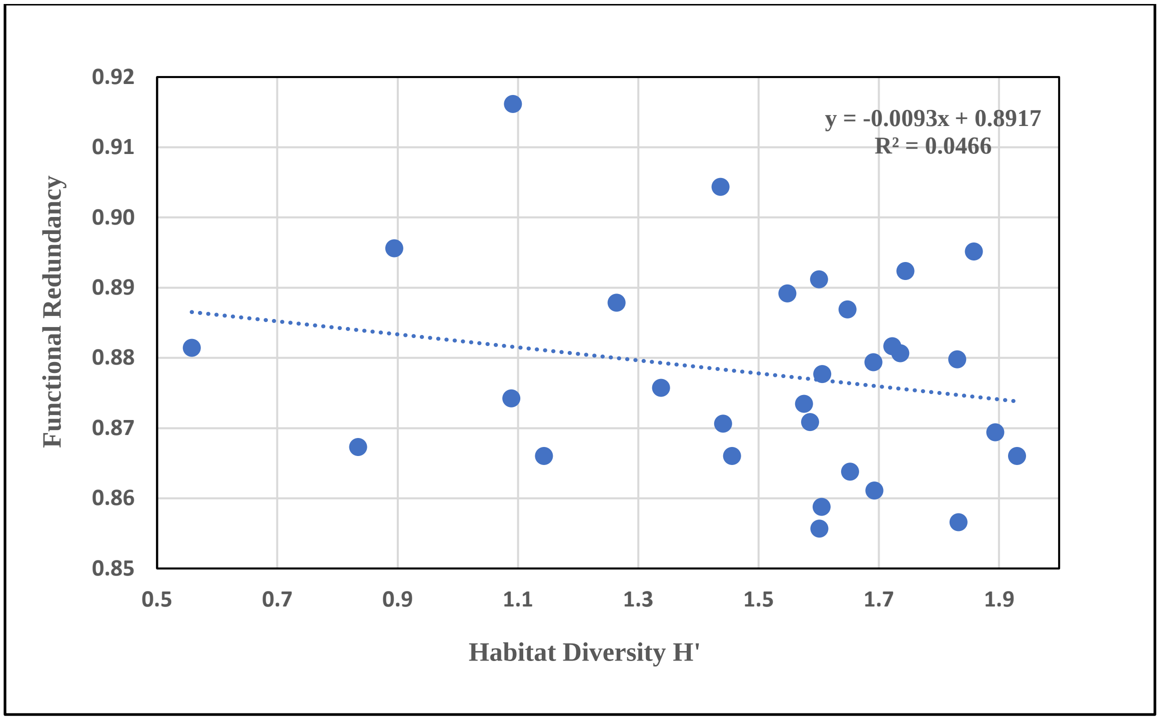


**Figure 3.8**. Functional redundancy by stream habitat diversity (H′). Functional redundancy declined with increases in stream habitat quality. (N=30 stream samples).

Riffles are important for taxa and trait richness, but other microhabitats are important as well. There were significant positive correlations between taxa richness and 5 microhabitats including pools, runs, backwater, leaf packs, and riffles (Table 3.3). There were significant positive correlations between taxa diversity (H′), and pools and leaf packs. There were significant positive correlations between trait richness, and runs, small wood, and riffles. There were significant positive correlations between trait diversity, and runs, leaf packs, small wood, and riffles.

**Table 3.3**. The Kendall Rank Correlation Test shows the correlation between Taxa and Trait Richness and Diversity (H′) with Microhabitats.

| **Taxa Metric** | **Kendall's rank correlation tau** | | | |
|---|---|---|---|---|
| **Microhabitats** | **Taxa Richness (S)** | **Taxa Shannon Diversity (H′)** | **Trait Richness (S)** | **Trait Shannon Diversity (H′)** |
| **Pool** | 0.3627821$^{**}$ | 0.2770079$^{*}$ | 0.2852978 | 0.2695513 |
| **Run** | 0.4225233$^{**}$ | 0.2504437 | 0.4456814$^{**}$ | 0.3121358$^{*}$ |
| **Backwater** | 0.3592008$^{**}$ | 0.1171394 | 0.172329 | 0.03176178 |
| **Root Wad** | 0.1862642 | 0.1177842 | 0.08895535 | 0.06936458 |
| **Undercut Bank** | 0.07084225 | 0.2412601 | 0.02756728 | 0.1360117 |
| **Leaf Pack** | 0.3454439$^{**}$ | 0.3569305$^{**}$ | 0.229133 | 0.29302$^{*}$ |
| **Small Wood** | 0.1169591 | 0.1879431 | 0.2904558$^{*}$ | 0.2741089$^{*}$ |
| **Large Wood** | 0.228241 | 0.2100592 | 0.1847922 | 0.1705775 |
| **Riffle Index** | 0.48947$^{**}$ | 0.2608741 | 0.5582309$^{***}$ | 0.3409778$^{**}$ |

Correlation coefficients significant p-values - * $\alpha < 0.05$, ** $\alpha < 0.01$, and *** $< 0.001$. Correlations range from -1 to 1 with -1 indicating a strong negative correlation between the 2 variables, 0 indicates no association between the 2 variables and 1 indicates a strong positive association between the 2 variables.

Regression models identified critical habitats for taxa and trait richness and diversity. Multiple linear regression analysis developed models that relate taxa richness, trait richness, taxa diversity and trait diversity to microhabitats observed in streams (Table 3.4). The regression model for taxa richness includes the riffle, large wood, run, and leaf pack microhabitats. The model for taxa diversity includes the leaf pack, riffle, and undercut bank habitats. The model for trait richness includes the riffle and small wood microhabitats and habitat diversity while the model for trait diversity includes the leaf pack and riffle microhabitats. All the models include riffles reflecting the importance of riffles developing and sustaining the aquatic insect community in streams. Leaf packs were also shown to be important microhabitats as they were included in 3 of the models.

**Table 3.4**. Multiple linear regression models for Taxa and Trait Richness and Diversity (H′). The initial set of parameters tested included: number of Pool, Run, Backwater, Root Wad, Undercut Bank, Leaf Pack, Small Wood, Large Wood, Riffle microhabitats, and Habitat Diversity (H′).

| | **Goodness of fit** | | **Regression model** |
|---|---|---|---|
| | **Adj. R2** | **p** | |
| **Taxa Richness** | **0.6737** | **1.301e-06** | **22.4426 + 2.9423 (RiffleInd) + 0.4861 (LgWood) - 6.7282 (Run) + 0.6970 (LeafPack)** |
| **Taxa Diversity (H′)** | **0.4145** | **0.0006875** | **2.248039 + 0.026279 (LeafPack) + 0.024478 (RiffleInd) + 0.009959 (UndercutBank)** |
| **Trait Richness** | **0.4099** | **0.0007586** | **48.73597 + 0.13697 (RiffleInd) + 2.56845 (HabitatH′) + 0.01428 (SmWood)** |
| **Trait Diversity (H′)** | **0.2608** | **0.006448** | **3.635762 + 0.003338 (LeafPack) + 0.003313 (RiffleInd)** |

### 3.5.2 MICROHABITATS

Taxa and trait diversity vary among microhabitats. The highest number of total taxa and total number of organisms were collected from riffles while the lowest numbers were found in sand and undercut banks. (Appendix Figure A14). The list of taxa collected from all sites are summarized in Appendix Table A7. Taxa diversity was lowest in sand compared to the other microhabitats although not significantly lower than taxa diversity in undercut banks and wood (Kruskal-Wallis $p<0.05$; Dunn test $p<0.05$; Figure 3.9). Trait diversity did not follow the same pattern across microhabitats as taxa diversity. Trait diversity was similar in the leaf pack, riffle, root wad, undercut bank, and woody debris microhabitats and higher than trait diversity in backwater and sand microhabitats (Kruskis-Wallis $p<0.05$; Dunn Test $p<0.05$; Figure 3.10).

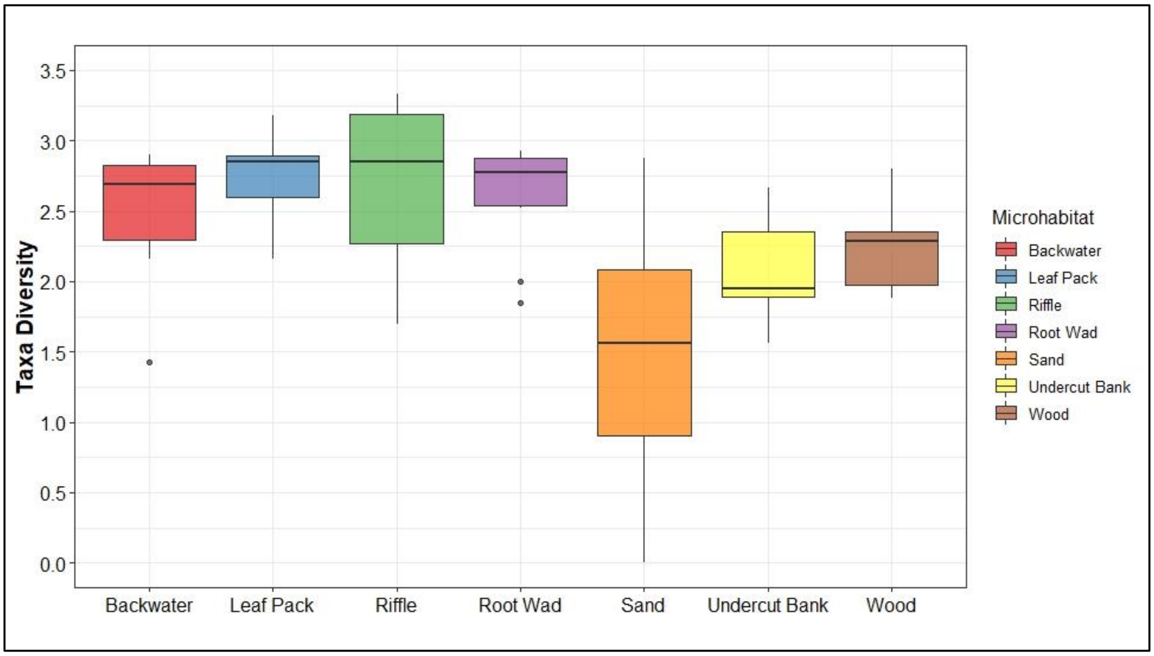


**Figure 3.9**. Box plots of taxa diversity across all microhabitats. Taxa diversity was lowest in sand compared to the other microhabitats although not significantly lower than taxa diversity in undercut banks and wood (Kruskal-Wallis $p<0.05$; Dunn test of comparisons $p<0.05$). The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

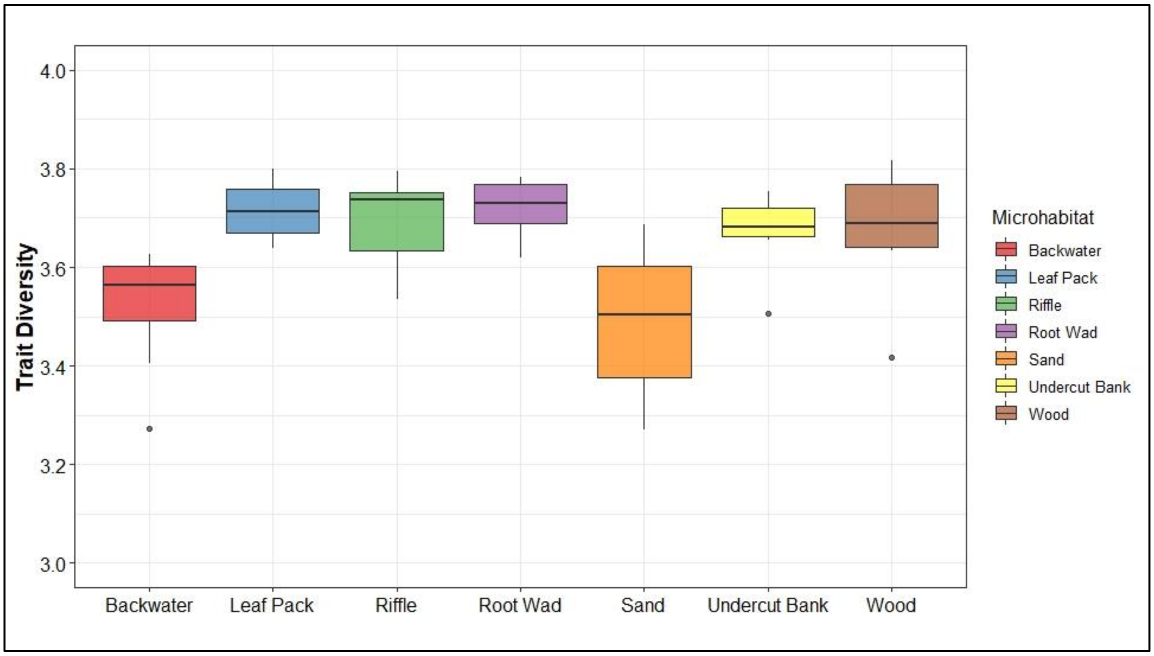


**Figure 3.10**. Box plots of trait diversity across all microhabitats. Trait diversity was similar in the leaf pack, riffle, root wad, undercut bank, and woody debris microhabitats and higher than trait diversity in backwater and sand microhabitats (Kruskis-Wallis $p<0.05$; Dunn test of comparisons $p<0.05$). The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

Taxa and traits differ across microhabitats. The NMDS plots for taxa by microhabitat indicate that taxa found in riffles are closer in composition to the taxa in leaf pack and woody debris. The taxa composition found in the root wad, undercut bank and backwater microhabitats were similar but differ from that in the riffle/leaf pack/woody debris microhabitats. The taxa found in the sand overlapped with the undercut bank and backwater microhabitats (Figure 3.11). The distribution of traits among the microhabitats followed a pattern like the distribution of the taxa. The NMDS plots for traits by microhabitat showed that the macroinvertebrate communities found in the riffle were similar to the traits found in the leaf packs and woody debris microhabitats. In contrast, the traits found in the undercut bank, backwater, and root wad microhabitats were less like the traits found in the riffles, leaf packs, and on woody debris (Figure 3.12).

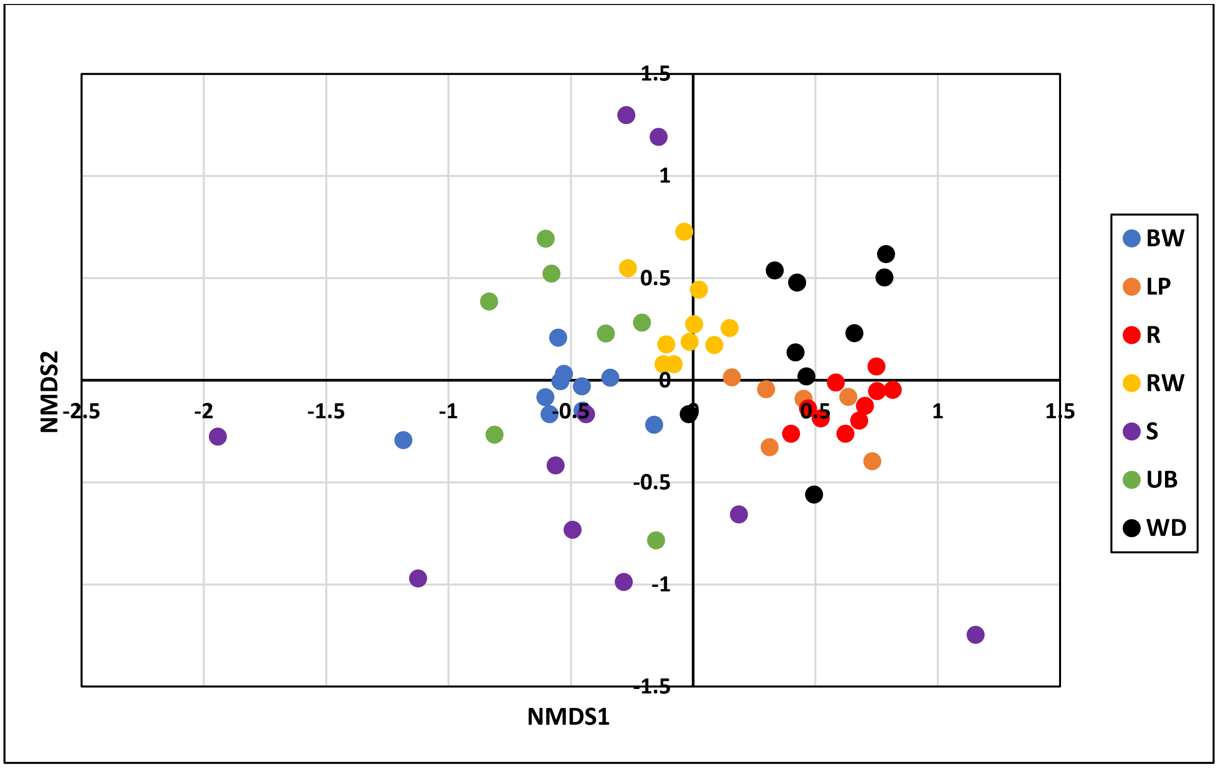


**Figure 3.11**. NMDS plot of taxa abundance across all microhabitats. Taxa found in riffles are more similar to the taxa found in leaf pack and woody debris microhabitats and are less similar to the taxa found in root wad, undercut bank, and backwater microhabitats. The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

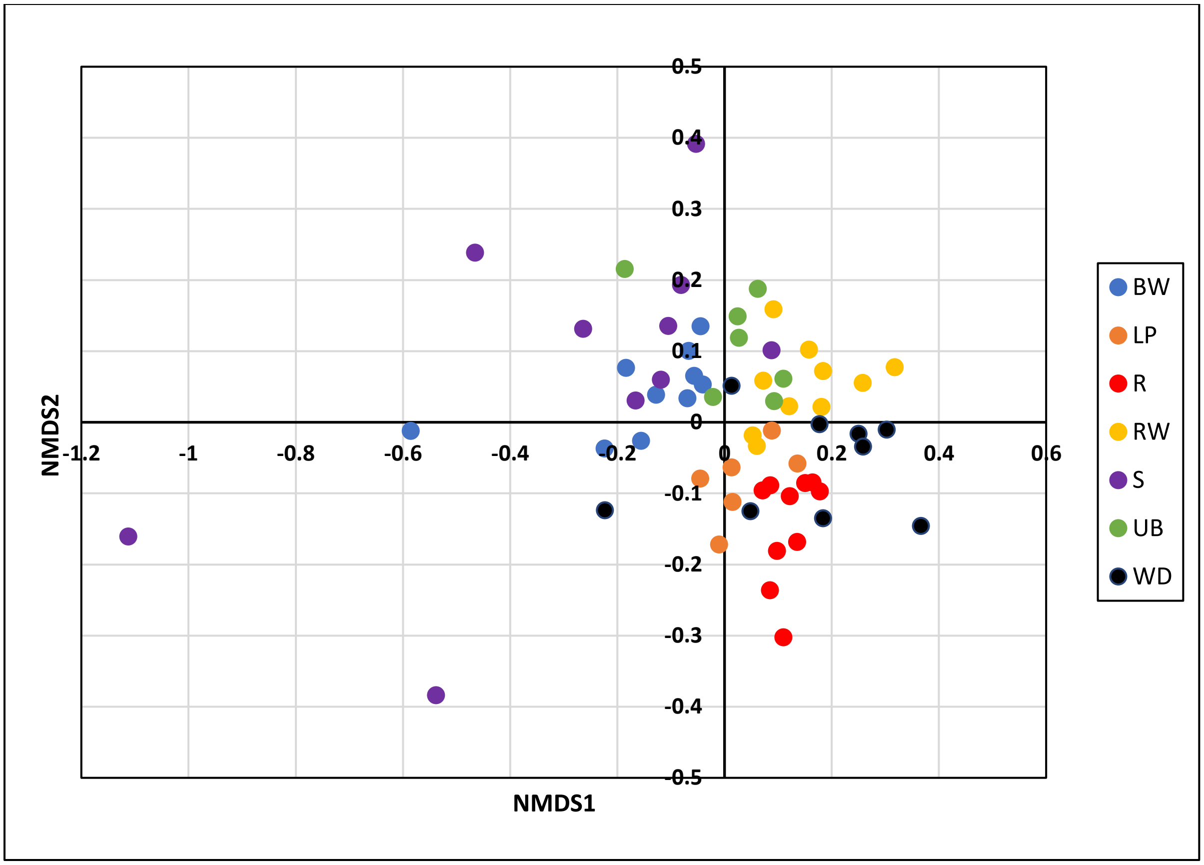


**Figure 3.12**. NMDS plot of trait abundance across all microhabitats. Traits found in riffles are more similar to the traits found in leaf pack and woody debris microhabitats and are less similar to the traits found in root wad, undercut bank, and backwater microhabitats. The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

Trait functional redundancy was highest in microhabitats with lower diversity of taxa and traits. The functional redundancy of traits was higher in the backwater and sand habitats, while the leaf pack, riffle, root wad, undercut bank, and woody debris microhabitats had similar levels of functional redundancy (Kruskis-Wallis $p<0.05$; Dunn Test $p<0.05$; Figure 3.13). There were fewer taxa found in both the backwater and sand habitats. These taxa shared several taxa traits with the other microhabitats.

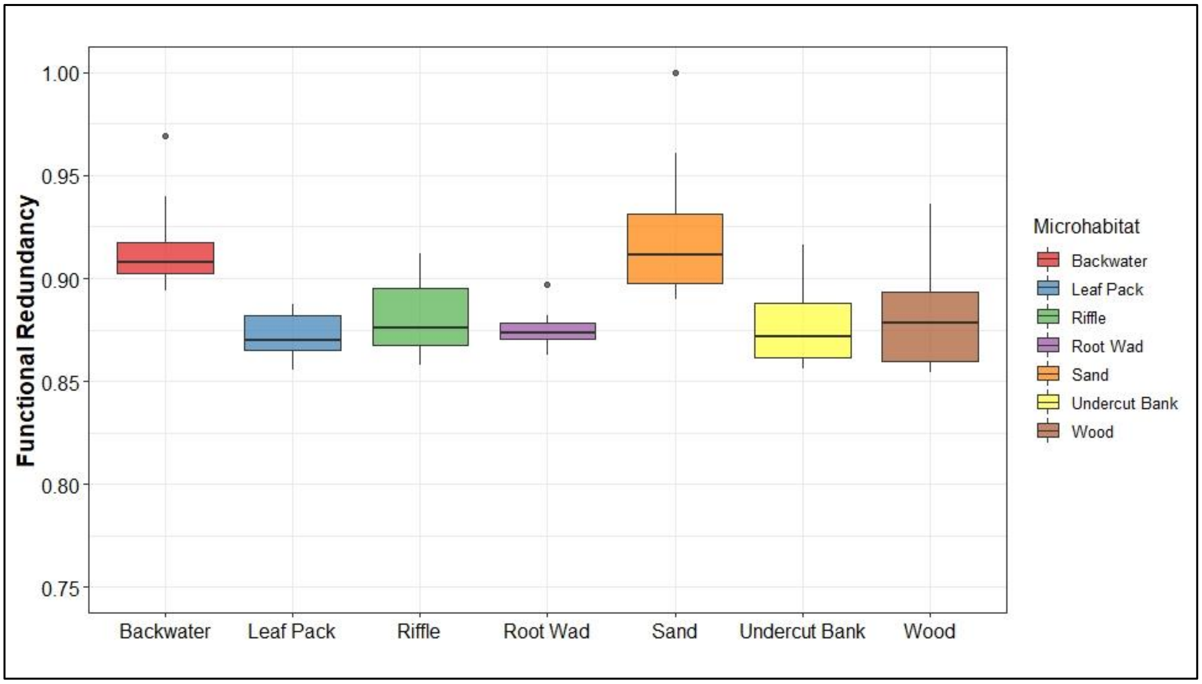


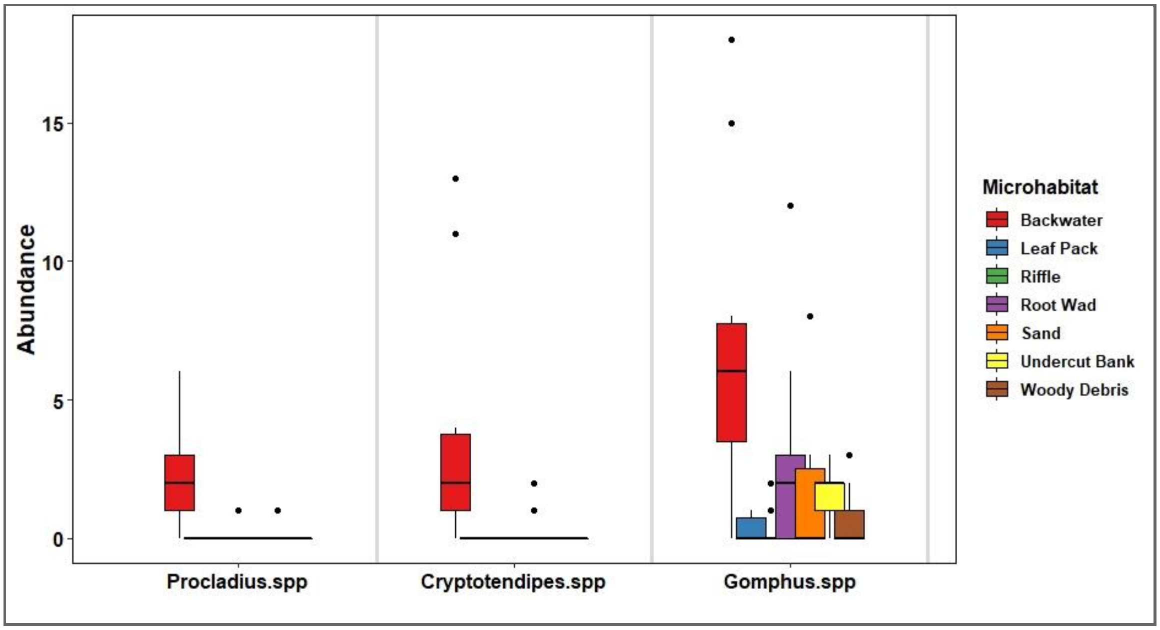
**Figure 3.13**. Box plots of functional redundancy across all microhabitats. The functional redundancy of traits was higher in the backwater and sand habitats than in the other microhabitats (Kruskis-Wallis p<0.05; Dunn test of comparisons p<0.05). The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

There were specific taxa and traits associated with specific microhabitats. Each taxon possesses a unique combination of traits, some of which can be shared with other taxa found in different microhabitats. However, no one taxon can possess all traits as sub-traits within each trait group describe different strategies to survive in the stream. For example, some taxa, like the Simuliidae, physically attach themselves to the substrate to filter food particles from areas with faster flow rates while other taxa swim from rock to rock in the same microhabitat looking for food.

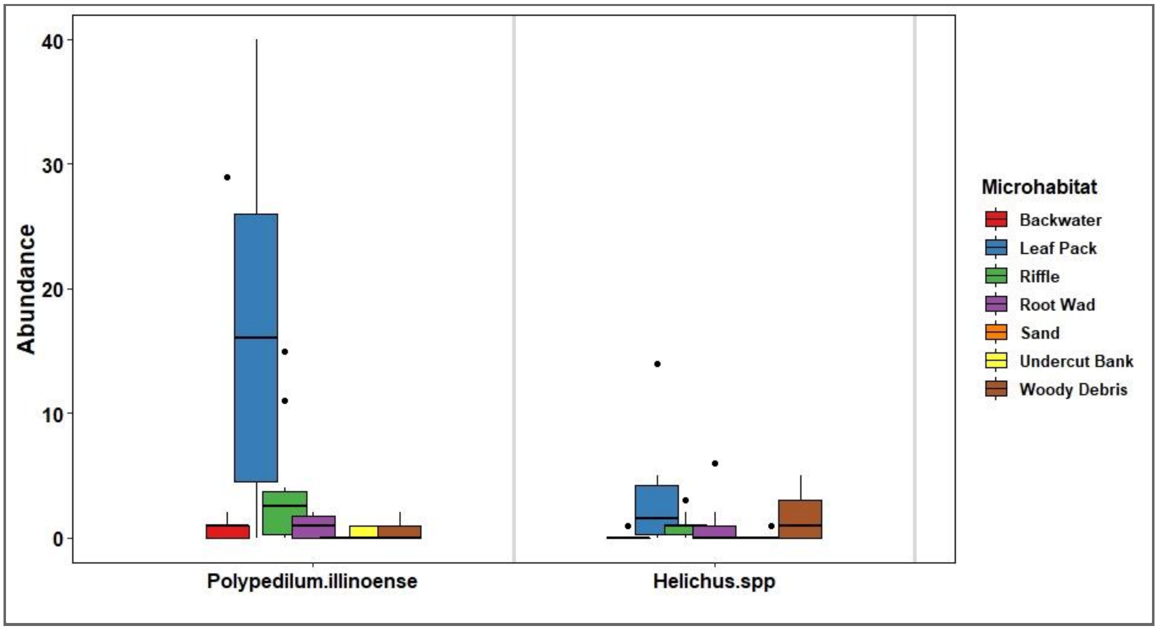
The percentage of traits found in each microhabitat ranged from zero to 16%, indicating that the taxa traits found in each microhabitat reflect the adaptations that taxa commonly possess in each microhabitat. Some of the traits were found in multiple microhabitats, providing multiple locations where similar traits and the corresponding ecosystem function can be found.

While most traits were found in each microhabitat, there were some traits that were more commonly found in one habitat than in the other microhabitats. Out of 156 taxa collected from the 10 sites, the Indicator Species Analysis identified 10 taxa associated with Backwater, 2 species with Leaf Packs, 33 taxa with Riffles, 3 taxa with Root Wads and 1 taxon with Woody Debris (Appendix Table A8). While certain species were associated with a specific habitat, they were also found in other microhabitats as well (Figures 3.14A through 3.14D). The Indicator Species Analysis also associated 13 taxa with combinations of microhabitats such as Leaf Packs and Riffles (Figure 3.15). Additional associations include Backwater and Leaf Packs, Backwater and Riffles, and Root Wads and Woody Debris (Appendix Table A8 and Appendix Figure A15).

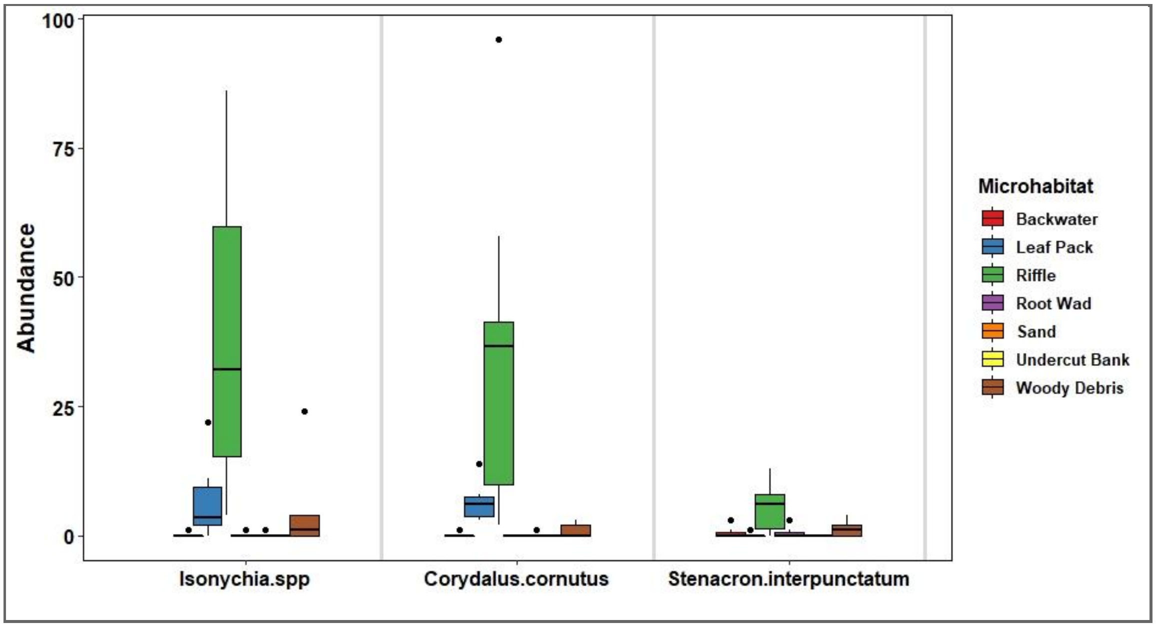
**A. Backwater Taxa**



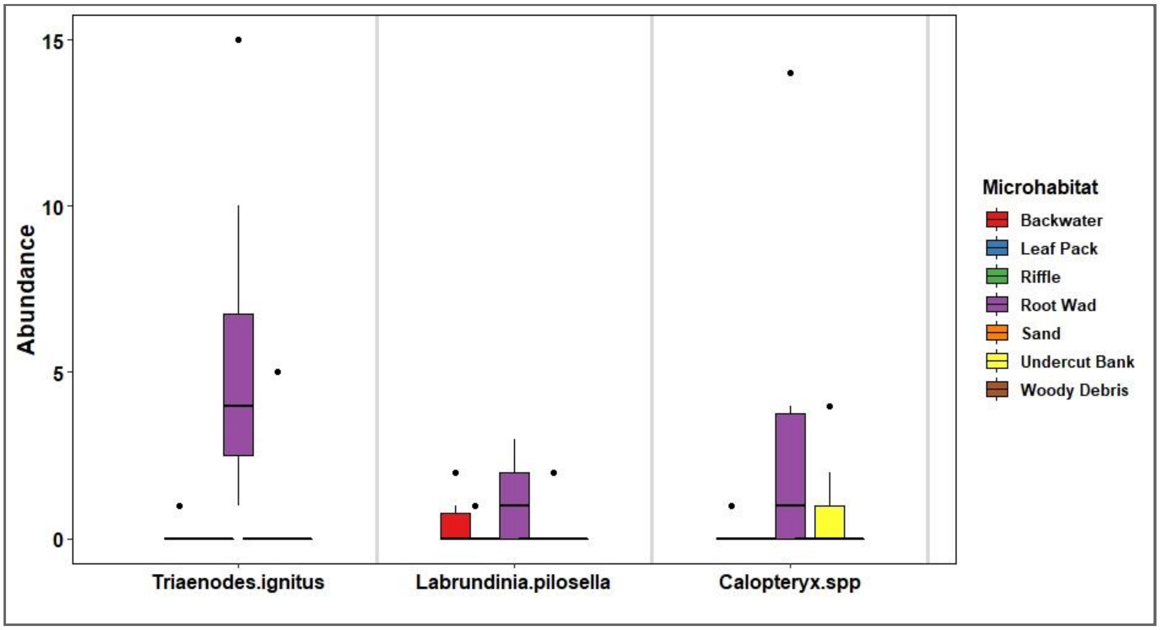
## B. Leaf Pack Taxa



## C. Riffle Taxa



### D. Root Wad Taxa



**Figures 3.14A-3.14D**. The Indicator Species Analysis identified 10 taxa associated with Backwater, 2 taxa with Leaf Packs, 33 taxa with Riffles, 3 taxa with Root Wads. The top 3 taxa associated with each microhabitat are shown. The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

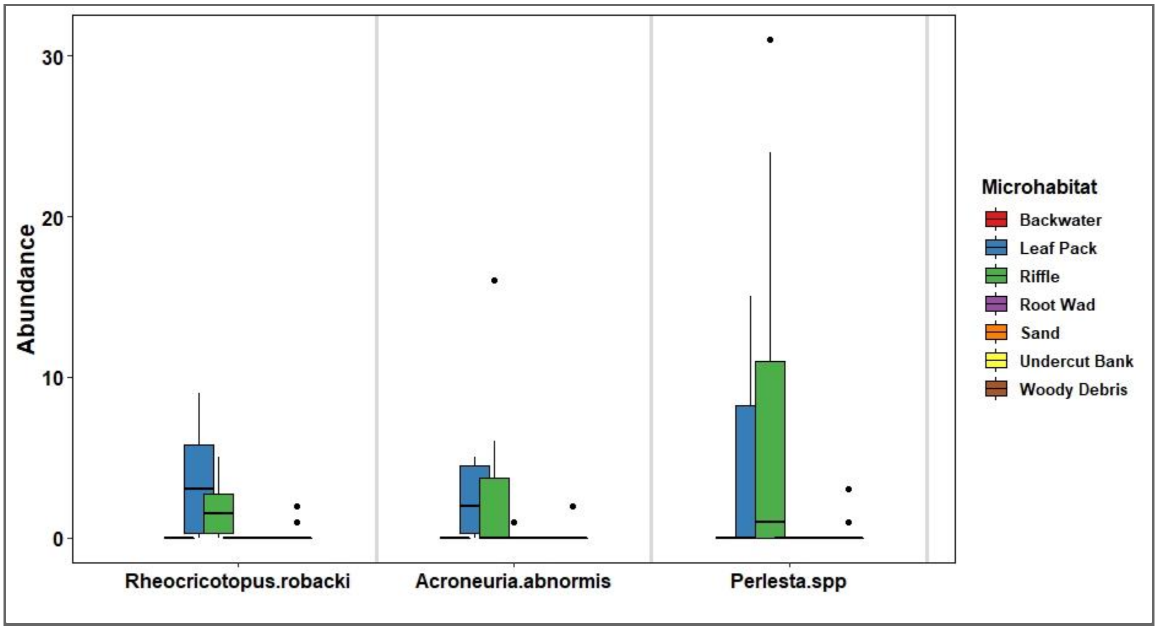


**Figure 3.15.** The Indicator Species Analysis identified 6 taxa associated with both Leaf Pack and Riffle microhabitats. The top 3 taxa are shown. The number of samples for each microhabitat = 10 except for Leaf Pack (6), Undercut Bank (7), and Woody Debris (9).

Out of 58 taxa traits possessed by the taxa collected from the 10 sites, the Indicator Species Analysis identified the majority of traits (46) were associated with taxa collected from riffles (Appendix Table A9). One trait, respiration through the plastron, was associated with leaf packs and 1 trait, climber, was associated with root wads (Figure 3.16). Three traits, ADRF, MV, and CLD, were associated with both leaf packs and riffles. Four traits were associated with combinations of 3 microhabitats such as BRW and DEP with backwater, leaf packs, and riffles, SPL with backwater, riffles, and root wads, and NSE with riffles, root wads, and woody debris.

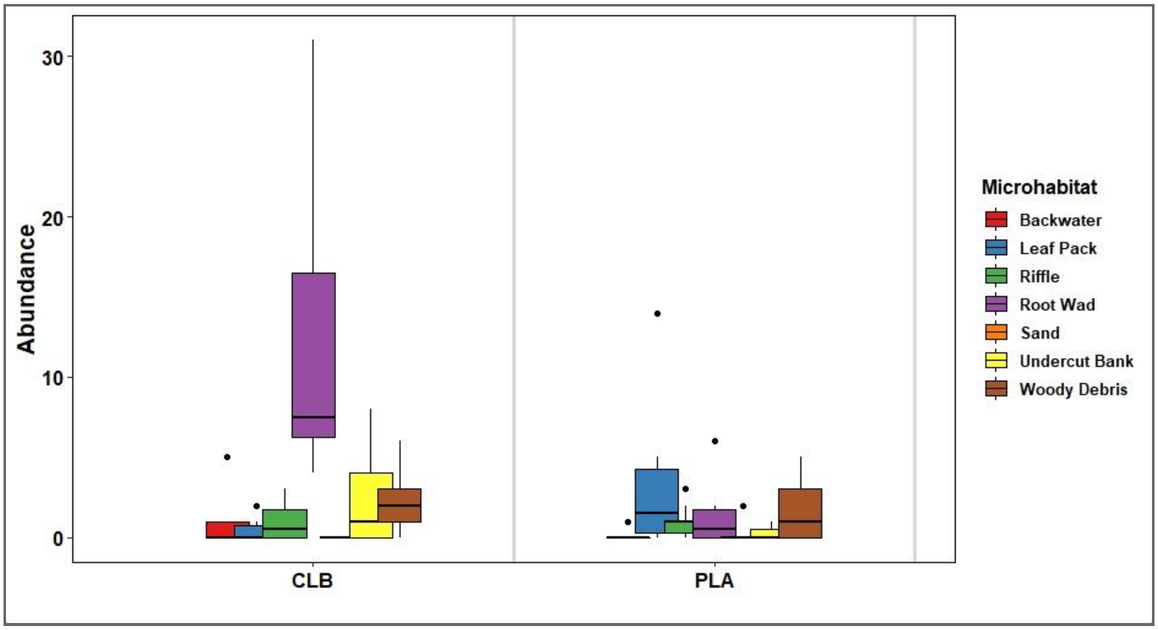


**Figure 3.16**. The Indicator Species Analysis identified 1 trait associated with Root Wads (CLB – climber habit) and 1 trait associated with Leaf Packs (PLA – respiration using a plastron).

## 3.6 DISCUSSION

My first objective was to examine the relationship between stream habitat diversity and aquatic insect taxa and trait richness and diversity by evaluating 30 streams in the Piedmont, North Carolina, spanning a gradient of good to poor habitat quality at the watershed scale. My second objective was to quantify the relationship between taxa and trait richness and diversity and microhabitats at the reach level in 10 streams with high habitat quality. Understanding the relationship between macroinvertebrate taxa diversity and trait diversity is important for the management and restoration of river ecosystems.

### 3.6.1 TAXA AND TRAIT PATTERNS AT THE WATERSHED SCALE

When comparing the 30 sites across a habitat gradient, supporting streams were more similar to each other than to partially supporting and impaired streams when described by taxa abundance; however, supporting and partially supporting streams were more similar when described by trait abundance. As EMHAP scores declined, habitat diversity had greater variability in the partially supporting and impaired sites. Some streams that were identified as having low EMHAP scores had diverse habitats. This variability may be due to different types of stressors across these watersheds including percent impervious cover, presence or absence of stormwater control measures, and pollution being sourced from different land use such as commercial and industrial versus residential (Walsh et al. 2005; Brown et al. 2009; Cuffney et al. 2010; Bell et al. 2012; Carlisle et al. 2013).

Urbanization results in degraded stream channels, reduced stream habitat diversity and impaired benthic macroinvertebrate communities (Center for Watershed Protection 2003; Coles et al. 2004; Alberti 2005; Walsh et al. 2005; Cuffney et al. 2010; Bell et al. 2012; Coles et al. 2012; Baumgartner and Robinson 2017). I found that taxa richness and diversity were significantly correlated with habitat diversity. The impaired streams had lower habitat diversity than both the partially supporting and supporting streams. The supporting streams' habitat were more heterogeneous, sustaining a more complex benthic macroinvertebrate community. Wang et al (1997) found that stream habitat quality was negatively correlated with percent of urban land area. Gage et al. (2004) found that benthic macroinvertebrate communities were less diverse in streams in urbanized watersheds than in streams in rural watersheds. While the correlation between traits and habitat diversity in my study was positive, the correlation was not significant. There was little to no change in trait richness and diversity as the habitat diversity improved. This does not fully support my hypothesis that traits are positively correlated with habitat diversity. This further supports my observation of the resiliency of trait composition within the aquatic insect community. Similar to my study, Peru and Doledec (2010) found that while taxa richness varied greatly in response to natural environmental gradients, trait diversity was fairly stable. Bêche et al. (2006) found that benthic macroinvertebrate trait composition changed very little during the different seasons of the year while both taxa assemblages and abundances changed significantly.

I found that both taxa richness and diversity declined at a greater rate than trait richness and diversity along an in-stream habitat diversity gradient. Trait richness and diversity changed very little with changes in habitat condition and support my hypothesis that the decline in total taxa richness would be greater than the decline in total trait diversity with decreasing habitat condition. My regression models identified riffles as a key microhabitat for diversity and richness (taxa and trait), reflecting the importance of riffles for developing and sustaining the aquatic insect community in streams. Leaf packs were also shown to be important microhabitats as they were included in 3 of the models. Several of the partially supporting and impaired streams lacked riffles and woody debris while others lacked undercut banks and root wads limiting the diversity of both taxa and trait richness and diversity.

Functional redundancy was higher in urban streams with lower habitat diversity which indicates that trait composition is not changing with habitat condition and reflects the resiliency of the community trait composition (Peru and Doledec 2010). Rosenfeld (2002) found that functional redundancy was higher in ecosystems in which taxa niches overlapped. Streams with lower habitat diversity would have more overlap of niches that are inhabited by species with similar traits (Rosenfeld 2002; Bêche and Statzner 2009; Lamothe et al. 2018). I found that most urban streams studied have poor habitat conditions which is typical of streams in watersheds with high impervious cover. The US Geological Survey conducted a series of studies on the impact of urbanization on streams and found that most urban streams had severely degraded channels and low habitat diversities (Coles et al. 2004; Brown et al. 2009; Cuffney et al. 2010; Bell et al. 2012; Coles et al. 2012). The habitat in these streams has homogenized, reducing the diversity of habitats available to the benthic macroinvertebrate community. As a result, streams that don't have a high diversity of habitats have high redundancy of traits since the benthic macroinvertebrates are living in the similar types of habitats that favor similar traits (Walker 1992; Rosenfeld 2002; Heatherly et al. 2007; Bêche and Statzner 2009; Flynn et al. 2009). Streams with higher habitat diversity are generally found in watersheds with lower development and lower percent impervious cover. These streams have more trait specialization, lower redundancy, and greater resiliency to withstand disturbance associated with urbanization (Milesi et al. 2016).

High habitat diversity and complexity is important for a healthy stream benthic macroinvertebrate community (Hynes 1970; Allan and Castillo 2007). I examined the relationship between microhabitats and the aquatic insect community at the watershed scale and found a number of different habitats were significantly positively correlated with taxa and trait richness and diversity (Table 3.3). Taxa richness was positively correlated with pools, runs, backwater, leaf packs, and riffles, while trait richness was positively correlated with runs, small wood, and riffles. Taxa diversity was positively correlated with pools, and leaf packs while trait diversity was positively correlated with runs, leaf packs, small wood, and riffles. Taxa richness was correlated with more microhabitats than trait richness reflecting the redundancy of traits that are shared by taxa living in similar microhabitats. The multiple linear regression models showed that riffles and leaf packs are particularly important microhabitats in the development of the macroinvertebrate community found in streams. In addition to riffles and leaf pack microhabitats, large wood, and runs were important for taxa richness; undercut banks were important for taxa diversity; and habitat diversity and small wood were important for trait richness. Watershed level analysis may not explain the taxa and trait variations in the aquatic insect assemblages due to microhabitat preferences compared to reach scale analysis (Richards et al. 1997; Dovciak and Perry 2002; Waite 2014; Krynak and Yates 2018). The aquatic insect assemblages within each microhabitat should be expected to differ as the assemblages are the result of environmental filters such as food resources, hydrology, and structural features of the microhabitat (Lamouroux et al. 2004; Bêche and Statzner 2009; Milesi et al. 2016).

### 3.6.2 TAXA AND TRAIT PATTERNS AT THE REACH SCALE

In my 30-site study I correlated taxa and trait diversity and richness to habitat parameters that led to the observation that there were microhabitats that were more important than others to contributing to diversity and richness. Thus, I specifically sampled multiple microhabitats in the 9 supporting and 1 partially supporting streams to determine the relationship between microhabitats and aquatic insect taxa and trait richness and diversity.

Using NMDS, I examined the relationship that individual microhabitats had on the distribution of taxa and traits and found similar taxa and traits in similar microhabitats. For example, the taxa and traits found in riffles and leaf packs were more similar than taxa and traits found in undercut banks and root wads. This may be due to the differences observed in the stream flow velocities within the microhabitats. While I did not quantify water velocity, riffles and leaf packs are generally found in stream reaches with faster stream velocities and the root wads and undercut bank areas are found along the stream edges where the stream velocities are often slower (Bisson et al. 2006). Flow conditions have been identified as one of the environmental filters, along with substrate particle size and water depth, determining the composition of benthic macroinvertebrate assemblages within microhabitats (Jowett and Richardson 1990; Wohl et al. 1995; Lamouroux et al. 2004; White et al. 2019; Forcellini et al. 2022). Scotti et al. (2020) found similar taxa and trait compositions in streams in grasslands and pastures where habitats were also similar. These results support my hypothesis that similar traits are found among the aquatic insect taxa residing in similar microhabitats within the same stream.

Taxa and trait diversity and richness generally followed a similar pattern to their distribution across microhabitats where the highest taxa diversity and richness were found in the riffles while the lowest were found in the sand and undercut banks. Functional redundancy varied among the different microhabitats and similar to my analysis at the watershed level, functional redundancy was related to the magnitude of taxa diversity and richness. That is, functional redundancy was highest in the backwater and sand microhabitats which also had the lowest taxa richness and diversity and the majority of taxa in these microhabitats had very similar trait compositions. Flynn et al. (2009) found that functional diversity reflects taxa richness and that communities with low functional diversity generally have high functional redundancy, which is what I observed with the benthic macroinvertebrate assemblages in the backwater and sand microhabitats. While I found practically every trait in the microhabitats sampled, the traits were not evenly distributed among the microhabitats. Higher percentages of most traits were found in the riffles. However, there were higher percentages of a small number of traits, such as good armoring, plastron respiration, and climber, which were found in other microhabitats like woody debris, root wads, and leaf packs.

At the habitat scale, I found that the highest taxa richness and diversity were in the riffles. Gregory (2005) and Wang et al. (2006) found that taxa richness of aquatic insects was slightly higher on woody debris than in riffles but had lower densities of organisms. Large woody debris can increase stream habitat complexity by altering the flow path in a stream resulting in the formation of pools, exposure of gravel and cobble substrates, enhance sediment deposition, increase the retention of leaves and other organic debris, and provide stable substrates for aquatic insects and periphyton (Coe et al. 2006; Cordova et al. 2007; Coe et al. 2009; Pilotto et al. 2016; de Brouwer et al. 2020; Entrekin et al. 2020). Large woody debris serves as refugia for fish and aquatic insects during high flows as well as stable substrate for both aquatic insects and periphyton (Coe et al. 2006; Huryn et al. 2008; White et al. 2019). Coe et al. (2009) reported that woody debris supports a unique community of aquatic insects.

Indicator species analysis revealed that there were specific taxa and traits that were associated with specific microhabitats or combinations of microhabitats. For example, *Triaenodes ignitus* (Walker 1852), *Calopteryx* Leach 1815, and *Labrundinia pilosella* (Loew 1866) were associated with root wads while *Isonychia* Eaton 1871, *Stenacron interpunctatum* (Say 1839), and *Corydalus cornutus* (Linnaeus 1758) were associated with riffles and *Rheocricotopus robacki* (Beck and Beck 1964), *Acroneuria abnormis* (Newman 1838), and *Perlesta* Banks 1906 were associated with both leaf packs and riffles. Taxa that were associated with specific microhabitats were not limited to utilizing that microhabitat alone. For example, *Gomphus* Leach, 1815, which was associated with the backwater microhabitat, was found in all microhabitats except riffles, and *Polypedilum illinoense* (Malloch 1915), which was associated with leaf packs, was found in all microhabitats except sand. Gregory (2005) found specific taxa associated with either woody debris or riffle microhabitats. Other studies showed that some taxa preferred specific substrates (Rabeni and Minshall 1997; Schröder et al. 2013) or combinations of substrate, water velocity, water depth, and benthic coarse particulate organic matter (Orth and Maughan 1983; Jowett and Richardson 1990; Lamouroux et al. 2004; Brooks et al. 2005; White et al. 2019). While most traits were associated with riffles, several traits were significantly associated with other microhabitats such as root wads (e.g., CLB) and leaf packs (e.g., PLA). Taxa and traits found in a specific stream reach or microhabitat are the result of numerous environmental filters such as physical (water velocity and habitat diversity), environmental (water chemistry, pollution, and temperature), and biological (food types, mobility, and life cycle requirements) (Lamouroux et al. 2004; Smith et al. 2015; White et al. 2019; Edegbene et al. 2020; Forcellini et al. 2022; Jordt and Taylor 2021).

## 3.7 APPLICATION TO STREAM RESTORATIONS

Stream restoration has become a multimillion-dollar industry as watershed managers respond to degradation of urban stream channels and stream biota by restoring streams to a more natural state. Unfortunately, the biological uplift expected through urban stream restoration has not occurred in most cases which may be due to the general focus on stream channel stabilization more than instream habitat restoration (Sudduth and Meyer 2006; Louhi et al. 2011; Violin et al. 2011; Ernst et al. 2012; Stranko et al. 2012).

My results show that all microhabitats contribute to the overall ecosystem function by providing habitat for a diverse aquatic insect community. I show that woody debris, leaf packs, undercut banks, and root wads support species that would otherwise not be found in the stream.

Woody debris has been shown to have an important role in creating and maintaining diverse flow patterns and heterogeneous habitats (Coe et al. 2006; Cordova et al. 2007; de Brouwer et al. 2020) as well as maintaining healthy food webs and promoting carbon assimilation (Coe et al. 2009; Entrekin et al. 2020). My results show that there are specific taxa that are associated with microhabitats such as root wads, backwater, and leaf packs, and traits associated with root wads and leaf packs. These taxa and traits would add to the stream biodiversity and ecosystem function.

Insect reproduction and adult emergence, two important stages of the aquatic insect life cycle, are often overlooked when stream restoration projects are planned (Merten et al. 2014; Jordt and Taylor 2021). Merten et al. (2014) showed that the abundance of substrates such as wood and boulders that have surfaces exposed to the air that allow adult aquatic insects to exit the stream are correlated with emerging insect biomass. They recommended that stream restoration designers should include habitat for aquatic insect emergence. Jordt and Taylor (2021) found a shortage of rocks suitable for oviposition in restored stream segments. Most boulders in restored stream segments are not stable and often roll during storm events making them unsuitable for successful oviposition limiting reproduction success.

If we want to improve biodiversity and ecosystem function in degraded urban streams, I recommend that stream restoration broaden the types of microhabitats included in restoration design. Increasing the diversity of microhabitats could increase the biodiversity of taxa and traits resulting in a more resilient ecosystem. It is possible that retention of woody debris and leaf pack and the occurrence of undercut banks and root wads would increase with restoration age. However, these microhabitats would surely develop if the restoration design encouraged these microhabitats.

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## 3.9 APPENDIX

**Table A6.** Environmental Data from Stream Sites in Piedmont, North Carolina

| Stream | Site | Canopy | Discharge ($m^3$/sec) | Temp | DO | Cond | pH |
|---|---|---|---|---|---|---|---|
| **Beaverdam Cr** | BC | 81.00 | 0.0147 | 19.9 | 6.77 | 84 | 6.69 |
| **Clear Cr** | CC | 86.17 | 0.0608 | 21.7 | 7.99 | 119 | 7.17 |
| **Indian Cr** | IC | 75.25 | 0.5975 | 21.5 | 8.95 | 65 | 7.02 |
| **Leeper's Cr** | LC | 67.25 | 0.2093 | 21.4 | 9.48 | 74 | 7.09 |
| **Long Cr** | LCM | 65.00 | 0.1026 | 25.3 | 7.64 | 171 | 7.18 |
| **L Hunting Cr** | LHC | 66.58 | 0.4365 | 22.8 | 8.13 | 79 | 7.02 |
| **L Indian Cr** | LIC | 75.42 | 0.0378 | 21.8 | 8.64 | 58 | 6.75 |
| **Long Cr** | MC14A | 76.83 | 0.3656 | 24.9 | 9.01 | 190 | 6.99 |
| **Paw Cr** | MC17 | 92.17 | 0.1009 | 24.5 | 6.31 | 292 | 7 |
| **McDowell Cr** | MC2 | 93.33 | 0.0467 | 27.2 | 6.67 | 131 | 6.95 |
| **Coffey Cr** | MC25 | 74.50 | 0.1821 | 20.9 | 7.07 | 106 | 7.09 |
| **Sugar Cr** | MC27 | 13.17 | 1.0922 | 24.5 | 9.22 | 413 | 7.46 |
| **Little Sugar Cr** | MC29A1 | 19.67 | 0.1613 | 27.3 | 7.74 | 275 | 6.93 |
| **McDowell Cr** | MC2A1 | 55.25 | 0.1545 | 26.5 | 7.02 | 111 | 6.75 |
| **Briar Cr** | MC33 | 50.33 | 0.0819 | 32 | 8.75 | 165 | 7.26 |
| **Irvins Cr** | MC36 | 70.42 | 0.0129 | 15 | 6.59 | 154 | 6.93 |
| **McDowell Cr** | MC4 | 90.25 | 0.3737 | 23.7 | 7.91 | 123 | 7.02 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Fourmile Cr** | MC40A | 88.25 | 0.0542 | 19.8 | 6.32 | 173 | 7.13 |
| **McMullen Cr** | MC42 | 59.50 | 0.0258 | 18.1 | 7.47 | 144 | 7.13 |
| **Steele Cr** | MC47A | 54.75 | 0.1236 | 24.5 | 6.77 | 263 | 7.11 |
| **Little Sugar Cr** | MC49A | 17.50 | 1.0609 | 24.1 | 7.52 | 500 | 7.39 |
| **Sixmile Cr** | MC51 | 85.33 | 0.1741 | 10.71 | 8.79 | 263 | 7.32 |
| **Clarke Cr** | MY10 | 83.17 | 0.2397 | 28.2 | 6.17 | 222 | 7.06 |
| **Mallard Cr** | MY11B | 77.92 | 0.3798 | 14.4 | 8.08 | 239 | 6.99 |
| **West Rocky R** | MY1B | 37.92 | 0.1755 | 24.8 | 6.98 | 152 | 6.71 |
| **McKee Cr** | MY7B | 91.92 | 0.0222 | 23.9 | 5.1 | 178 | 6.69 |
| **Goose Cr** | MY9 | 88.25 | 0.0466 | 20.1 | 8.62 | 112 | 7.02 |
| **Pott Cr** | PC | 88.17 | 0.0906 | 22.3 | 5.22 | 65 | 6.28 |
| **Rocky Cr J** | RCJ | 65.83 | 0.3650 | 18.5 | 8.73 | 52 | 6.59 |
| **Rocky Cr M** | RCM | 63.33 | 0.3568 | 19.6 | 8.91 | 45 | 6.75 |

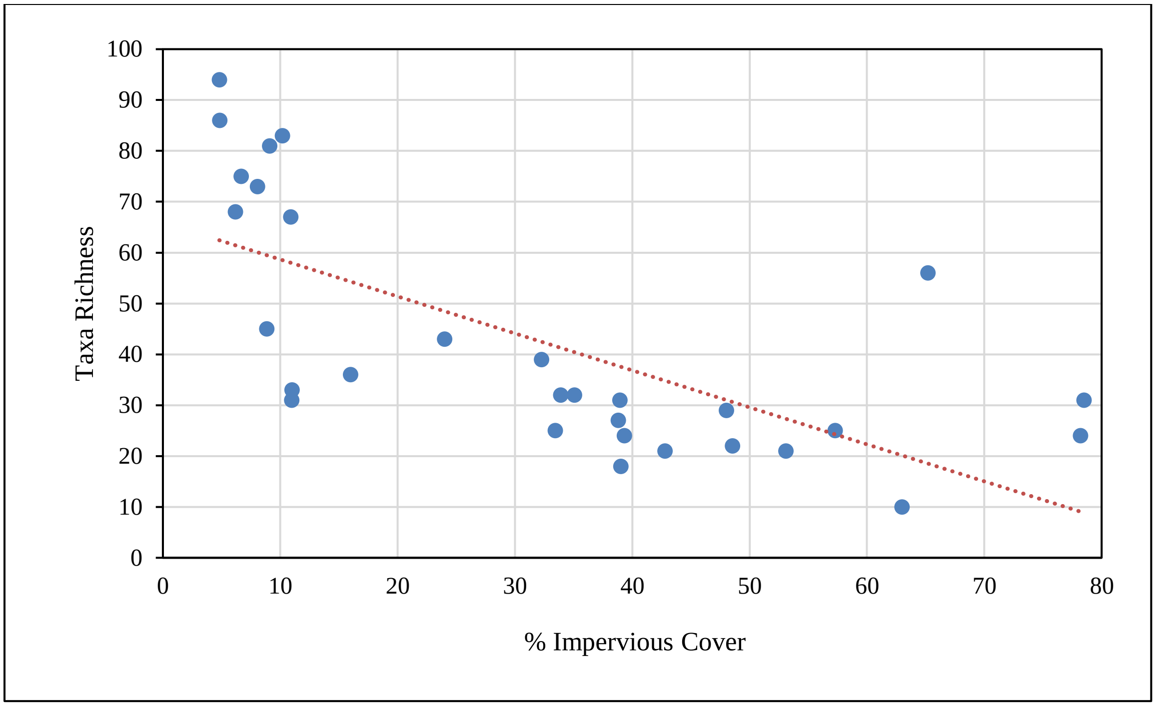

**Figure A12**. Taxa richness declines as percent impervious cover increases.

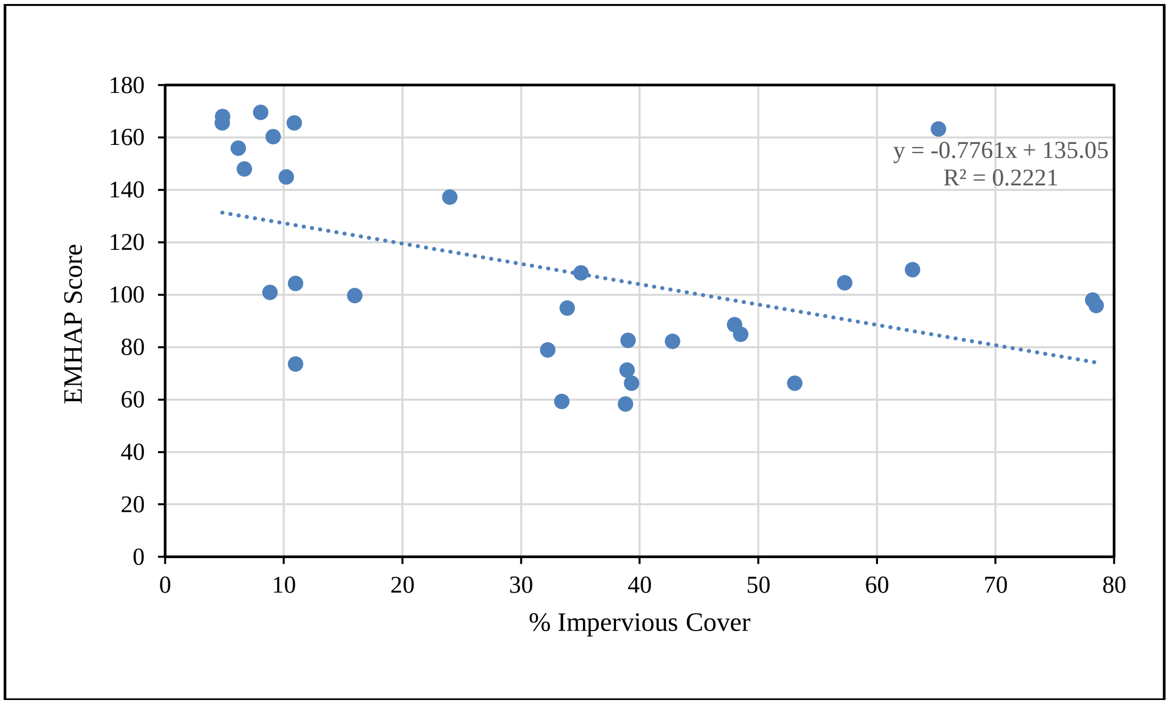

**Figure A13**. Stream habitat expressed as EMHAP scores declines as percent impervious cover increases.

**Table A7.** List of taxa collected from the 30 study sites. The * indicates taxa whose traits are unknown or partially known and removed from the analysis.

| **Class** | **Order** | **Family** | **Genus Species** | **Taxa with traits unknown** |
|---|---|---|---|---|
| Bivalvia | Veneroica | Corbiculidae | *Corbicula fluminea* | * |
| Bivalvia | Veneroica | Sphaeriidae | *Sphaerium* spp. | * |
| Crustacea | Amphipoda | Gammaridae | *Gammarus* spp. | * |
| Crustacea | Isopoda | Asellidae | *Asellus* spp. | * |
| Gastropoda | Basommatophora | Ancylidae | *Ferrissia* spp. | * |
| Gastropoda | Basommatophora | Lymnaeidae | *Pseudosuccinea columella* | * |
| Gastropoda | Basommatophora | Lymnaeidae | *Stagnicola* spp. | * |
| Gastropoda | Basommatophora | Physidae | *Physa* spp. | * |
| Gastropoda | Basommatophora | Planorbidae | *Helisoma anceps* | * |
| Gastropoda | Basommatophora | Planorbidae | *Menetus dilatatus* | * |
| Gastropoda | Neotaenioglossa | Pleuroceridae | *Elimia* spp. | * |
| Hirudinea | Arhynchobdellida | Erpobdellidae | *Erpobdella/Mooreobdella* spp. | * |
| Hirudinea | Rhynchobdellida | Glossiphoniidae | *Helobdella triserialis* | * |
| Insecta | Coleoptera | Dryopidae | *Helichus* spp. | |
| Insecta | Coleoptera | Dytiscidae | *Acilius fraternus* | |
| Insecta | Coleoptera | Dytiscidae | *Neoporus* spp. | |
| Insecta | Coleoptera | Elmidae | *Ancyronyx variegatus* | |
| Insecta | Coleoptera | Elmidae | *Dubiraphia vittata* | |
| Insecta | Coleoptera | Elmidae | *Macronychus glabratus* | |
| Insecta | Coleoptera | Elmidae | *Promoresia elegans* | |
| Insecta | Coleoptera | Elmidae | *Stenelmis* spp. | |
| Insecta | Coleoptera | Gyrinidae | *Dineutus* spp. | * |

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|---|---|---|---|---|
| Insecta | Coleoptera | Haliplidae | *Peltodytes* spp. | |
| Insecta | Coleoptera | Hydrophilidae | *Berosus* spp. | * |
| Insecta | Coleoptera | Hydrophilidae | *Helophorus* spp. | * |
| Insecta | Coleoptera | Hydrophilidae | *Hydrochus* spp. | * |
| Insecta | Coleoptera | Hydrophilidae | *Laccobius* spp. | * |
| Insecta | Coleoptera | Hydrophilidae | *Sperchopsis tessellatus* | * |
| Insecta | Coleoptera | Hydrophilidae | *Tropisternus* spp. | * |
| Insecta | Coleoptera | Psephenidae | *Ectopria nervosa* | |
| Insecta | Coleoptera | Psephenidae | *Psephenus herricki* | |
| Insecta | Coleoptera | Ptilodactylidae | *Anchytarsus bicolor* | * |
| Insecta | Diptera | Athericidae | *Atherix lantha* | |
| Insecta | Diptera | Ceratopogonidae | *Atrichopogon* spp. | |
| Insecta | Diptera | Ceratopogonidae | *Palpomyia complex* | |
| Insecta | Diptera | Chironomidae | *Ablabesmyia annulata* | |
| Insecta | Diptera | Chironomidae | *Ablabesmyia mallochi* | |
| Insecta | Diptera | Chironomidae | *Ablabesmyia rhamphe* | |
| Insecta | Diptera | Chironomidae | *Ablabesmyia* spp. | |
| Insecta | Diptera | Chironomidae | *Brillia* spp. | |
| Insecta | Diptera | Chironomidae | *Chironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Cladotanytarsus* spp. | |
| Insecta | Diptera | Chironomidae | *Clinotanypus* spp. | |
| Insecta | Diptera | Chironomidae | *Corynoneura* spp. | |
| Insecta | Diptera | Chironomidae | *Cricotopus annulator (complex)* | |
| Insecta | Diptera | Chironomidae | *Cricotopus bicinctus* | |
| Insecta | Diptera | Chironomidae | *Cricotopus* spp. | |

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|---|---|---|---|---|
| Insecta | Diptera | Chironomidae | *Cryptochironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Cryptotendipes* spp. | |
| Insecta | Diptera | Chironomidae | *Dicrotendipes fumidus* | |
| Insecta | Diptera | Chironomidae | *Dicrotendipes neomodestus* | |
| Insecta | Diptera | Chironomidae | *Dicrotendipes* spp. | |
| Insecta | Diptera | Chironomidae | *Diplocladius cultriger* | |
| Insecta | Diptera | Chironomidae | *Endochironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Eukiefferiella* spp. | |
| Insecta | Diptera | Chironomidae | *Glyptotendipes* spp. | |
| Insecta | Diptera | Chironomidae | *Goeldichironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Labrundinia pilosella* | |
| Insecta | Diptera | Chironomidae | *Larsia* spp. | |
| Insecta | Diptera | Chironomidae | *Microtendipes* spp. | |
| Insecta | Diptera | Chironomidae | *Nanocladius* spp. | |
| Insecta | Diptera | Chironomidae | *Natarsia* spp. | |
| Insecta | Diptera | Chironomidae | *Nilotanypus* spp. | |
| Insecta | Diptera | Chironomidae | *Nilothauma* spp. | |
| Insecta | Diptera | Chironomidae | *Orthocladius dubitatus* | |
| Insecta | Diptera | Chironomidae | *Orthocladius* spp. | |
| Insecta | Diptera | Chironomidae | *Parachironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Parakiefferiella* spp. | |
| Insecta | Diptera | Chironomidae | *Paralauterborniella nigrohalteralis* | |
| Insecta | Diptera | Chironomidae | *Paramerina* spp. | |
| Insecta | Diptera | Chironomidae | *Parametriocnemus* spp. | |

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|---|---|---|---|---|
| Insecta | Diptera | Chironomidae | *Paratanytarsus* spp. | |
| Insecta | Diptera | Chironomidae | *Paratendipes* spp. | |
| Insecta | Diptera | Chironomidae | *Phaenopsectra* spp. | |
| Insecta | Diptera | Chironomidae | *Polypedilum fallax* | |
| Insecta | Diptera | Chironomidae | *Polypedilum flavum* | |
| Insecta | Diptera | Chironomidae | *Polypedilum halterale* | |
| Insecta | Diptera | Chironomidae | *Polypedilum illinoense* | |
| Insecta | Diptera | Chironomidae | *Polypedilum scalaenum* | |
| Insecta | Diptera | Chironomidae | *Polypedilum* spp. | |
| Insecta | Diptera | Chironomidae | *Procladius* spp. | |
| Insecta | Diptera | Chironomidae | *Pseudochironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Rheocricotopus robacki* | |
| Insecta | Diptera | Chironomidae | *Rheotanytarsus* spp. | |
| Insecta | Diptera | Chironomidae | *Robackia demeijerei* | |
| Insecta | Diptera | Chironomidae | *Stelechomyia perpulchra* | |
| Insecta | Diptera | Chironomidae | *Stenochironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Stictochironomus* spp. | |
| Insecta | Diptera | Chironomidae | *Sublettea coffmani* | |
| Insecta | Diptera | Chironomidae | *Tanytarsus* spp. | |
| Insecta | Diptera | Chironomidae | *Thienemanniella* spp. | |
| Insecta | Diptera | Chironomidae | *Thienemannimyia group* | |
| Insecta | Diptera | Chironomidae | *Tribelos jucundum* | |
| Insecta | Diptera | Chironomidae | *Tvetenia discoloripes (group)* | |
| Insecta | Diptera | Chironomidae | *Xylotopus par* | |
| Insecta | Diptera | Chironomidae | *Zavrelimyia* spp. | |

| | | | | |
|---|---|---|---|---|
| Insecta | Diptera | Culicidae | *Anopheles* spp. | * |
| Insecta | Diptera | Dixidae | *Dixella indiana* | * |
| Insecta | Diptera | Empididae | *Hemerodromia* spp. | |
| Insecta | Diptera | Simuliidae | *Simulium* spp. | |
| Insecta | Diptera | Syrphidae | *Eristalis* spp. | * |
| Insecta | Diptera | Tabanidae | *Chrysops* spp. | * |
| Insecta | Diptera | Tipulidae | *Antocha spp.* | |
| Insecta | Diptera | Tipulidae | *Hexatoma* spp. | |
| Insecta | Diptera | Tipulidae | *Limonia* spp. | |
| Insecta | Diptera | Tipulidae | *Pilaria* spp. | |
| Insecta | Diptera | Tipulidae | *Pseudolimnophila spp.* | |
| Insecta | Diptera | Tipulidae | *Tipula* spp. | |
| Insecta | Ephemeroptera | Baetidae | *Acentrella nadineae* | |
| Insecta | Ephemeroptera | Baetidae | *Acentrella parvula* | |
| Insecta | Ephemeroptera | Baetidae | *Baetis flavistriga* | |
| Insecta | Ephemeroptera | Baetidae | *Baetis intercalaris* | |
| Insecta | Ephemeroptera | Baetidae | *Baetis pluto* | |
| Insecta | Ephemeroptera | Baetidae | *Centroptilum* spp. | |
| Insecta | Ephemeroptera | Baetidae | *Heterocloeon curiosum* | |
| Insecta | Ephemeroptera | Baetidae | *Labiobaetis propinquus* | |
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes fleeki* | |
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes minutus* | |
| Insecta | Ephemeroptera | Baetidae | *Paracloeodes* spp. | |
| Insecta | Ephemeroptera | Baetidae | *Plauditus cestus* | |
| Insecta | Ephemeroptera | Baetidae | *Plauditus dubius (group)* | |
| Insecta | Ephemeroptera | Baetiscidae | *Baetisca carolina* | |

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|---|---|---|---|---|
| Insecta | Ephemeroptera | Caenidae | *Brachycercus* spp. | |
| Insecta | Ephemeroptera | Caenidae | *Caenis* spp. | |
| Insecta | Ephemeroptera | Caenidae | *Cerobrachys* spp. | |
| Insecta | Ephemeroptera | Ephemerellidae | *Serratella serratoides* | |
| Insecta | Ephemeroptera | Ephemerellidae | *Telagonopsis deficiens* | |
| Insecta | Ephemeroptera | Ephemeridae | *Ephemera blanda* | |
| Insecta | Ephemeroptera | Ephemeridae | *Ephemera* spp. | |
| Insecta | Ephemeroptera | Ephemeridae | *Hexagenia* spp. | |
| Insecta | Ephemeroptera | Heptageniidae | *Epeorus vitreous* | |
| Insecta | Ephemeroptera | Heptageniidae | *Heptagenia marginalis* | |
| Insecta | Ephemeroptera | Heptageniidae | *Leucrocuta* spp. | |
| Insecta | Ephemeroptera | Heptageniidae | *Stenonema modestum* | |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron carolina* | |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron interpunctatum* | |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron pallidum* | |
| Insecta | Ephemeroptera | Isonychiidae | *Isonychia* spp. | |
| Insecta | Ephemeroptera | Leptohyphidae | *Tricorythodes* spp. | |
| Insecta | Ephemeroptera | Leptophlebiidae | *Habrophlebiodes* spp. | |
| Insecta | Heteroptera | Corixidae | *Sigara* spp. | |
| Insecta | Heteroptera | Nepidae | *Ranatra* spp. | * |
| Insecta | Heteroptera | Notonectidae | *Notonecta* spp. | * |
| Insecta | Lepidoptera | Pyralidae | *Petrophila* spp. | |
| Insecta | Megaloptera | Corydalidae | *Corydalus cornutus* | |
| Insecta | Megaloptera | Corydalidae | *Nigronia fasciatus* | |
| Insecta | Megaloptera | Corydalidae | *Nigronia serricornis* | |
| Insecta | Megaloptera | Sialidae | *Sialis* spp. | |

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|---|---|---|---|---|
| Insecta | Odonata | Aeshnidae | *Basiaeschna janata* | |
| Insecta | Odonata | Aeshnidae | *Boyeria vinosa* | |
| Insecta | Odonata | Calopterygidae | *Calopteryx* spp. | |
| Insecta | Odonata | Coenagrionidae | *Argia* spp. | |
| Insecta | Odonata | Coenagrionidae | *Enallagma* spp. | |
| Insecta | Odonata | Coenagrionidae | *Ischnura* spp. | |
| Insecta | Odonata | Cordulegastridae | *Cordulegaster* spp. | |
| Insecta | Odonata | Corduliidae | *Epitheca* spp. | |
| Insecta | Odonata | Corduliidae | *Somatochlora* spp. | |
| Insecta | Odonata | Gomphidae | *Gomphus* spp. | |
| Insecta | Odonata | Gomphidae | *Hagenius brevistylus* | |
| Insecta | Odonata | Gomphidae | *Lanthus* spp. | |
| Insecta | Odonata | Gomphidae | *Ophiogomphus* spp. | |
| Insecta | Odonata | Gomphidae | *Progomphus obscurus* | |
| Insecta | Odonata | Gomphidae | *Stylogomphus albistylus* | |
| Insecta | Odonata | Libellulidae | *Libellula* spp. | * |
| Insecta | Odonata | Macromiidae | *Macromia georgina* | |
| Insecta | Odonata | Macromiidae | *Macromia* spp. | |
| Insecta | Plecoptera | Capniidae | *Allocapnia* spp. | |
| Insecta | Plecoptera | Leuctridae | *Leuctra* spp. | |
| Insecta | Plecoptera | Peltoperlidae | *Tallaperla* spp. | * |
| Insecta | Plecoptera | Perlidae | *Acroneuria abnormis* | |
| Insecta | Plecoptera | Perlidae | *Acroneuria arenosa* | |
| Insecta | Plecoptera | Perlidae | *Agnetina annulipes* | |
| Insecta | Plecoptera | Perlidae | *Eccoptura xanthenes* | |
| Insecta | Plecoptera | Perlidae | *Paragnetina fumosa* | |

| | | | | |
|---|---|---|---|---|
| Insecta | Plecoptera | Perlidae | *Paragnetina ichusa/media* | |
| Insecta | Plecoptera | Perlidae | *Perlesta* spp. | |
| Insecta | Plecoptera | Pteronarcyidae | *Pteronarcys biloba* | |
| Insecta | Plecoptera | Pteronarcyidae | *Pteronarcys dorsata* | |
| Insecta | Plecoptera | Pteronarcyidae | *Pteronarcys proteus* | |
| Insecta | Trichoptera | Brachycentridae | *Brachycentrus nigrosoma* | |
| Insecta | Trichoptera | Brachycentridae | *Brachycentrus spinae* | |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche bronta* | |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche morosa* | |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche sparna* | |
| Insecta | Trichoptera | Hydropsychidae | *Cheumatopsyche* spp. | |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche betteni* | |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche venularis* | |
| Insecta | Trichoptera | Hydroptilidae | *Hydroptila* spp. | |
| Insecta | Trichoptera | Hydroptilidae | *Leucotrichia pictipes* | |
| Insecta | Trichoptera | Leptoceridae | *Ceraclea ancylus* | |
| Insecta | Trichoptera | Leptoceridae | *Ceraclea transversa* | |
| Insecta | Trichoptera | Leptoceridae | *Nectopsyche exquisita* | |
| Insecta | Trichoptera | Leptoceridae | *Oecetis persimilis* | |
| Insecta | Trichoptera | Leptoceridae | *Oecetis* spp. | |
| Insecta | Trichoptera | Leptoceridae | *Setodes* spp. | |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes ignitus* | |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche guttifer* | |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche lepida* | |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche scabripennis* | |
| Insecta | Trichoptera | Philopotamidae | *Chimarra* spp. | |

| | | | | |
|---|---|---|---|---|
| Insecta | Trichoptera | Philopotamidae | *Dolophilodes distincta* | |
| Insecta | Trichoptera | Polycentropodidae | *Polycentropus* spp. | |
| Insecta | Trichoptera | Psychomyiidae | *Lype diversa* | |
| Insecta | Trichoptera | Psychomyiidae | *Psychomyia flavida* | |
| Insecta | Trichoptera | Psychomyiidae | *Psychomyia nomada* | |
| Insecta | Trichoptera | Rhycophilidae | *Rhyacophila formosa* | |
| Insecta | Trichoptera | Rhycophilidae | *Rhyacophila fuscula* | |
| Insecta | Trichoptera | Thremmatidae | *Neophylax oligius* | |
| Oligochaeta | Haplotaxida | Naididae | *Nais* spp. | * |
| Oligochaeta | Haplotaxida | Naididae | *Pristina* spp. | * |
| Oligochaeta | Haplotaxida | Tubificidae | *Branchiura sowerbyi* | * |
| Oligochaeta | Haplotaxida | Tubificidae | *Limnodrilus hoffmeisteri* | * |
| Oligochaeta | Haplotaxida | Tubificidae | *Limnodrilus* spp. | * |
| Oligochaeta | Haplotaxida | Tubificidae | *Tubifex* | * |
| Oligochaeta | Lumbriculida | Lumbriculidae | Lumbriculidae | * |

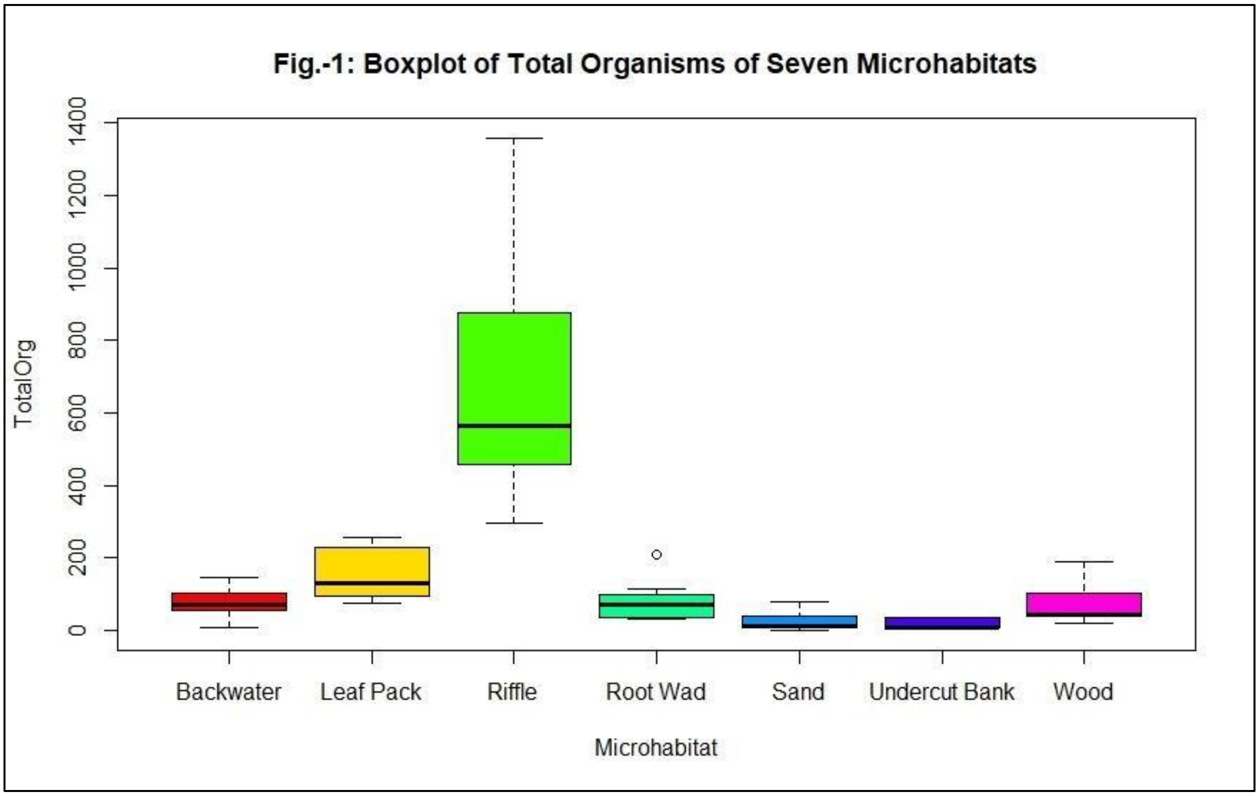

**Figure A14.** Boxplot of Total Organisms by Microhabitat. Riffles supported the highest number of total organisms compared to the other microhabitats.

**Table A8.** Taxa Associated with Microhabitats

| **Backwater** | stat | p.value |
|---|---|---|
| *Procladius*.spp. | 0.768 | 0.0001*** |
| *Cryptotendipes*.spp. | 0.610 | 0.0006*** |
| *Gomphus*.spp. | 0.599 | 0.0006*** |
| *Paratendipes*.spp. | 0.552 | 0.0026 ** |
| *Hexagenia*.spp. | 0.512 | 0.0051 ** |
| *Tribelos.jucundum* | 0.510 | 0.0073 ** |
| *Ablabesmyia.annulata* | 0.487 | 0.0156 * |
| *Centroptilum*.spp. | 0.461 | 0.0197 * |
| *Cladotanytarsus*.spp. | 0.443 | 0.0195 * |

| | | |
|---|---|---|
| *Chironomus*.spp. | 0.432 | 0.0194 * |
| **Leaf Pack** | stat | p.value |
| *Polypedilum.illinoense* | 0.629 | 0.0004*** |
| *Helichus*.spp. | 0.440 | 0.0213 * |
| **Riffle** | stat | p.value |
| *Isonychia*.spp. | 0.750 | 0.0001 *** |
| *Corydalus.cornutus* | 0.723 | 0.0001 *** |
| *Stenacron.interpunctatum* | 0.702 | 0.0002 *** |
| *Chimarra*.spp. | 0.698 | 0.0001 *** |
| *Ophiogomphus*.spp. | 0.681 | 0.0002 *** |
| *Acentrella nadineae* | 0.675 | 0.0001 *** |
| *Stenonema.modestum* | 0.674 | 0.0002 *** |
| *Baetis.intercalaris* | 0.621 | 0.0001 *** |
| *Ceraclea.ancylus* | 0.603 | 0.0036 ** |
| *Polypedilum.flavum* | 0.599 | 0.0006 *** |
| *Hydropsyche.sparna* | 0.595 | 0.0015 ** |
| *Leucrocuta*.spp. | 0.587 | 0.0009 *** |
| *Cheumatopsyche*.spp. | 0.553 | 0.0015 ** |
| *Psephenus.herricki* | 0.547 | 0.0007 *** |
| *Thienemannimyia.group* | 0.547 | 0.0011 ** |
| *Heptagenia.marginalis* | 0.545 | 0.0017 ** |
| *Hexatoma*.spp. | 0.542 | 0.0036 ** |
| *Stenelmis*.spp. | 0.527 | 0.0036 ** |
| *Antocha*.spp. | 0.524 | 0.0026 ** |

| | | |
|---|---|---|
| *Simulium*.spp. | 0.520 | 0.0055 ** |
| *Cricotopus.annulator* | 0.511 | 0.0047 ** |
| *Lanthus*.spp. | 0.510 | 0.0041 ** |
| *Nanocladius*.spp. | 0.505 | 0.0036 ** |
| *Rheotanytarsus*.spp. | 0.496 | 0.0051 ** |
| *Neophylax.oligius* | 0.472 | 0.0359 * |
| *Rhyacophila.fuscula* | 0.446 | 0.0209 * |
| *Nigronia.serricornis* | 0.431 | 0.0309 * |
| *Baetis.flavistriga* | 0.425 | 0.0084 ** |
| *Parametriocnemus*.spp. | 0.421 | 0.0448 * |
| *Stenacron.carolina* | 0.421 | 0.0315 * |
| *Hydroptila*.spp. | 0.418 | 0.0476 * |
| *Leuctra*.spp. | 0.401 | 0.0288 * |
| *Hydropsyche.betteni* | 0.382 | 0.0356 * |
| **Root Wad** | stat | p.value |
| *Triaenodes.ignitus* | 0.743 | 0.0001*** |
| *Labrundinia.pilosella* | 0.504 | 0.0058** |
| *Calopteryx*.spp. | 0.469 | 0.0083** |
| **Woody Debris** | stat | p.value |
| *Pycnopsyche.guttifer* | 0.397 | 0.0476 * |
| **Backwater+Leaf Pack** | stat | p.value |
| *Polypedilum.scalaenum* | 0.478 | 0.0122 * |
| **Backwater+Riffle** | stat | p.value |
| *Tanytarsus*.spp. | 0.416 | 0.0411 * |

| **Backwater+Root Wad** | stat | p.value |
|---|---|---|
| *Ablabesmyia.mallochi* | 0.475 | 0.01 ** |
| **Backwater+Sand** | stat | p.value |
| *Cryptochironomus*.spp. | 0.513 | 0.0069** |
| **Leaf Pack+Riffle** | stat | p.value |
| *Rheocricotopus.robacki* | 0.602 | 0.0004*** |
| *Acroneuria.abnormis* | 0.496 | 0.0050 ** |
| *Perlesta*.spp. | 0.480 | 0.0132 * |
| *Corynoneura*.spp. | 0.479 | 0.0166 * |
| *Nilotanypus*.spp. | 0.475 | 0.0135 * |
| *Brillia*.spp. | 0.431 | 0.0431 * |
| **Leaf Pack+Woody Debris** | stat | p.value |
| *Paragnetina.fumosa* | 0.522 | 0.0045** |
| **Riffle+Woody Debris** | stat | p.value |
| *Macronychus.glabratus* | 0.501 | 0.0068** |
| **Root Wad+Woody Debris** | stat | p.value |
| *Boyeria.vinosa* | 0.59 | 0.0011** |
| **Backwater+Root** | stat | p.value |
| *Macromia.georgina* | 0.413 | 0.0486 * |

Significance codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

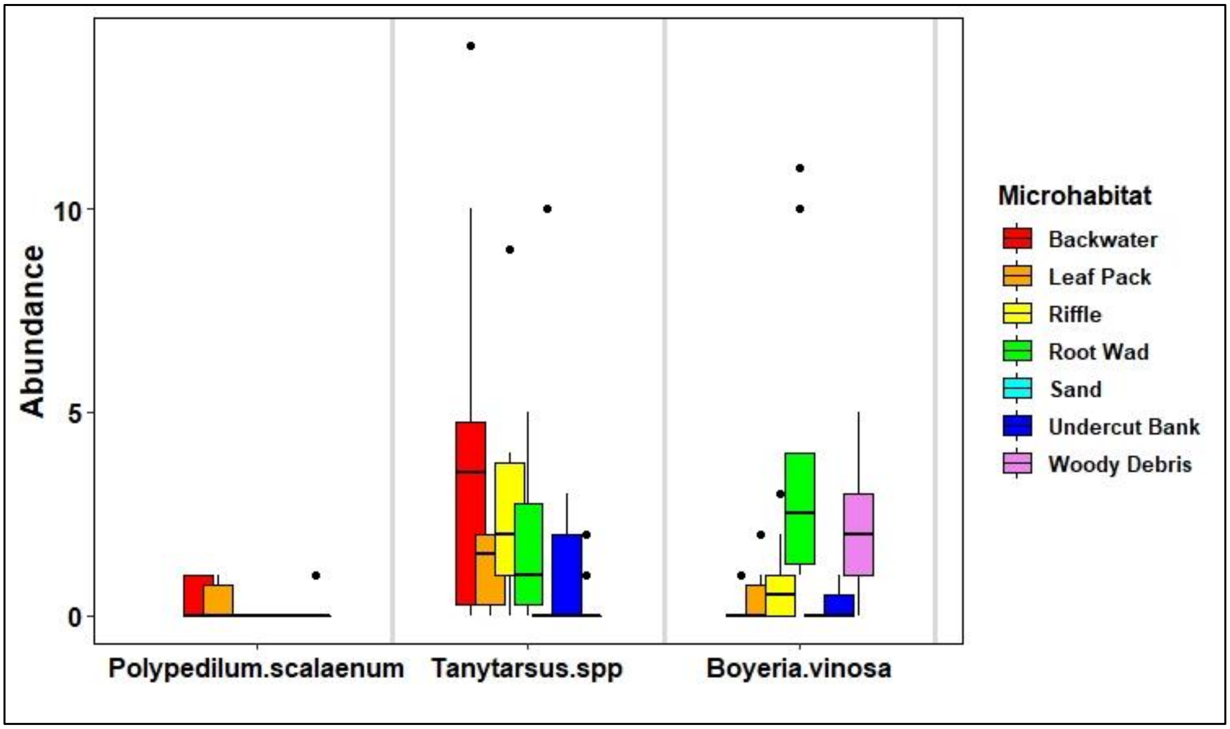

**Figure A15**. The Indicator Species Analysis identified *Polypedilum scalaenum* associated with Backwater and Leaf Pack microhabitats; *Tanytarsus* spp. with Backwater and Riffle microhabitats; and *Boyeria vinosa* with Root Wad and Woody Debris microhabitats.

**Table A9**. Traits Associated with Microhabitats

| **Leaf Pack** | **stat** | **p.value** |
|---|---|---|
| PLA | 0.427 | 0.0266 * |
| **Riffle** | stat | p.value |
| LDIS | 0.913 | 1e-04 *** |
| TEG | 0.893 | 1e-04 *** |
| RDRF | 0.855 | 1e-04 *** |
| WFLY | 0.853 | 1e-04 *** |
| PARM | 0.848 | 1e-04 *** |
| HCW | 0.846 | 1e-04 *** |
| STR | 0.844 | 1e-04 *** |
| EAB | 0.838 | 1e-04 *** |

| | | |
|---|---|---|
| SAB | 0.835 | 1e-04 *** |
| SSE | 0.830 | 1e-04 *** |
| WSY | 0.829 | 1e-04 *** |
| COL | 0.828 | 1e-04 *** |
| CLG | 0.817 | 1e-04 *** |
| NOSTR | 0.813 | 1e-04 *** |
| NATT | 0.812 | 1e-04 *** |
| DE | 0.811 | 1e-04 *** |
| STSW | 0.809 | 1e-04 *** |
| NSW | 0.806 | 1e-04 *** |
| UV | 0.806 | 1e-04 *** |
| PSY | 0.804 | 1e-04 *** |
| WKSW | 0.804 | 1e-04 *** |
| SWM | 0.803 | 1e-04 *** |
| NARM | 0.795 | 1e-04 *** |
| SLF | 0.791 | 1e-04 *** |
| ERO | 0.786 | 1e-04 *** |
| GIL | 0.778 | 1e-04 *** |
| SM | 0.778 | 1e-04 *** |
| SV | 0.771 | 1e-04 *** |
| PD | 0.770 | 1e-04 *** |
| MD | 0.764 | 1e-04 *** |
| CF | 0.757 | 1e-04 *** |
| HB | 0.757 | 1e-04 *** |

| | | |
|---|---|---|
| SATT | 0.755 | 1e-04 *** |
| VSLF | 0.750 | 1e-04 *** |
| CDRF | 0.739 | 1e-04 *** |
| LCW | 0.737 | 1e-04 *** |
| VLCW | 0.731 | 1e-04 *** |
| FSE | 0.720 | 1e-04 *** |
| SFLY | 0.710 | 1e-04 *** |
| HDIS | 0.703 | 1e-04 *** |
| LG | 0.702 | 3e-04 *** |
| EPR | 0.685 | 3e-04 *** |
| SH | 0.671 | 1e-04 *** |
| CG | 0.655 | 1e-04 *** |
| LLF | 0.634 | 2e-04 *** |
| SPR | 0.574 | 2e-04 *** |
| **Root Wad** | stat | p.value |
| CLB | 0.716 | 1e-04 *** |
| **Leaf Pack+Riffle** | stat | p.value |
| ADRF | 0.697 | 1e-04 *** |
| MV | 0.647 | 1e-04 *** |
| CLD | 0.610 | 2e-04 *** |
| **Backwater+Leaf** | stat | p.value |
| BRW | 0.656 | 1e-04 *** |
| DEP | 0.616 | 1e-04 *** |
| **Backwater+Riffle+Root** | stat | p.value |

| | | |
|---|---|---|
| SPL | 0.56 | 6e-04 *** |
| **Riffle+Root Wad+Woody** | stat | p.value |
| NSE | 0.48 | 0.0094 ** |

**Significance codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1**

# CHAPTER 4: EVALUATION OF THE IMPACT OF STORMWATER ON BENTHIC MACROINVERTEBRATE DIVERSITY AND STREAM ECOSYSTEM FUNCTION IN A PIEDMONT STREAM IN NORTH CAROLINA

## 4.1 ABSTRACT

As Percent Impervious Cover (%IC) increases with development, the increasing stormwater runoff volume and intensity negatively impacts stream geomorphology, natural hydrologic regime, water quality, and aquatic biota. However, the extent to which the increased stormwater flow from unmitigated stormwater impacts benthic macroinvertebrate taxa and trait richness, diversity, and function is not well documented. To better understand stormwater impacts on benthic macroinvertebrates assemblages, I took advantage of a natural field experiment to compare macroinvertebrate taxa and trait richness and diversity in 2 adjacent headwater tributaries that received stormwater runoff through different processes. One received stormwater runoff directly via 3 stormwater outfalls (TI) while the adjacent tributary received stormwater via natural overland and subsurface processes (TF). I investigated: 1) if the pattern of taxa and trait richness and diversity differ between the 2 tributaries receiving stormwater from different sources; and 2) if the carbon sources available to benthic macroinvertebrate assemblages differ between these 2 tributaries. I hypothesized that both taxa and trait richness and diversity would be lower in the stormwater impacted (TI) tributary than in the forest (TF) tributary due to the increased stormwater runoff from the adjacent development**.** I hypothesized that the carbon sources available to benthic macroinvertebrate food webs would be altered in the stormwater impacted tributary (TI) with changes in food sources. I predicted that the carbon sources impacted by stormwater flow will have fewer taxa in each trophic group and will have different carbon sources than the less impacted stream.

I sampled benthic macroinvertebrates from 3 sites in each tributary every 3 months from July 2016 through October 2017. During each sampling event I quantified the stream habitat quality using the Mecklenburg Habitat Assessment Protocols (EMHAP). To quantify benthic macroinvertebrate trophic relationships in the 2 tributaries, an analysis of carbon sources and functional feeding groups (FFGs) was conducted using $\delta^{13}C$ isotopes.

The habitat condition in the stormwater impacted tributary (TI), which had higher predicted shear stress than the forested tributary (TF) for the same storm event, was impaired and had EMAHP scores significantly lower than in the TF tributary. Taxa richness was significantly higher in the TF tributary than in the TI tributary. Taxa diversity and trait richness were also significantly higher in the TF tributary. Trait diversity was not significantly different between tributaries. The summer carbon $\delta^{13}C$ values of the periphyton and leaf pack food sources were distinctly different in the TF tributary while they were very similar and closer to allochthonous source $\delta^{13}C$ values in the TI tributary. The carbon $\delta^{13}C$ values of the collector-gatherers, herbivore-scrapers, predators, and shedders were closer to the carbon $\delta^{13}C$ values of the rock scrub in both tributaries. In the winter, the carbon $\delta^{13}C$ values of the rock scrub (periphyton) and leaf pack food sources were distinctly different in both tributaries. All the FFG trait richness except for the collector-gatherer were similar between tributaries. The collector-gatherer richness was significantly greater in the TF tributary. Collector-gatherers have been found to be the most abundant benthic macroinvertebrate feeding groups in impaired urban streams like TI.

## 4.2 INTRODUCTION

As population growth and development transforms watersheds from rural to more urban settings, the percent impervious cover of these watersheds increases, limiting the amount of precipitation that naturally infiltrates into the soil while increasing the amount of runoff that reaches a stream. It is well documented that stormwater runoff negatively impacts urban stream geomorphology, the natural hydrologic regime, water quality, and the aquatic biota (Center for Watershed Protection 2003; Coles et al. 2004; Walsh et al. 2005; Brown et al. 2009; Cuffney et al. 2010; Coleman et al 2011; Anim et al. 2018; Erba et al. 2020; Maloney et al. 2021). Walsh et al. (2005) described a group of predictable negative impacts due to the increased stormwater inputs to streams associated with urbanization as "*the Urban Stream Syndrome*". Stream channels are degraded by the increased volume and intensity of stormwater runoff. Bank erosion widens stream channels while channel bed scour deepens the channel. Sediment from eroding stream banks and adjacent development is deposited on the streambed, degrading benthic macroinvertebrate habitat (Allan 2004; Coleman et al. 2011; Coles et al. 2012; Booth et al. 2016; Roy et al. 2016; Sterling et al. 2016; Vietz et al. 2016) resulting in a decline of sensitive and an increase in tolerate benthic macroinvertebrate and fish species (Walsh et al. 2005; Gresens et al. 2007; Cuffney et al. 2010; Ntloko et al. 2021; Zerega et al. 2021; Bower et al. 2022). In addition to habitat impacts, nutrient and pollutant concentrations, and temperature increase in urban streams  (Dewson et al. 2007; Walsh et al. 2005) with negative impacts on in-stream communities (Carlisle et al. 2013; Juvigny-Khenafou 2021; Miller et al. 2019; Paul and Meyer. 2001; Serra et al. 2019).

In addition to in-stream impacts, urbanization negatively impacts the stream riparian zone by either thinning or removing the terrestrial plants that are essential for a healthy stream ecosystem (Paul and Meyer 2001; Roy et al. 2003; Gage et al. 2004; Alberti 2005; Walsh et al. 2007; Segura and Booth 2010; Coles et al. 2012; Sterling et al. 2016). The River Continuum Concept (Vannote et al. 1980) suggests that allochthonous carbon sources drive the energy flow in forested headwater streams. As the stream becomes larger in the downstream portions of the watershed, the riparian canopy cover thins allowing more sunlight to reach the stream while at the same time reducing the contribution of allochthonous material shifting the primary carbon source from allochthonous to autochthonous (algae) sources. The loss of riparian vegetation along small headwater streams due to urbanization may impact the energy sources available to the benthic macroinvertebrate assemblage impacting the benthic macroinvertebrate food webs found in headwater streams.

Numerous studies have shown that the use of stable-carbon isotopes in food web studies can identify the sources of carbon flowing through the ecosystem (Fry 1991, 2006; Mihuc and Toetz 1994; Finlay 2001; Zah et al. 2001; Post 2002; Ulseth and Hershey 2005; Reid et al. 2008; Winemiller et al. 2010; Layman et al. 2012; Baumgartner and Robinson 2017; Smucker et al. 2018). The type of carbon isotope ($^{13}C$ or $^{12}C$) incorporated in the photosynthetic process depends on the type of plant. $C_3$ plants, including trees, shrubs, and many grasses typically found in the riparian zone adjacent to streams, have lower $\delta^{13}C$ values that average near $-28^{o}/_{oo}$ (Fry 1991, 2006) while $C_4$ plants, such as freshwater algae, have higher $\delta^{13}C$ values ranging from -47 to $-27^{o}/_{oo}$ (Finlay 2001), thus enabling the distinction between allochthonous and autochthonous carbon sources.

The majority of studies of the effects of urbanization on streams and benthic macroinvertebrate assemblage focus on the collective impact of increased stormwater runoff on taxa richness (Walsh et al. 2005) and do not specifically address the impact of stormwater on benthic macroinvertebrate taxa trait diversity and food webs (Walsh et al. 2001; Roy et al. 2003; Gage et al. 2004; Wenger et al. 2009; Cuffney et al. 2010; O'Driscoll et al. 2010; King and Baker 2011; García et al. 2017; Mackintosh et al. 2017; Schmera et al. 2017; Laigle et al. 2018). While several stable-isotope studies have examined the impact of anthropogenic sources of nitrogen (for example, wastewater treatment plant discharges, sewer leaks, and lawn fertilizers) on stream food webs (Ulseth and Hershey 2005; Baumgartner and Robinson 2017; Smucker et al. 2018), the impact of increased stormwater volume and intensity on urban stream carbon sources has received little attention.

## 4.3 STUDY OBJECTIVES AND RESEARCH QUESTIONS

To better understand stormwater impacts on macroinvertebrates assemblages, I took advantage of a natural field experiment to compare macroinvertebrate taxa and trait diversity and richness in 2 tributaries that received stormwater runoff from different sources. Changes in biodiversity can impact ecosystem function by altering taxa traits and food web interactions(Loreau et al. 2001; Thébault and Loreau 2003, 2006; Woodward 2009; Thompson et al. 2012a, 2012b; Singer and Battin 2007; García et al. 2017; Mackintosh et al. 2017; Laigle et al. 2018). The two tributaries of Torrence Creek in this study are identical in all characteristics except for stormwater inputs. The stormwater impacted (TI) tributary receives direct stormwater runoff via stormwater infrastructure from impervious areas within the adjacent subdivision while the forested (TF) tributary receives stormwater runoff through more natural overland and subsurface processes. I assumed that the tributary receiving direct stormwater inputs has an altered (e.g., flashiness, peak flow, time to peak flow) hydrology compared to the TF tributary.

**Q4.1. How does the pattern of taxa and trait diversity and richness differ between 2 tributaries receiving stormwater from different sources?** I hypothesized that both taxa and trait richness and diversity would be lower in the stormwater impacted (TI) tributary than in the forest (TF) tributary due to increased stormwater runoff from the adjacent development.

**Q4.2. How do the carbon sources available to benthic macroinvertebrate assemblages differ between 2 tributaries receiving stormwater from different sources?** I hypothesized that the carbon sources available to benthic macroinvertebrate food webs were altered in the stormwater impacted tributary (TI) with changes in food sources. I predicted that the carbon sources impacted by stormwater flow will have fewer taxa in each trophic group and will have different carbon sources than the less impacted stream.

## 4.4 METHODS

### 4.4.1 STUDY SITES

A natural experimental site exists in the headwaters of Torrence Creek in Huntersville, NC (Figure 4.1A, 35.3848, -80.8442) where two adjacent tributaries were the focus of this study (Figure 4.1B). One tributary (Stormwater Impacted (TI)) is adjacent to a subdivision, which was built in the late 1990s, and receives stormwater runoff from the development through storm drainage infrastructure that is piped directly to the stream via three outfalls. Two stormwater outfalls drain directly to the stream above sites TI1 and TI3. The third stormwater outfall drains into a stormwater ditch between several houses and enters the TI tributary above site TI2. The second tributary (Forested Tributary (TF)) receives stormwater runoff through the riparian zone via natural overland and subsurface processes. The TI tributary watershed is small (0.257 $km^2$) and has 18.8% impervious cover (USGS StreamStats; https://streamstats.usgs.gov/ss/). TI is incised and does not have a well-defined riffle-pool structure. The stream substrate is covered with sand and coarse sediment. The TF tributary watershed is also small (0.345 $km^2$) and has 16.1 % impervious cover. The upper portion of the TF watershed is occupied by J.M. Alexander Middle School (1961) which has 51,200 $m^2$ of impervious area or 2.74% of the watershed. There were no storm drains connecting this impervious area directly to the forested tributary. A Duke Energy Power Line crosses the upper half of the watershed. Most of the watershed is covered with second growth hardwoods (oak, sweetgum, and tulip poplar are the dominant trees). TF has a well-defined riffle-pool configuration resembling a more natural stream ecosystem. The nearest USGS stream gauge on Torrence Creek to the study sites is approximately 3.4 km downstream. The annual precipitation in Mecklenburg County in 2016–2017 was 99.4 cm, which was 6.4 cm below normal. However, this rainfall total was slightly lower than the average rainfall of 122.4 inches for the previous 3 years.  The annual average temperature in

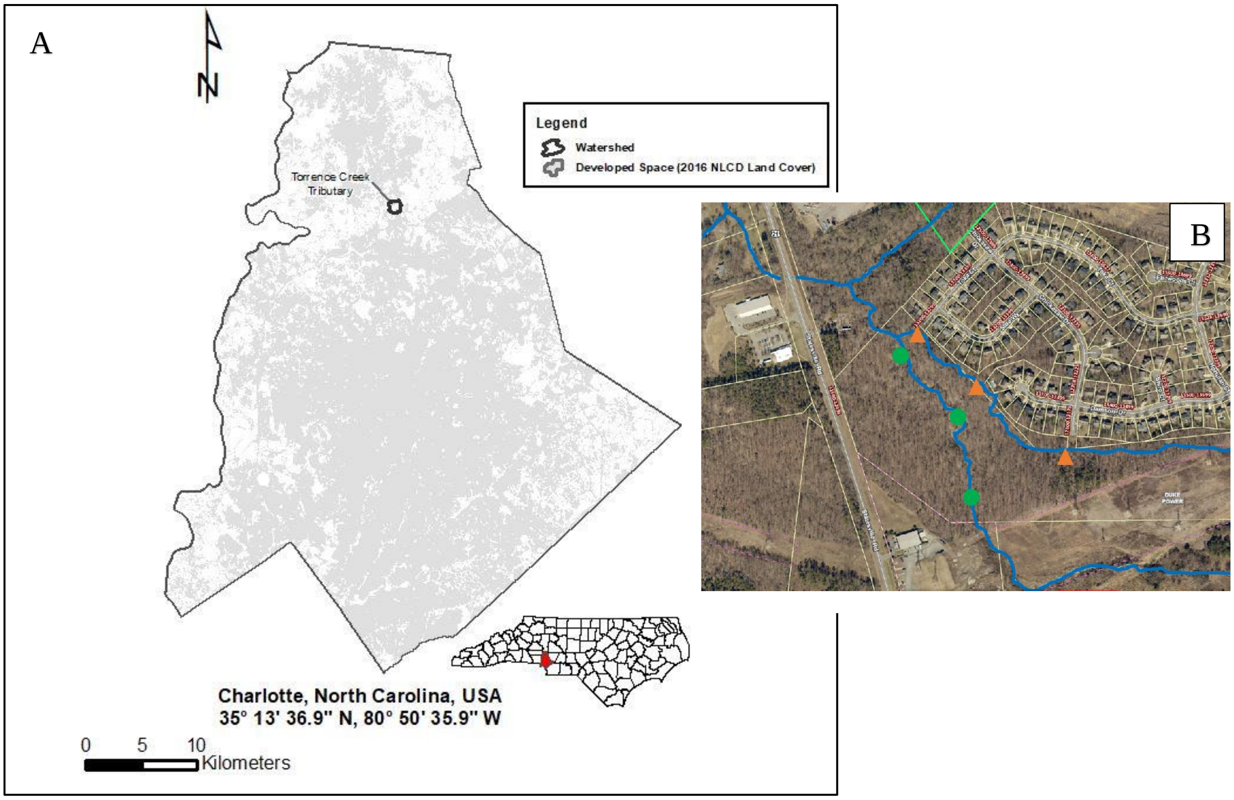

**Figure 4.1.** (**A**) Map of Mecklenburg County, NC, showing the location of the two Torrence Creek Tributaries near Huntersville. (**B**) Aerial photo showing the stormwater impacted tributary (TI) adjacent to a 200-home development and the forested tributary (TF) flowing through a forest dominated watershed with a middle school occupying a small area in the upper portion of the watershed. The orange triangles show the locations of the TI tributary sites (I1, I2, I3) and the green circles show the locations of the TF tributary sites (F1, F2, F3).

Mecklenburg County in 2016–2017 was 17.2$^{\circ\mathrm{C}}$ which was approximately normal (15.5°$^{\mathrm{C}}$) (https://www.weather.gov/gsp/cltcli).

There were three monitoring sites in each tributary: one at the lower end above the confluence of the 2 tributaries (I1, F1; Figures 4.1B, 4.2, 4.3), one near the middle of each tributary reach (I2, F2), and one located in the upper section(I3, F3). Each site (I1, I2, I3) on the TI tributary was located immediately downstream of a stormwater outfall (Figure 4.4). Each monitoring site was 50 m in length.

**Figure 4.2**. Forested Torrence Creek tributary (TF) Site F1 (looking upstream) located at the downstream end of the study reach.

**Figure 4.3.** Stormwater impacted Torrence Creek tributary (TI) Site I1 (looking upstream) located at the downstream end of the study reach.

**Figure 4.4**. Storm drain located at the upper end of site I1 on the stormwater impacted tributary (TI).

### 4.4.2 SITE GEOMORPHOLOGY, SEDIMENT SIZE, AND SHEAR STRESS

For each tributary, the stream channel geomorphology was surveyed in July 2016, and cross section maps for each monitoring site was created by the Charlotte-Mecklenburg Stormwater Services (CMSWS unpublished data). The sediment transport capacity of each tributary was modeled by CMSWS using the FLOWSED-POWERSED model using the RIVERMorph software (Version 5.2.0; Stantec, 2021).

The 100-particle Wolman pebble counts were conducted on 27 February 2018 in a representative riffle at sites TF1 and TI1 (Wolman 1954; Harrelson et al. 1994). The pebble count began at a randomly selected point at one side of the riffle. A substrate particle was selected by picking up the first particle touched by the tip of your index finger at the toe of your boot while averting your gaze. The particles were measured using a gravelometer for particles 4 to 362 mm. For particles less than 2.0 mm (silt to very coarse sand), a W. F. McCollough Sand-Gauge card was used to determine the sand size. For particles greater than 512 mm, the particle's intermediate axis was measured. Embedded particles or those too large to be moved were measured in place. For these, the smaller of the two exposed axes was measured. The next particle was measured by taking one step across the channel in the direction of the opposite bank and then selecting and measuring the substrate particle as previously described. This process is repeated until 100 substrate particles are measured. If additional measurements are needed to complete the 100 particle measurements when the opposite bank is reached, an additional transect is started by moving upstream or downstream randomly. The pebble count data were used to calculate each stream's $d_{50}$ using The Reference Reach Spreadsheet Pebble Count software (Mecklenburg 2006; Appendix Figures A16 and A17), which were then used in the FLOWSED-POWERSED model. Macroinvertebrates and environmental data were collected from each site once per meteorological season from July 2016 through October 2017.

### 4.4.3 BACKGROUND ENVIRONMENTAL DATA

Field measurements of conductivity, dissolved oxygen (DO), pH, and temperature were taken using a YSI Pro DSS hand-held multi-probe meter (Table 4.1) during each benthic macroinvertebrate sampling event at each stream site in each tributary. Base flow water quality was monitored from the center of the channel at the lowest sampling point on each tributary (I1 and F1) during each meteorological season from December 2016 through November 2017. The parameters measured, including nutrients, fecal coliform bacteria, suspended solids, and turbidity, were similar to each other in all seasons (Appendix Table A10). All water samples were analyzed by the Charlotte Water Utility's Laboratory Services except for TOC and DOC samples which were processed at the Hydrology and Biogeochemical Lab at the University of North Carolina Charlotte (Appendix Table A11). All parameters were within average ranges for Mecklenburg County, NC streams.

**Table 4.1.** Mean and standard deviation of field measurements of temperature, dissolved oxygen, conductivity, and pH taken at each site during the benthic macroinvertebrate sampling from July 2017 through October 2017. Number of samples = 3 for each date.

| **Tributary** | **Date** | **Temp °C** | **DO mg/l** | **DO %** | **Conductivity µS/cm** | **pH** |
|---|---|---|---|---|---|---|
| Forested | 7/27/2016 | 24.35±0.22 | 7.46±0.37 | 89.2±4.53 | 132.33±2.52 | 7.08±0.27 |
| Impacted | 7/21/2016 | 21.84±1.49 | 6.88±0.93 | 78.57±12.92 | 99.67±6.66 | 6.76±0.25 |
| Forested | 12/7/2016 | 10.47±1.16 | 8.93±0.70 | 79.87±4.47 | 126.67±9.50 | 6.51±0.18 |
| Impacted | 11/28/2016 | 10.22±2.06 | 8.80±1.25 | 80.77±15.57 | 103.67±8.51 | 6.79±0.015 |
| Forested | 2/9/2017 | 11.13±0.40 | 10.24±0.23 | 100.77±0.65 | 111.13±1.03 | 7.39±0.15 |
| Impacted | 2/8/2017 | 13.83±0.90 | 9.99±1.13 | 96.5±10.19 | 99.8±9.59 | 7.15±0.39 |
| Forested | 5/15/2017 | 18.47±0.74 | 8.48±0.55 | 90.87±4.11 | 127.5±2.43 | 7.25±0.14 |
| Impacted | 5/2/2017 | 17.6±1.35 | 7.74±0.18 | 81.0±2.43 | 101.38±11.38 | 6.86±0.36 |
| Forested | 7/13/2017 | 22.87±1.51 | 7.82±0.33 | 91.07±4.24 | 132.7±1.92 | 7.27±0.11 |
| Impacted | 7/10/2017 | 20.73±1.17 | 7.76±0.10 | 86.63±3.12 | 102.57±9.07 | 6.94±0.29 |
| Forested | 10/31/2017 | 11.63±1.46 | 9.77±0.61 | 91.53±2.97 | 132.1±0.96 | 7.29±0.078 |
| Impacted | 10/31/2017 | 11.6±2.17 | 8.80±0.51 | 80.7±2.49 | 105.27±9.79 | 6.9±0.13 |

### 4.4.4 BENTHIC MACROINVERTEBRATE SAMPLING

Benthic macroinvertebrate assemblages were collected from each of the three 50-meter sites on each tributary using the Standard Qualitative Method developed by NCDEQ Biological

Assessment Unit (NCDEQ 2016) and described in the Mecklenburg County Bioassessment Standard Operating Procedures (CMSWS 2017). The Standard Qualitative Method collections consists of 2 kick net riffle samples, 3 sweep-net samples from microhabitats found within runs, glides, and pool such as root wads, soft sediment in undercut bank areas, woody debris, macrophyte beds, and overhanging vegetation, 1 leaf-pack sample, 2 rock and/or log wash samples, and visual collections. The benthic macroinvertebrates in these samples were sorted in the field and preserved in glass vials containing 95% ethanol. The samples were collected from each site once per meteorological season beginning in July 2016. Meteorological seasons were defined as: Winter (December-February); Spring (March-May); Summer (June-August); Fall (September-November). All benthic macroinvertebrates collected were identified to the lowest possible taxonomic level (List of taxa collected is in Appendix Table A12).

### 4.4.5 CARBON SOURCES TO FUNCTIONAL FEEDING GROUPS

To quantify benthic macroinvertebrate trophic relationships in the 2 tributaries, an analysis of carbon sources and functional feeding groups (FFGs) was conducted using $\delta^{13}C$ isotopes. Samples of benthic macroinvertebrate taxa from each trophic group (Predators, Shredders, Collector-Gatherer, Collector-Filterer, Herbivore-scraper; Poff et al. 2006) were collected from each tributary in June 2017 and February 2018. To characterize potential food sources, fine particulate organic matter (FPOM), sediment, algae, and leaf pack samples were collected at the same time as the benthic macroinvertebrate taxa. Surface water was collected in plastic liter jars and filtered through a GF/F filter to collect FPOM suspended in the water column. FPOM suspended on the surface of the substrate was collected using an aspirator. Sediment samples were collected from the top 2 cm of the substrate using a plastic corer. Rocks were scrubbed to collect periphyton samples. Leaf packs that have been conditioned by colonization by bacteria and fungi (slimy to the touch), if present, were also collected. Samples were dried and prepared following McNeely et al. (2006) and analyzed by the Utah State University Stable Isotope Laboratory lab for $\delta^{13}C$ analysis. Each taxa collected was individually analyzed for $\delta^{13}C$.

### 4.4.6 STREAM HABITAT ASSESSMENT

Stream habitat conditions were assessed at all 6 sites using the Enhanced Mecklenburg Stream Habitat Assessment Protocols (EMHAP; Barbour et al. 1999; CMSWS 2020a; CMSWS 2020b) during each sampling event. The EMHAP evaluates 10 habitat condition parameters including instream cover, epifaunal substrate, embeddedness, channel alteration, sediment deposition, frequency of riffles, channel flow status, bank vegetation protection, bank stability, and vegetative riparian zone width using a combination of visual assessments and physical measurements. The EMHAP scores range have been associated with stream habitat conditions ranging from degraded (<60) to fully supporting (≥160). At each site, the number of microhabitats, including riffles, pools, runs, root wads, undercut banks, woody debris, leaf packs, backwater, macrophyte beds, and sandy areas found in the 50-meter study reach were counted. In addition to the EMHAP assessment at each site, 4 transects across the stream were set up at 17 meters intervals starting the beginning of each 50-meter study. At each transect, both the active-channel width (generally baseflow channel) and wetted-channel width were measured using a measuring tape. USGS (2004) defines the active channel as “the width of the active channel measured perpendicular to streamflow.” The active channel is usually much narrower than the bankfull channel and is measured from a point along the bank near the edge of permanent vegetation closest to the stream (Figure 4.5). The substrate was assessed along each transect by taking an abbreviated Wolman particle count by measuring substrate particle sizes at 10 equally spaced intervals.

**Figure 4.5.** A transect across the channel showing the locations of the active- and wetted-channels (CMSWS 2020a).

Headwater streams in undisturbed watersheds usually have a denser canopy than higher order streams (Vannote et al. 1980) driving the primary food source in headwater streams to be primarily of allochthonous origins (Cummins 1974; Vannote et al. 1980; Allan and Castillo 2007). As a result, benthic macroinvertebrate assemblages in headwater streams were dominated by collector-gatherer and shredder trophic groups (Cummins 1974; Vannote et al. 1980; Allan and Castillo 2007; Merritt et al. 2008). To compare the canopy cover of the two tributaries, the canopy cover was quantified at each study site using a spherical densiometer (Lemmon 1956; Pleus and Schuett-Hames 1998). Measurements were taken at the 0-, 25-, and 50-meter points within the 50-meter stream segment. The three measurements were averaged together for the percent canopy cover score for the study reach.

## 4.5 DATA ANALYSIS

### 4.5.1 STREAM HABITAT CONDITION AND CANOPY COVER

The stream habitat conditions as measured by the EMHAP and percent canopy cover in the two tributaries were compared using the Kruskal-Wallis rank sum test to test the null hypothesis that the EMHAP scores and percent canopy cover at each site were the same in each tributary. The Dunn Test for multiple comparisons was used to evaluate pairwise comparisons between each tributary and season for each parameter to identify any significant interactions. A two-way Anova was run to test for tributary-season interactions (*aov* function in the ‘stats’ package in R; R Core Team 2020).

### 4.5.2 BENTHIC MACROINVERTEBRATES

I used non-metric multidimensional scaling (NMDS) analysis to evaluate the relationship of taxa and trait distribution among the two tributaries (metaMDS function in the ‘vegan’ package; Oksanen et al. 2020).

Taxa and trait diversity were calculated as Shannon-Wiener Diversity (H′) indices (*diversity* function in the ‘vegan’ package; Oksanen et al. 2020). I tested all data for normality using the Shapiro-Wilks Normality test and found that the macroinvertebrate taxa richness and taxa and trait diversity data had normal distribution. The trait richness data were significantly different from normal and were transformed using Tukey’s Ladder of Powers (*transformTukey* function in the ‘rcompanion’ package; Mangiafico 2016). I used the ANOVA model to test the null hypothesis that taxa and trait diversity and richness were the same in the TF and TI tributaries. I first tested for differences among the three sites in each tributary. Finding no significant differences among the sites in each tributary (Appendix Table A13), the three sites per tributary were treated as pseudoreplicates for each season in subsequent analyses. I then used the two-way ANOVA model to determine if significant interactions existed between multiple factors impacting taxa and trait diversity and richness such as stream type (TF vs. TI) and seasonality (*aov* function in the 'stats' package in R; R Core Team 2020). The *TukeyHSD* function in the 'stats' package in R was used to evaluate pairwise comparisons between streams and seasons to identify any significant interactions.

Preliminary analyses of Functional Feeding Group (FFG) taxa richness using the Kruskal-Wallis rank sum test showed and the Dunn Test for multiple comparisons showed that the FFG taxa richness at each site in each tributary was not significantly different. As a result, FFG taxa richness samples in each tributary were used as pseudoreplicates for each season. Comparison of richness the two tributaries were conducted using the Student T-test (t.test function in the 'stats' package R; R Core Team 2020). To examine how taxa and traits change along a stream habitat gradient, a correlation analysis was conducted on taxa and trait richness and diversity with EMHAP (*cor.test* function in the 'stats' package in R; R Core Team 2020).

### 4.5.3 CARBON SOURCE ANALYSES

I tested all data for normality using the Shapiro-Wilks Normality test and found that the carbon $\delta^{13}C$ data did not have normal distributions. I used the Kruskal-Wallis rank sum test to test the null hypothesis that the $\delta^{13}C$ values were the same for each functional feeding group taxa and food source in each tributary. The Dunn Test for multiple comparisons was used to evaluate pairwise comparisons between each tributary and season for each parameter to identify any significant interactions.

I evaluated the $\delta^{13}C$ data for inconsistencies or signs of analytical errors prior to plotting the $\delta^{13}C$ data to evaluate the food web differences between the two tributaries in the summer and in the winter. Inconsistencies or apparent analytical errors were found with $\delta^{13}C$ data in several samples including all sediment samples, two summer Forest tributary Leaf Pack samples and the FPOM-water sample from the winter Forest sample. Analytical errors were seen in several of the benthic macroinvertebrate specimens analyzed where the $\delta^{13}C$ was not close to any carbon source. These errors generally occurred when the amount of carbon in the sample was small and were removed from the analysis.

## 4.6 RESULTS

### 4.6.1 GEOMORPHOLOGY, SEDIMENT SIZE, AND SHEAR STRESS

The cross sections of the TF and TI tributaries show valley shape differences between the 2 tributaries as well as differences in the floodplain connectivity (Figures 4.6 and 4.7; CMSWS unpublished data). The TI tributary has greater floodplain connectivity while the TF tributary has a steeper valley configuration. The FLOWSED-POWERSED model results show that shear stress was predicted to be greater in the TI tributary indicating that greater amounts of sediment were transported during a bankfull storm event than in the TF tributary (Figure 4.8). The $d_{50}$ particle size in the TI tributary was 18 mm smaller (14 mm) than the $d_{50}$ in the TF tributary (32 mm) supporting the FLOWSED-POWERSED model's prediction that more sediment is transported by the TI than by the TF tributary during the same storm event (Appendix Figures A16 and A17).

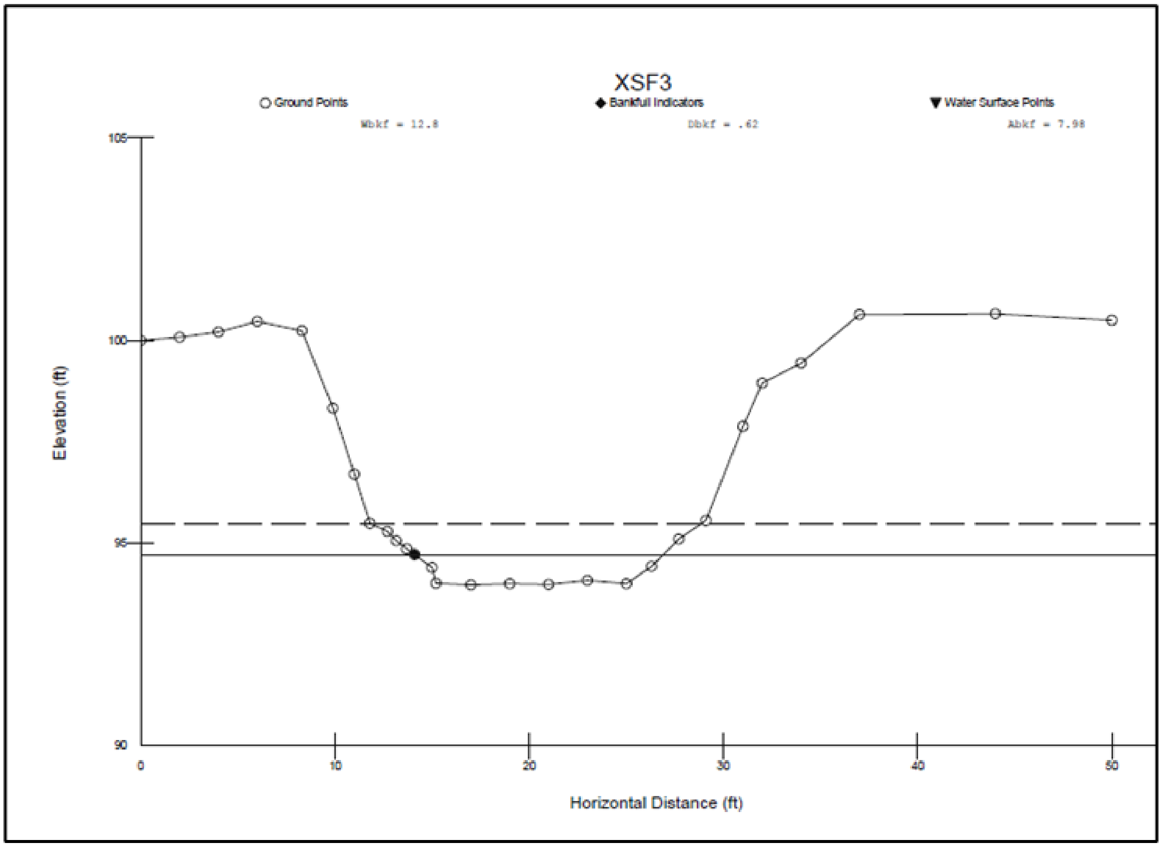

**Figure 4.6.** Cross section of Forested tributary (TF) at site F1.

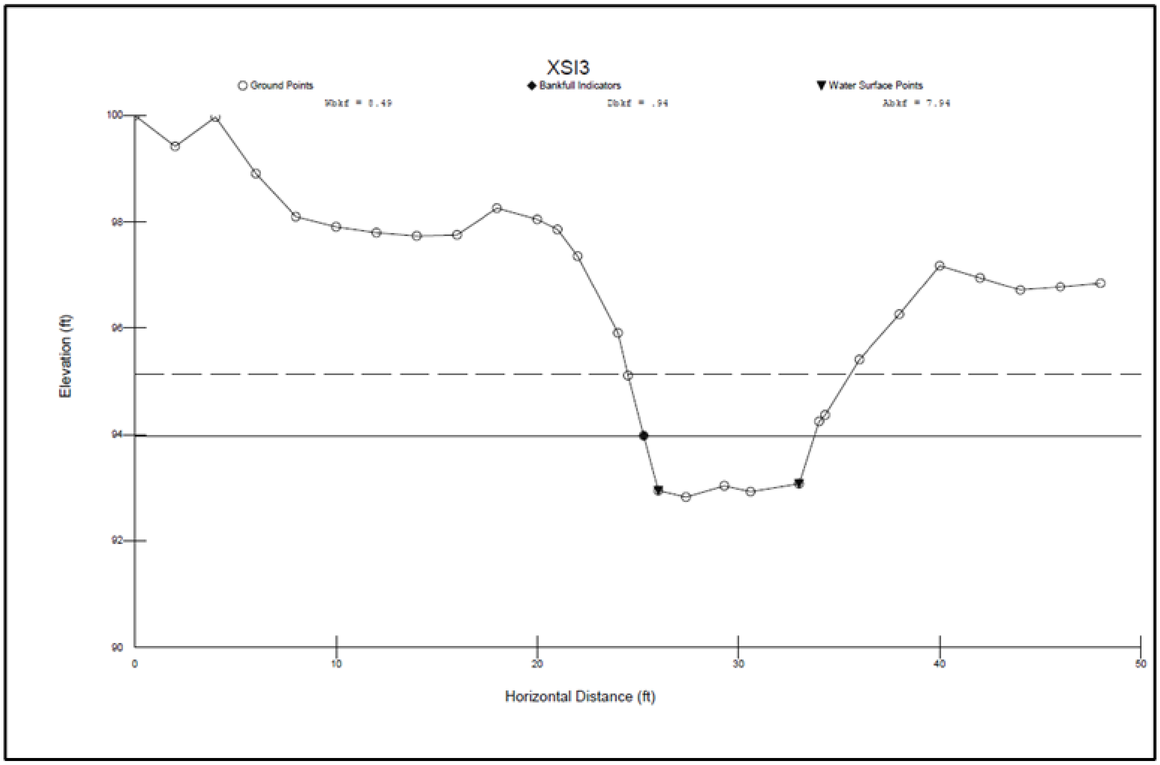

**Figure 4.7**. Cross section of Impacted tributary (TI) at site I1.

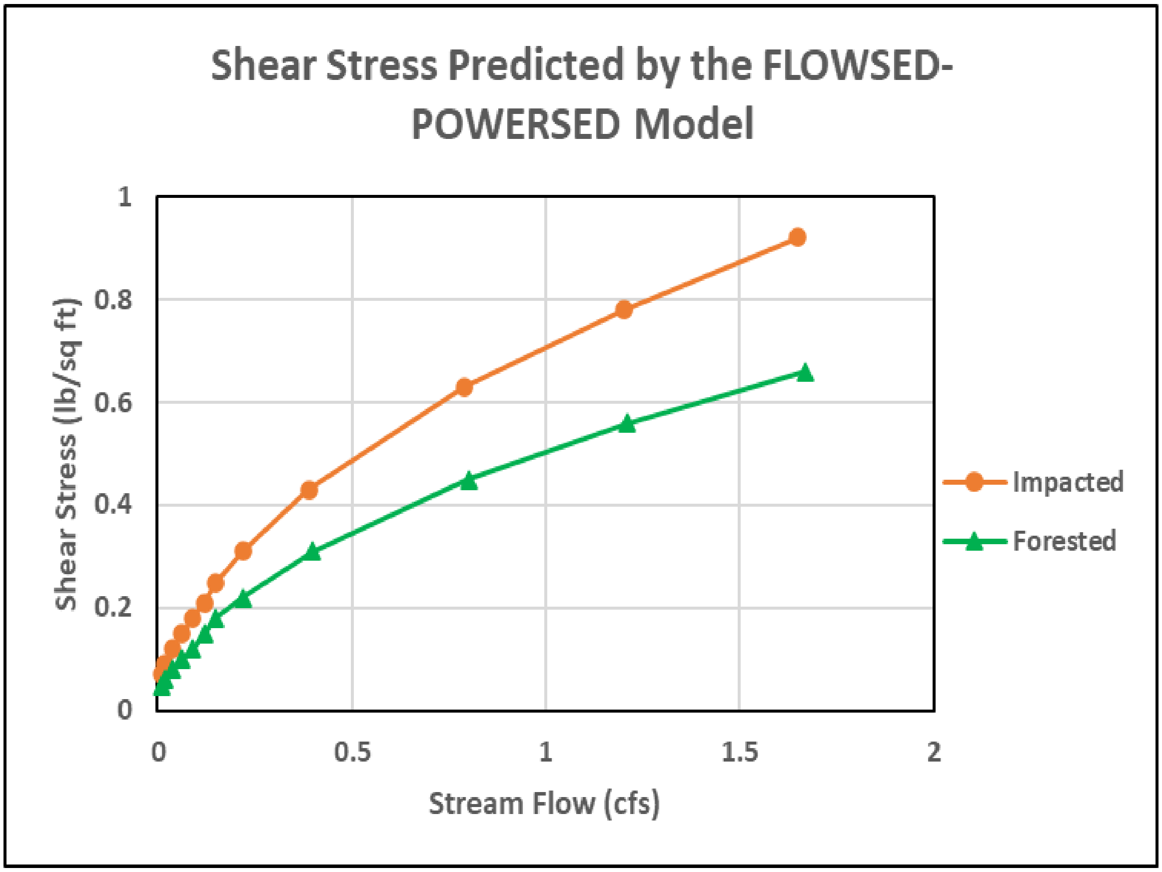

**Figure 4.8.** FLOWSED-POWERSED model results show that Shear Stress is predicted to be greater in the stormwater impacted (brown) than the forested (green) tributary for the same bankfull event.

### 4.6.2 HABITAT CONDITIONS

The EMHAP scores for the TF tributary ranged 113 - 139 while the EMHAP scores in the TI tributary ranged 80 -104. These scores rate the instream habitat in the TF tributary as partially supporting and in the TI tributary as impaired (Barbour et al. 1999). The instream habitat in the TF tributary was significantly higher than in the TI tributary (Two-Way ANOVA streams $p<0.001$; Figure 4.9). In each tributary, the EMHAP scores were not significantly different among seasons (Two-Way ANOVA seasons $p>0.05$ ; Figure 4.9). There was no stream:season interaction ($p>0.05$).

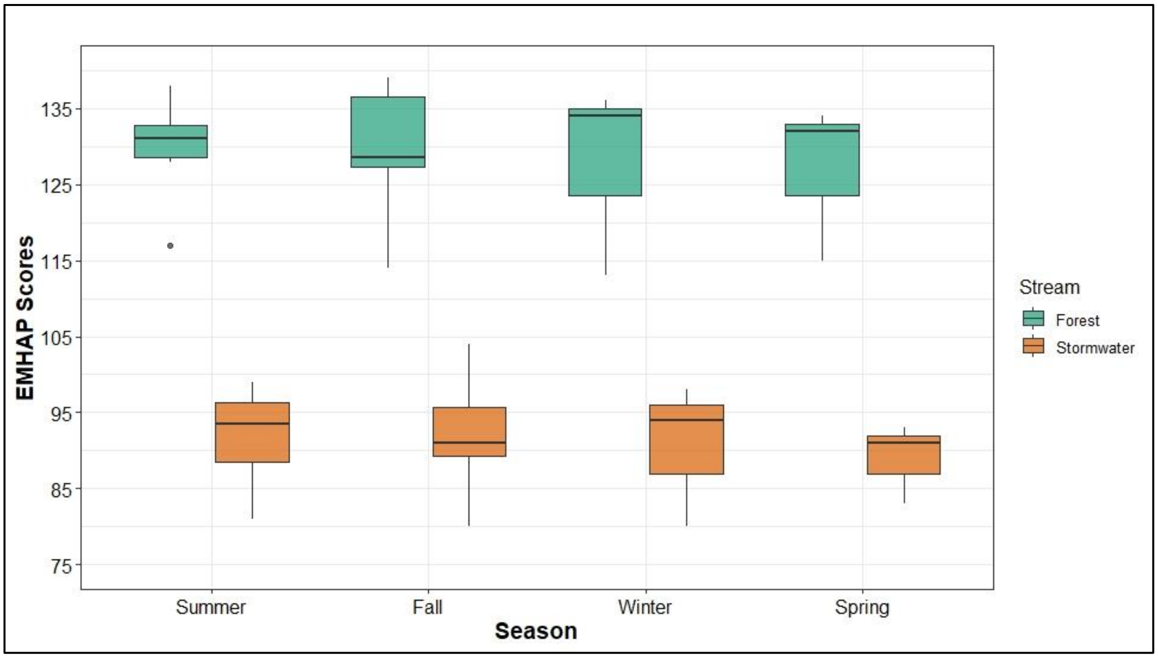

**Figure 4.9**. Box plots of EMHAP scores in each tributary across seasons. The EMHAP scores were significantly higher in the forested tributary (green) than in the stormwater impacted tributary (brown) in each tributary each season. Each season represents 3 replicates per stream.

Percent canopy cover in each tributary was not significantly different (Two-Way ANOVA streams $p>0.05$; Figure 4.10). However, the tree canopy varied among seasons with the percent canopy cover significantly less in winter (Two-Way ANOVA seasons $p<0.002$; Figure 4.10). There was no stream:season interaction ($p>0.05$).

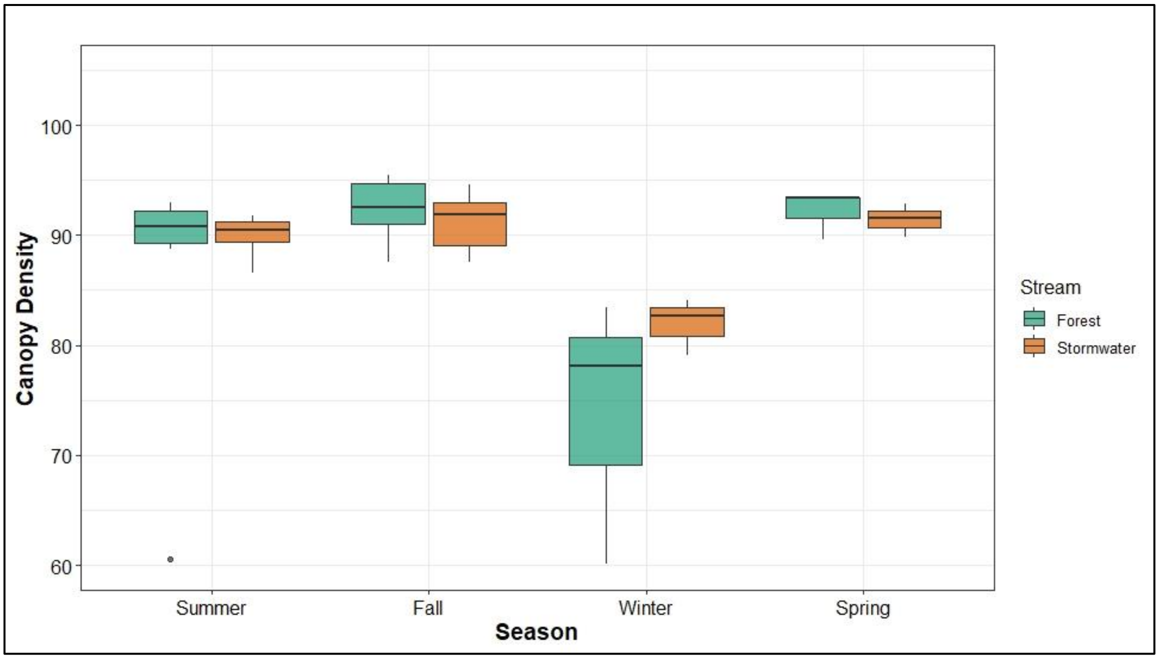

**Figure 4.10.** Box plots of percent canopy cover in each tributary across seasons. Percent canopy cover was similar in each tributary each season and was lowest during the winter season. Each season represents 3 replicates per stream.

### 4.6.3 BENTHIC MACROINVERTEBRATE

Taxa richness was greater in the TF tributary than in the TI tributary and was significantly positively correlated with EMHAP scores (r=0.531, p<0.001; Figure 4.11). Trait richness was similar in the two tributaries and was not significantly correlated with EMHAP scores (r=0.152, p>0.05). Taxa diversity was higher in the TF tributary than in the TI tributary but was not significantly correlated with EMHAP scores (r=0.0958, p>0.05; Figure 4.12). Trait diversity was similar in both tributaries and was not significantly correlated with EMHAP scores (r=-0.083, p>0.05).

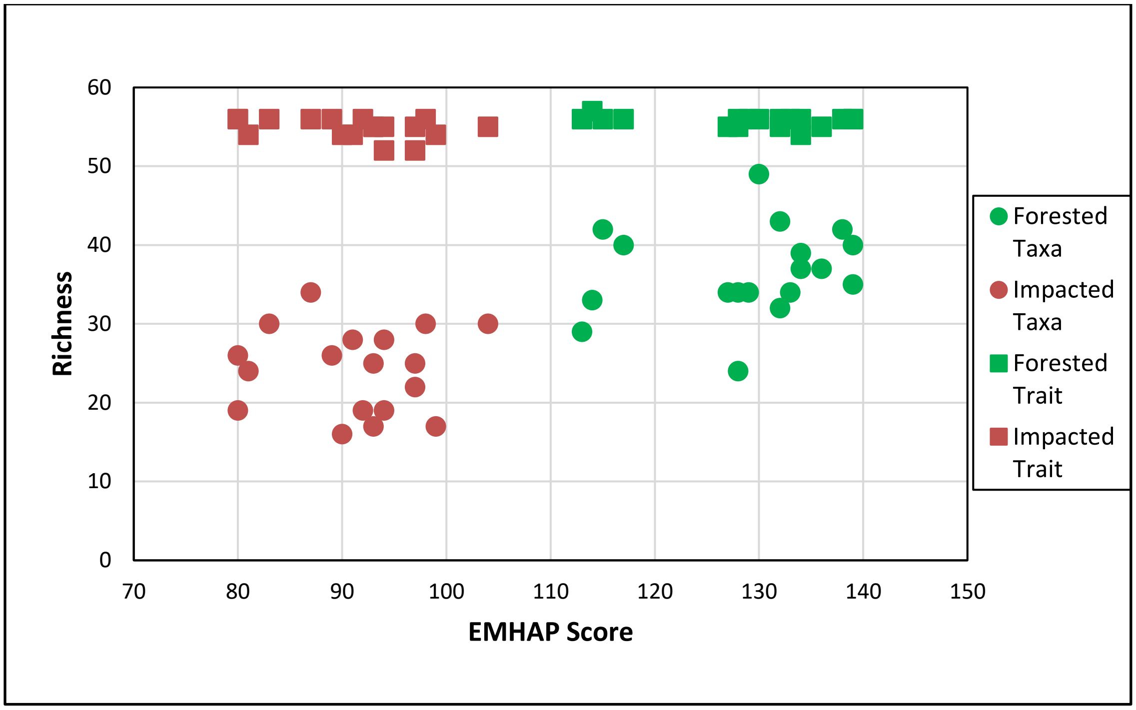

**Figure 4.11**. Changes in taxa (circles) and trait (squares) richness in the forested (green) and stormwater impacted (brown) tributaries with changes in EMHAP. Taxa richness was significantly positively correlated with EMHAP scores while trait richness was not correlated with EMHAP scores.

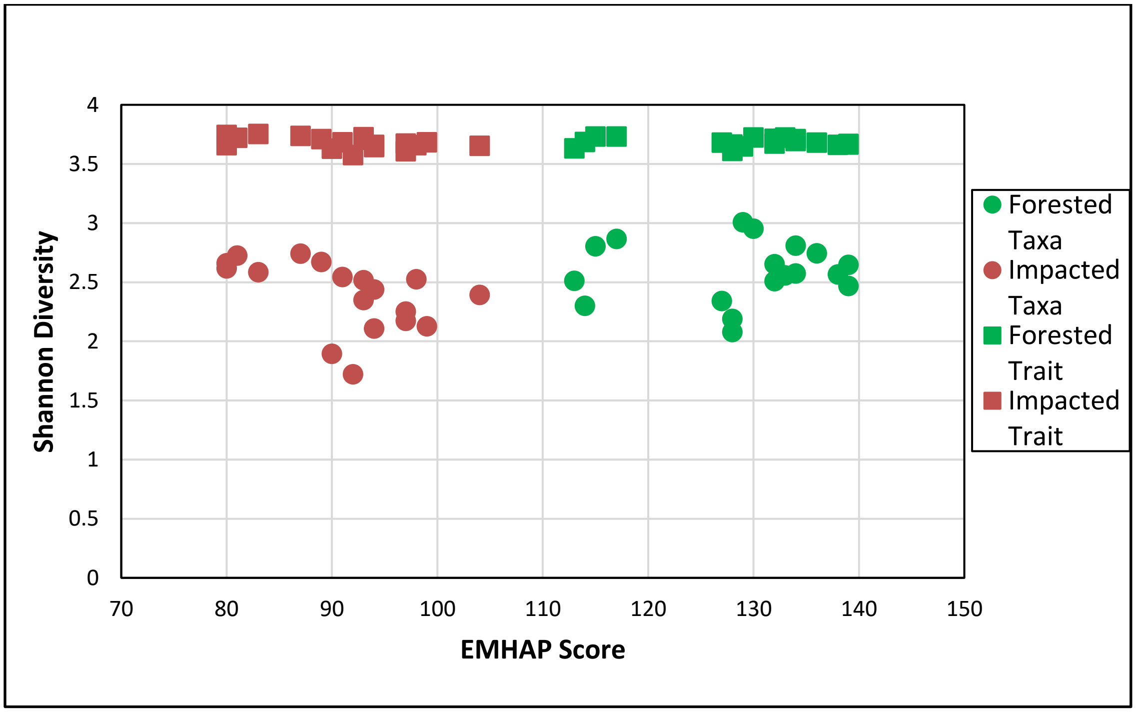

**Figure 4.12**. Taxa (circles) and trait (squares) diversity were not significantly correlated with EMHAP scores.

Taxa found in the TF tributary were more similar throughout the year than the taxa found in the TI tributary (Figure 4.13). Like the distribution of taxa, the traits found in the TF tributary were more similar than the traits found in the TI tributary (Figure 4.14).

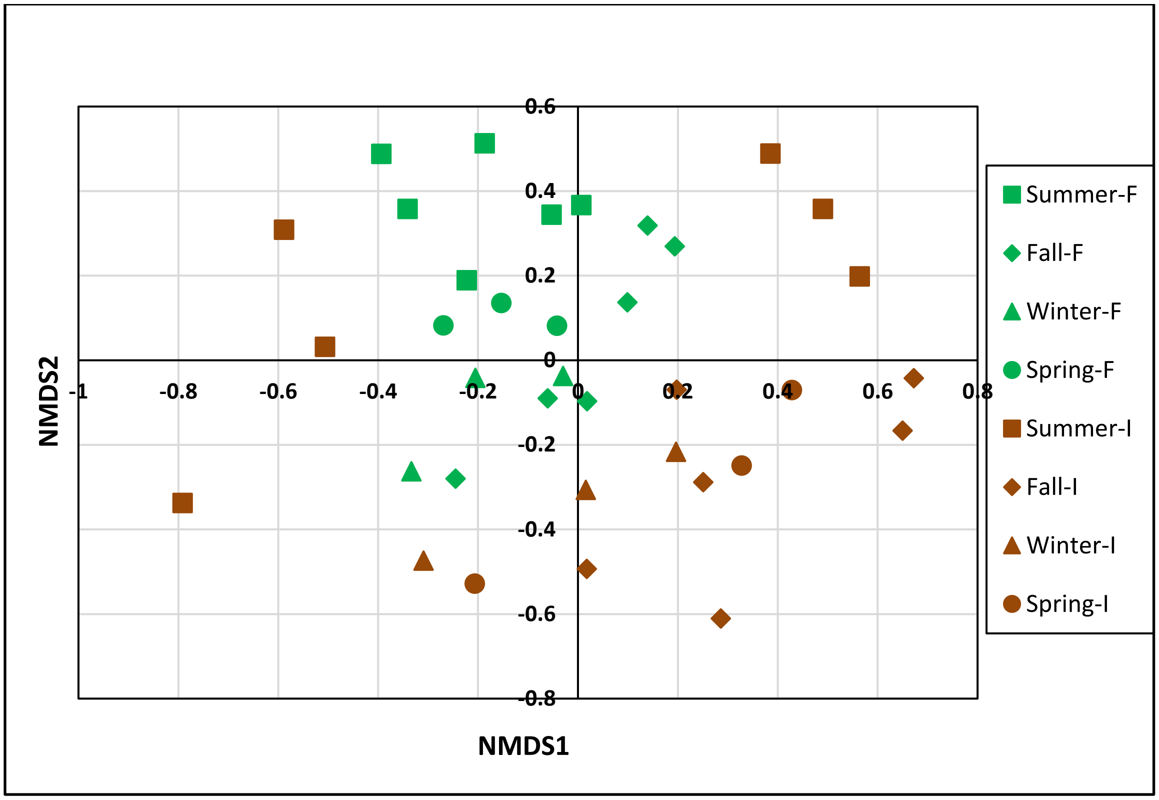

**Figure 4.13**. NMDS of taxa across seasons and tributaries. Taxa were more similar throughout the year in the forested tributary (green) than in the stormwater impacted tributary (brown).

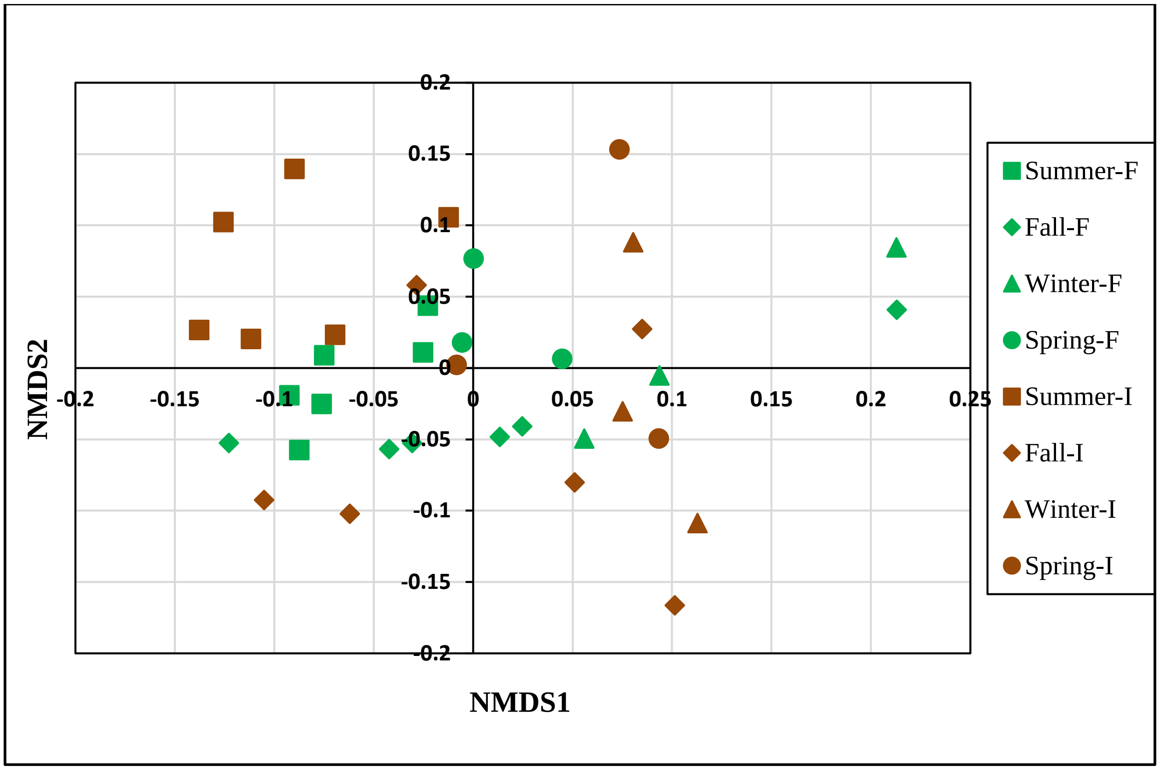

**Figure 4.14**. NMDS of traits across seasons and tributaries. Traits in the forested tributary (green) were more similar throughout the year than in the stormwater impacted tributary (brown).

Taxa richness was significantly higher in the TF than the TI tributary (Two-Way ANOVA streams p<0.001; Figure 4.15) but was not significantly different among seasons (Two-Way ANOVA seasons p>0.05). There was no stream:season interaction (p>0.05). Taxa diversity was significantly higher in the FI tributary than the TI tributary (Two-Way ANOVA streams p<0.05; Figure 4.16). Taxa diversity was not significantly different among seasons (Two-Way ANOVA seasons p>0.05). There was no stream:season interaction (p>0.05).

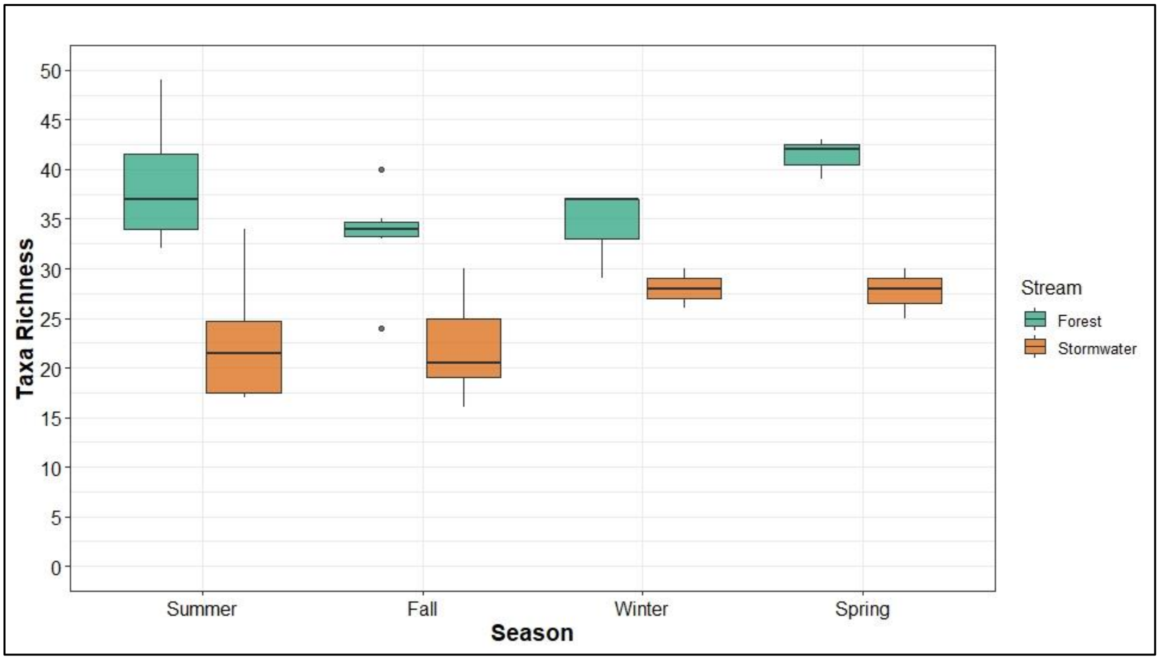

**Figure 4.15.** Box plot of taxa richness across seasons in the forested (green) and stormwater impacted (brown) tributaries. Taxa richness was significantly higher in the forested tributary than in the stormwater impacted tributary (Two-Way ANOVA streams p<0.001) but did not vary across seasons (Two-Way ANOVA seasons p>0.05). Each season represents 3 replicates per tributary.

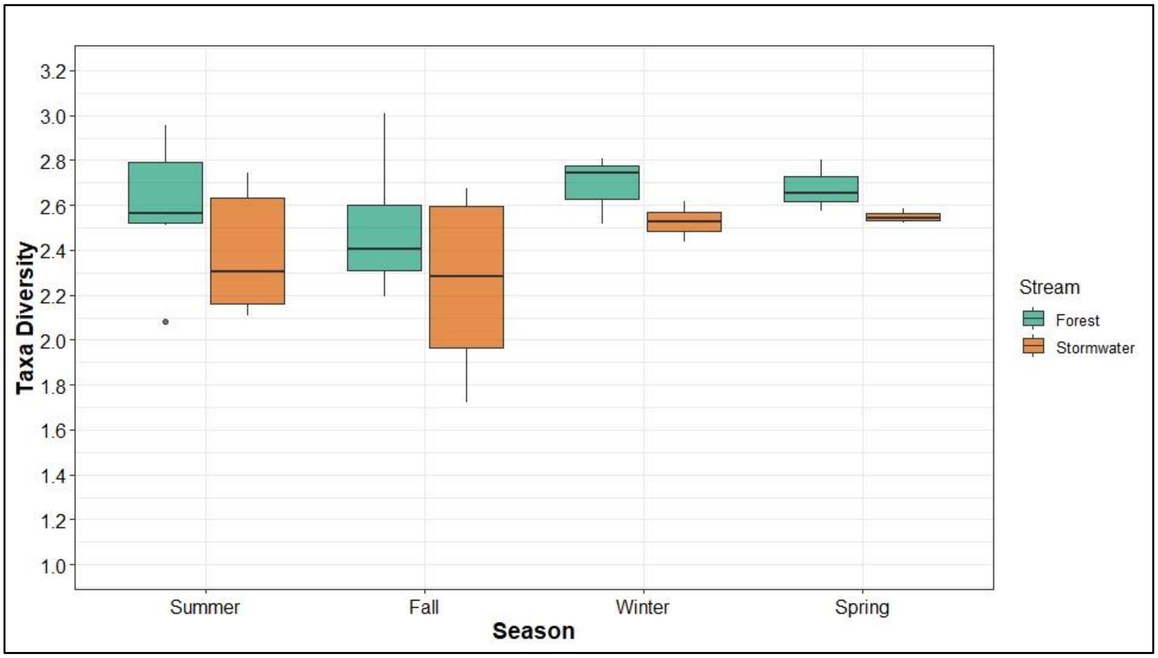

**Figure 4.16**. Box plot of taxa diversity across seasons in the forested (green) and stormwater impacted (brown) tributaries. Taxa diversity was significantly higher in the forested tributary than in the stormwater tributary (Two-Way ANOVA streams p<0.05) but did not vary across seasons (Two-Way ANOVA seasons p>0.05). Each season represents 3 replicates per tributary.

Trait richness was significantly different in the two tributaries (Two-Way ANOVA streams $p<0.05$; Figure 4.17). Trait richness was not significantly different among seasons (Two-Way ANOVA seasons $p>0.05$). There was no stream:season interaction ($p>0.05$). Trait diversity was not significantly different between the two tributaries (Two-Way ANOVA streams $p>0.05$; Figure 4.18). However, trait diversity was significantly different among seasons (Two-Way ANOVA seasons $p<0.05$). There was no stream:season interaction ($p>0.05$).

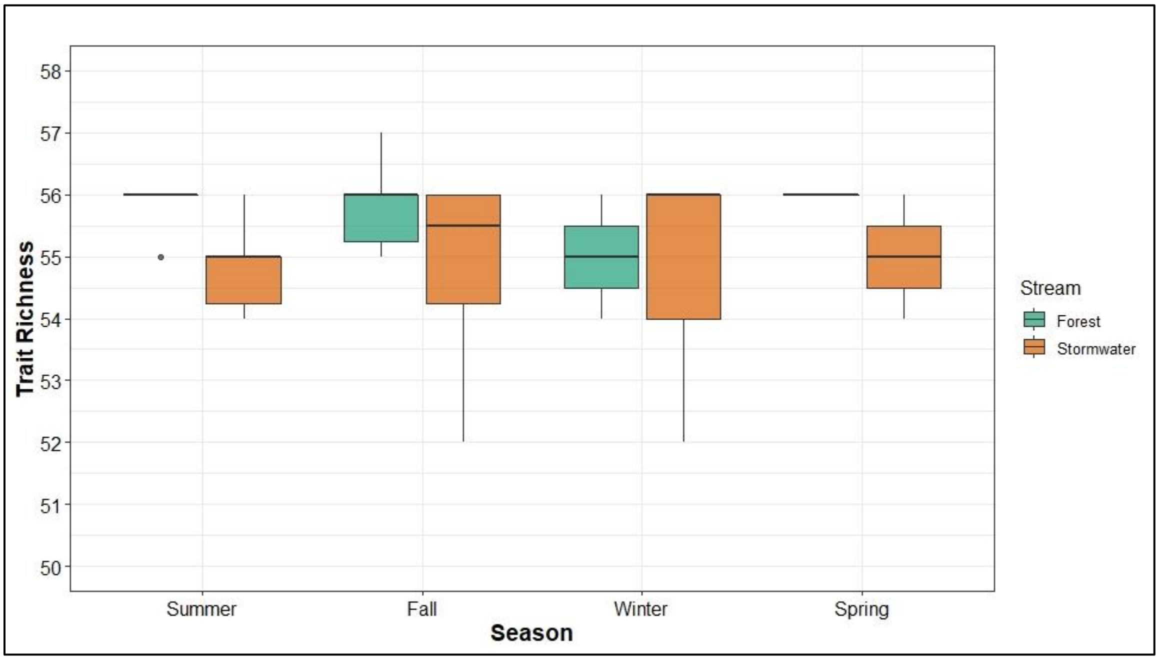

**Figure 4.17.** Box plot of trait richness across seasons in the forested (green) and stormwater impacted (brown) tributaries. Trait richness was significantly different between tributaries (Two-Way ANOVA streams $p<0.05$) but was not significantly different among seasons (Two-Way ANOVA seasons $p<0.05$). Each season represents 3 replicates per tributary.

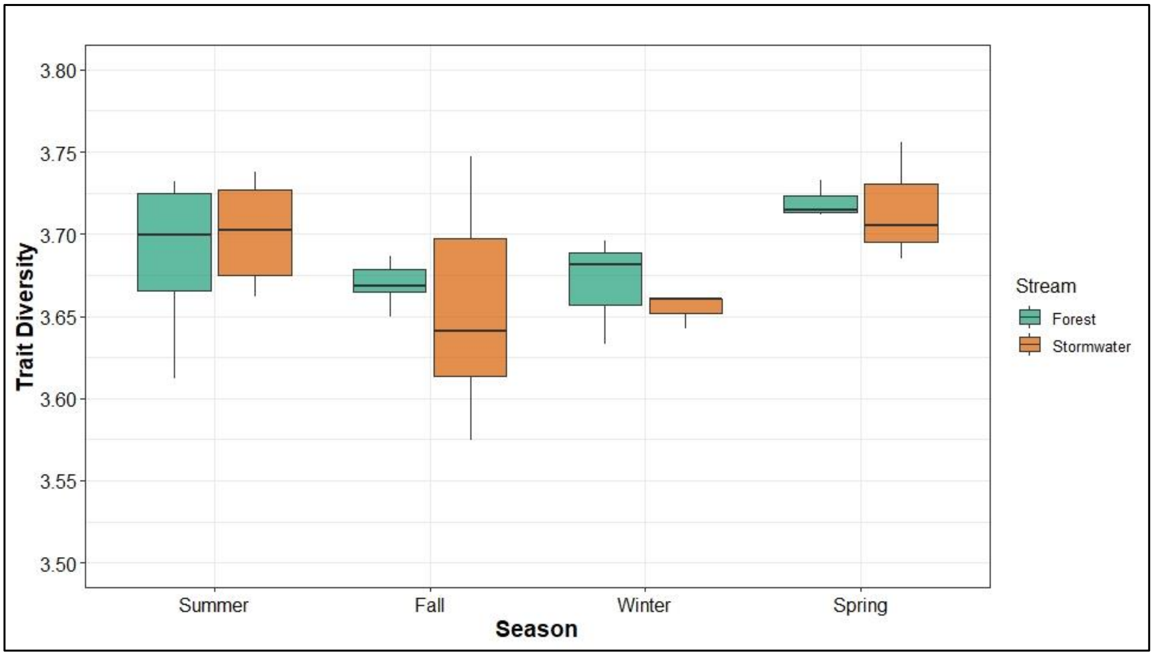

**Figure 4.18.** Box plot of trait diversity across seasons in the forested (green) and stormwater impacted (brown) tributaries. Trait diversity was not significantly different between the two tributaries (Two-Way ANOVA streams p>0.05) but was significantly different among seasons (Two-Way ANOVA seasons p<0.05). Each season represents 3 replicates per tributary.

Functional feeding group (FFG) richness were significantly different among the two tributaries (p<0.0001; Figure 4.19). Student T-test comparisons between each FFG showed that the taxa richness for all FFGs except Shredders was significantly higher in the TF tributary (Collector-Filterer p<0.05; Collector-Gatherer p<0.05; Herbivore-Scraper p<0.001; Predator p<0.0015; Shredder p>0.05).

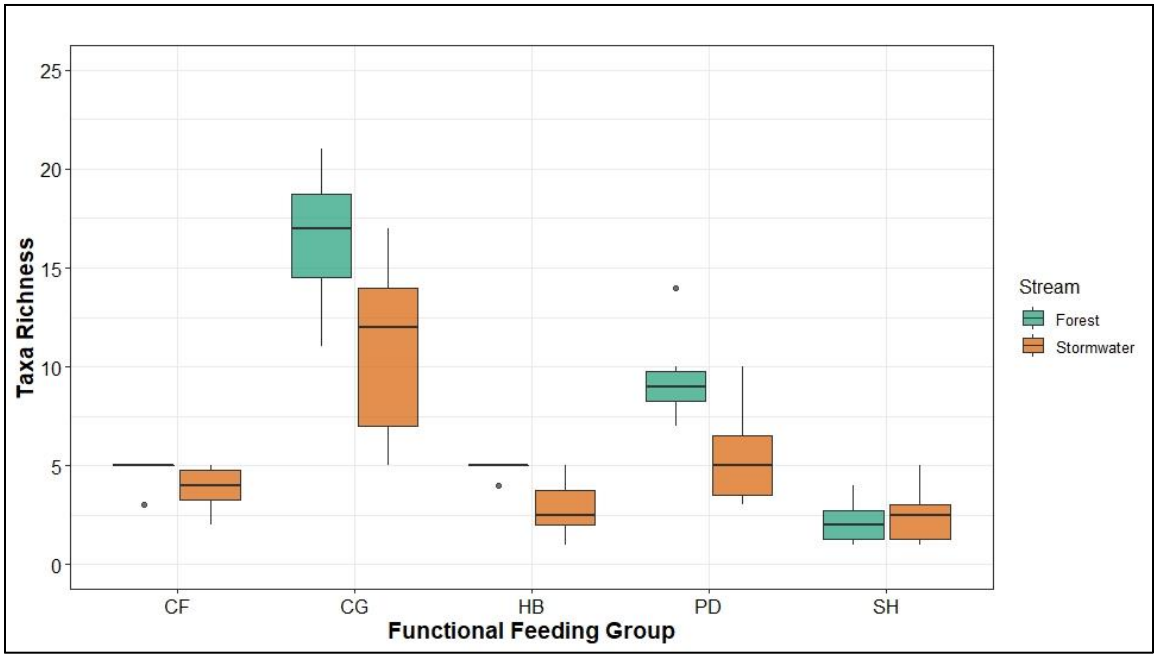

**Figure 4.19**. Box plot of taxa richness for each functional feeding group. Taxa richness for all Functional Feeding Groups (FFG) except shredders was significantly higher in the forested tributary. CF = collector filterers; CG = collector gatherers; HB = herbivore-scrapers; PD = predators; SH = shredders. Data for all seasons were combined for the forested (green) and stormwater (brown) tributaries.

### 4.6.4 CARBON SOURCE ANALYSES

The $\delta^{13}$C values of periphyton (rock scrub) and leaf pack food sources differ in the TF tributary during the summer (Figures 4.20). In the TF tributary, the periphyton $\delta^{13}$C was -27.6 ⁰/₀₀ while the leaf pack was -31.6 ⁰/₀₀. In the TI tributary, the $\delta^{13}$C values of periphyton and leaf pack were very similar (-29.7 ⁰/₀₀ for the periphyton and -29.9 ⁰/₀₀ for the leaf pack; Figure 4.21). The $\delta^{13}$Cvalues of the FPOM-silt and FPOM-water in both the TF and TI tributaries were very similar (FPOM-silt -29.4 and -29.0 ⁰/₀₀ respectively; FPOM-water -29.8 and -29.7 ⁰/₀₀ respectively).

In the TF tributary, the collector-gathers, herbivore-scrapers, predators, and shredders were closer to the periphyton $\delta^{13}$C while the collector-filterers were between the $\delta^{13}$C of the FPOM-water and -sediment and the periphyton. The $\delta^{13}$C value (-32.28 ⁰/₀₀) of one of the herbivore-scrapers, *Psephenus herricki* (DeKay 1844) (Coleoptera, Psephenidae), was closer to the leaf pack carbon source that the periphyton carbon source. The δ$^{13}$C values of the collector-gathers, herbivore-scrapers, predators, and shredders in the TI tributary were like those seen in the TF tributary.

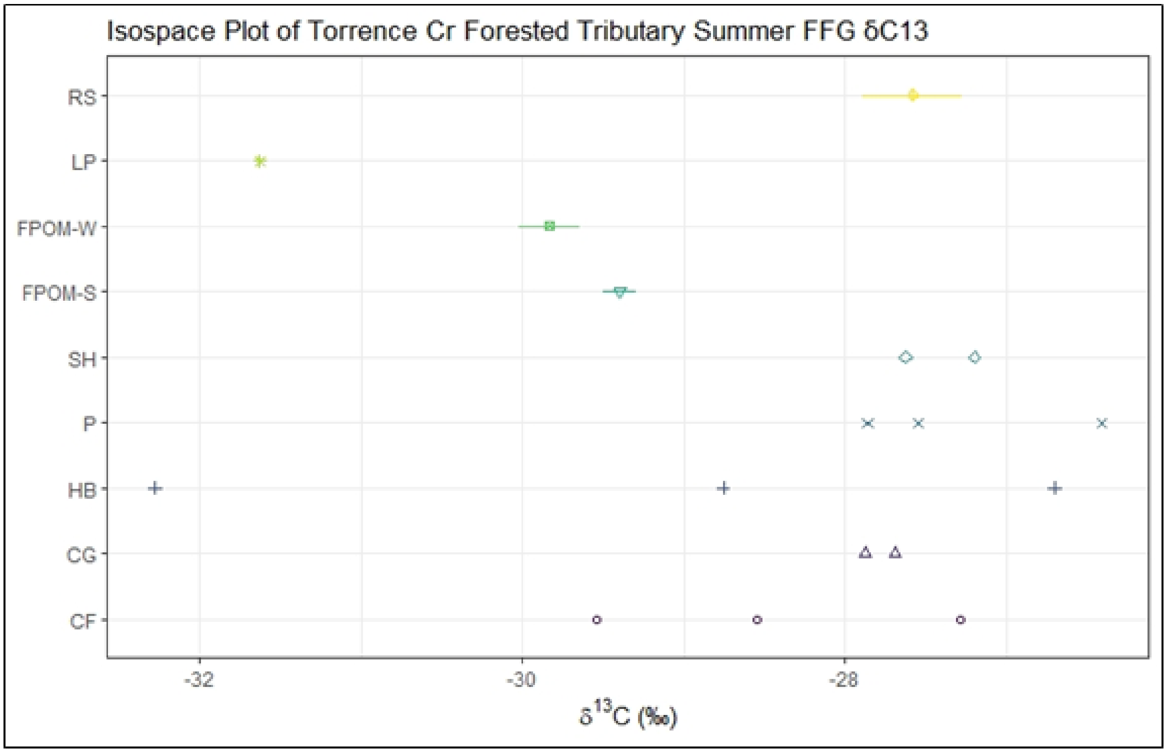

**Figure 4.20.** Torrence Creek Forested tributary summer stable δ$^{13}$C values for carbon sources rock scrub (periphyton), leaf pack, FPOM-water, and FPOM-silt, and for benthic macroinvertebrate functional feeding groups, shredders (SH), predators (P), herbivore-scrapers (HB), collector-gatherers (CG), and collector-filterers (CF).

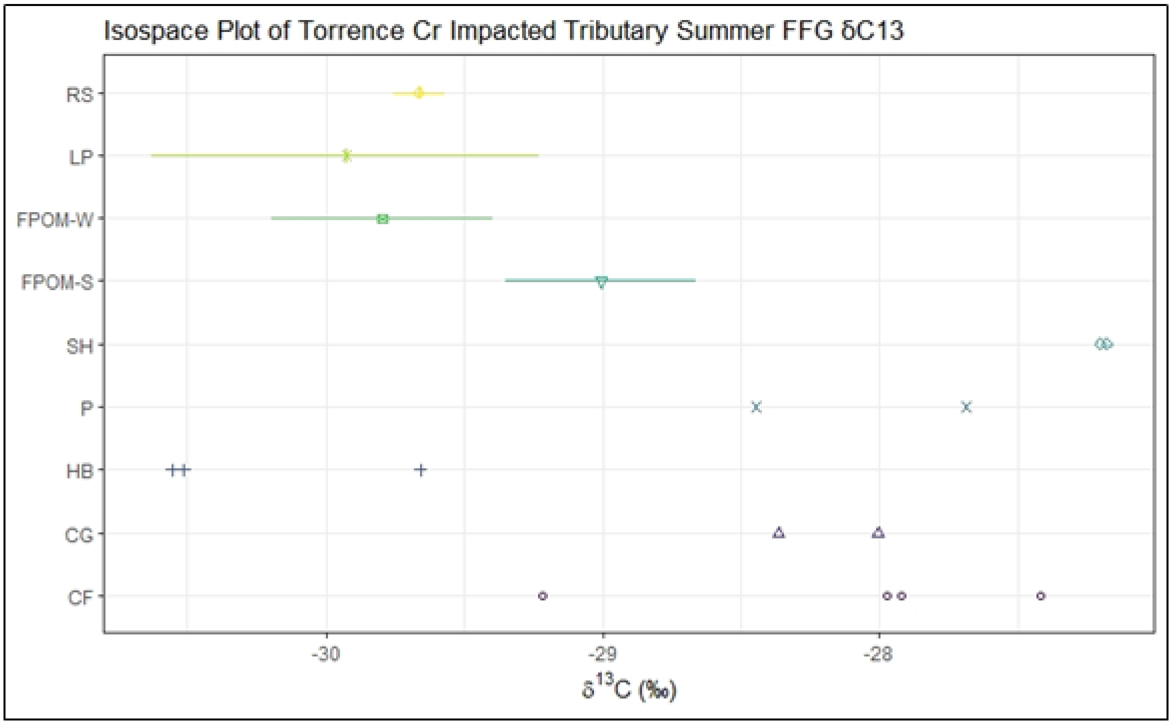

**Figure 4.21.** Torrence Creek Stormwater Impacted tributary summer δ$^{13}$C values for carbon sources rock scrub (periphyton), leaf pack, FPOM-water, and FPOM-silt, and for benthic macroinvertebrate functional feeding groups, shredders (SH), predators (P), herbivore-scrapers (HB), collector-gatherers (CG), and collector-filterers (CF).

During the winter, the δ$^{13}$C of periphyton was -27.9 ⁰/₀₀ while the leaf pack was -31.1 ⁰/₀₀ in the TF tributary (Figure 4.22). These δ$^{13}$C values were similar to the δ$^{13}$C values observed in the TI tributary (-28.3 ⁰/₀₀ for the periphyton and -31.7 ⁰/₀₀ for the leaf pack, Figure 4.23). The δ$^{13}$C of the FPOM-silt in both the TF and impacted tributaries were very similar (FPOM-silt -30.3 and -29.8 ⁰/₀₀ respectively).

In the TF tributary, the δ$^{13}$C of most of the herbivore-scrapers were closer to the leaf pack carbon δ$^{13}$C while the collector-gathers, predators, and shredders δ$^{13}$C was closer to the periphyton carbon source. The collector-filterers were located between the carbon δ$^{13}$C of the FPOM-silt and the periphyton. The δ$^{13}$C of the herbivore-scrapers, predators, and shredders in the TI tributary were closer to the periphyton δ$^{13}$C and similar to those seen in the TF tributary. Collector-filters were located midway between the FPOM-water and -silt and the periphyton

$\delta^{13}$C while the collector-gatherers were located between the leaf pack and periphyton $\delta^{13}$C. The biggest difference between the TF and TI tributaries in the winter was the $\delta^{13}$C of the herbivore-scrapers being closer to the $\delta^{13}$C of the leaf pack in the TF tributary and with the periphyton in the TI tributary.

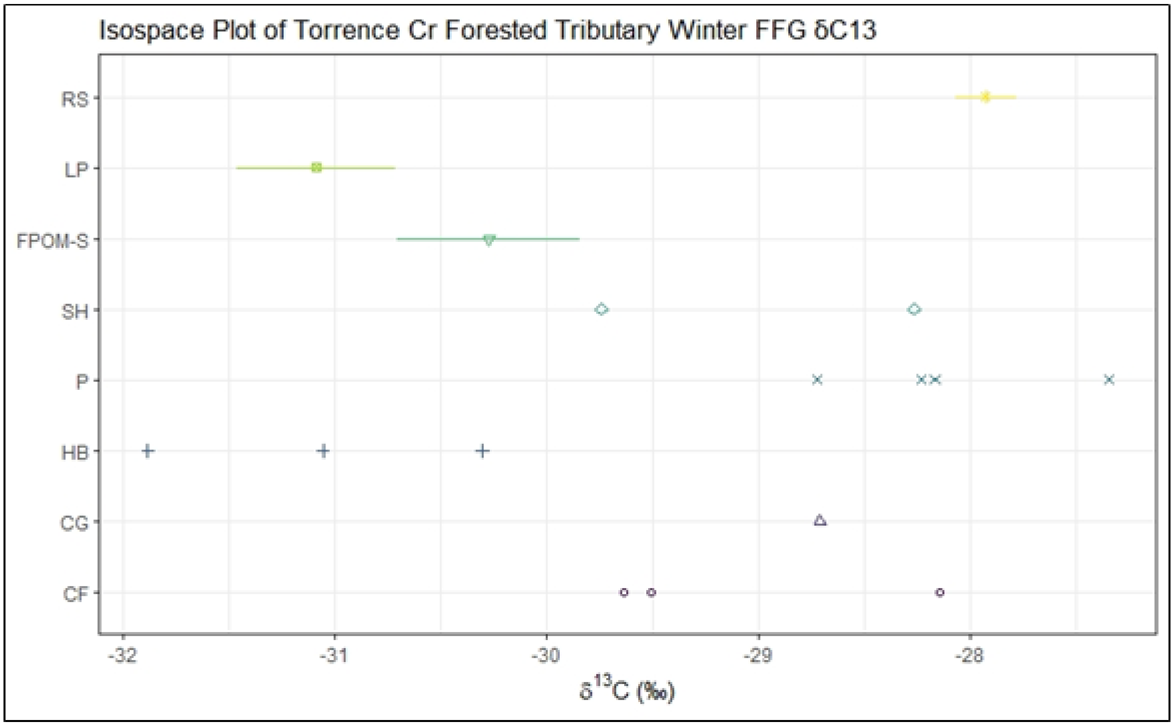

**Figure 4.22**. Torrence Creek Forested tributary winter $\delta^{13}$C values for carbon sources rock scrub (periphyton), leaf pack, and FPOM-silt and for benthic macroinvertebrate functional feeding groups, shredders (SH), predators (P), herbivore-scrapers (HB), collector-gatherers (CG), and collector-filterers (CF).

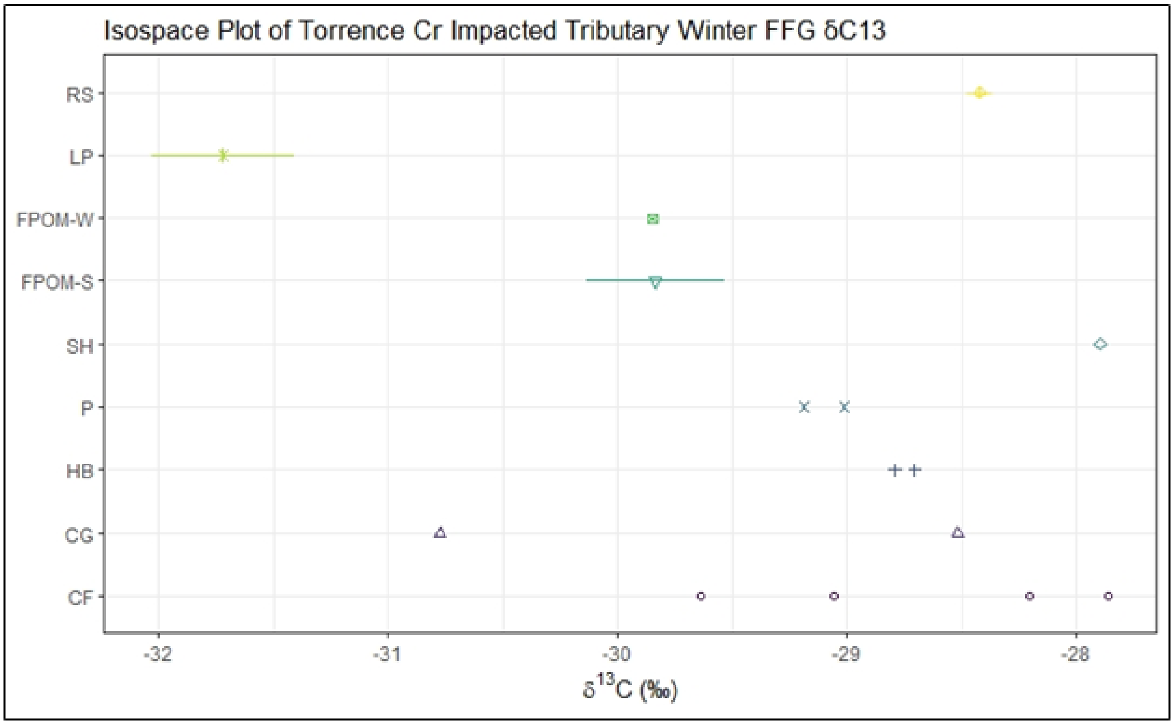

**Figure 4.23**. Torrence Creek Stormwater Impacted tributary winter $\delta^{13}$C values for carbon sources rock scrub (periphyton), leaf pack, FPOM-water, and FPOM-silt, and for benthic macroinvertebrate functional feeding groups, shredders (SH), predators (P), herbivore-scrapers (HB), collector-gatherers (CG), and collector-filterers (CF).

## 4.7 DISCUSSION

My first objective was to examine how taxa and trait richness and diversity respond to increases in stormwater runoff by evaluating two adjacent tributaries in the Piedmont, North Carolina that receive stormwater from different sources. My second objective was to determine how carbon sources available to benthic macroinvertebrate assemblages are impacted by urban stormwater runoff.

The two Torrence Creek tributaries are adjacent to one another and are physically similar in most ways except the stormwater input process to each tributary. They share the same tree canopy and have similar percent canopy coverage. Being in close proximity, the tributaries also receive the same amount of rainfall. However, stormwater runoff reaches the TF tributary through more natural processes while the TI tributary receives stormwater runoff from the adjacent neighborhood via stormwater infrastructure. Increases in the percentage of rainfall that reaches an urban stream can be expected when stormwater runoff is directly connected to the stream via stormwater infrastructure (Shuster et al. 2005; Walsh et al. 2005; Schueler et al. 2009; Cuffney et al. 2010; Zerega et al. 2021; Bower et al. 2022) resulting in increases in the frequency and magnitude of storm flows (Walsh et al. 2012). The increased flashiness in urban streams often results in channel bank erosion, streambed scouring and downstream sedimentation (Walsh et al. 2005; Coleman et al. 2011; Walsh et al. 2012; Zerega et al. 2021; Bower et al. 2022) negatively impacting the quality of habitats available to benthic macroinvertebrates (Bunn and Arthington 2002; Shuster et al. 2005; Walsh et al. 2005; Poff and Zimmerman 2010; Coleman et al. 2011; Utz and Hilderbrand 2011; Anim et al. 2018; Hayes et al 2018; Lundquist and Zhu 2019; Erba et al. 2020; Maloney et al. 2021).

Shear stress, the result of shear forces that are parallel to the stream bed, is an important factor impacting benthic macroinvertebrate assemblages in streams (Statzner et al. 1988; Mérigoux and Dolédec 2004; Hawley et al. 2016; Anim et al. 2018; Black 2019). Mérigoux and Dolédec (2004) found that increases in shear stress often led to reductions in the availability of suitable microhabitats for some taxa as well as a negative correlation between taxa richness and shear stress. Black (2019) found that the number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) sensitive taxa declined while the number of tolerant taxa increased as shear stress increased. The CMSWS FLOWSED-POWERSED model results show that shear stress was greater in the TI tributary indicating that greater amounts of sediment are transported during a storm event than in the TF tributary. The $d_{50}$ particle size in the TI tributary was less than half the size of the $d_{50}$ particle size in the TF tributary supporting the FLOWSED-POWERSED model's prediction that more sediment is transported by the TI than by the TF tributary during the same storm event. This indicates that the substrate is less stable in the TI tributary, thus reducing the stability of in-stream habitat available for the benthic macroinvertebrate. The lower EMHAP scores in the TI tributary support this assessment.

Benthic macroinvertebrate taxa richness and diversity and trait richness were greater in the TF than in the TI tributary. Streamflow conditions are important environmental filters, along with water depth, water chemistry, food sources, and substrate particle size, in forming the composition of benthic macroinvertebrate assemblages within stream habitats (Cummins and Lauff 1969; Erman and Erman 1984; Jowett and Richardson 1990; Wohl et al. 1995; Lamouroux et al. 2004; White et al. 2019; Forcellini et al. 2022; Salmaso et al. 2021). Recent studies found that altered stormwater flows in urban areas degrade physical habitat conditions in streams and negatively impact benthic macroinvertebrate assemblages (Castro and Reckendorf 1995; Kaller and Hartman 2004; Coleman et al. 2011; Jones et al. 2012; Carlisle et al. 2014; Kennen et al. 2014; Erba et al. 2020; Maloney et al. 2021; Bower et al. 2022). In this study, the habitat condition in the TI tributary, which had higher predicted shear stress than the TF tributary for the same bankfull storm event, was impaired and was significantly lower than in the TF tributary. EMHAP scores were also significantly correlated with taxa richness. Along with the correlation between declines in taxa richness and increases in shear stress, Merigoux and Dolédec (2004) found that the percentage of collector-filterers in the benthic macroinvertebrate assemblage increased with increases in shear stress while the percentages of collector-gathers declined. I found similar declines with collector-gathers in the TI tributary but did not see an increase in the collector-filterers. There may have been other stressors associated with the stormwater running off the adjacent neighborhood that may account for the decline in the collector filterers and other taxa in the TI tributary. Increases in stream conductivity, stormwater pollutants, fine sediments, low dissolved oxygen concentrations, and changes in stream temperature and water depth have been shown to have negative impacts on benthic macroinvertebrate assemblages (Castro and Reckendorf 1995; Coles et al. 2004; Kaller and Hartman 2004; Walsh et al. 2005; Gresens et al. 2007; Jones et al. 2012;  Zergega et al. 2021).

Recent studies have shown that substrate composition and habitat quality strongly influence benthic macroinvertebrate assemblage composition (Allan and Castillo 2007; Castro et al. 2017; Castro et al. 2018). In my study, habitat impairment in the TI tributary may explain the significantly higher benthic macroinvertebrate taxa richness found in the TF tributary. Taxa diversity and trait richness were also significantly higher in the TF tributary but were weakly correlated with EMHAP. Trait diversity was not significantly different between tributaries. This may be due to functional redundancy which occurs when more than one taxon shares one or more functional traits (Lamothe et al. 2018). The taxa in the two tributaries were similar in their trait composition allowing most traits to remain in the invertebrate assemblage in the TI tributary even with the loss of several taxa. While many traits are negatively impacted by habitat degradation, some traits are enhanced. For example, traits which are adapted to coarse particulate organic matter (CPOM), such as collector-filterers, clingers, sprawlers, and high sensitivity to low oxygen conditions, are negatively impacted by high levels of fine sediment that generally accompanies stream bank erosion occurring during high flow condition (Rabeni et al. 2005; Jones et al. 2012; Ntloko et al. 2021;) while traits such as burrowers, climbers, and high tolerance to low oxygen conditions are tolerant to fine particulate organic matter (Rabeni et al. 2005; Lundquist and Zhu 2018; Ntloko et al. 2021). These results support my hypothesis that taxa richness and diversity and trait richness will be lower in the tributary that receives increased amounts of stormwater runoff while not supporting the same hypothesis regarding trait diversity.

The $\delta^{13}C$ of periphyton and leaf pack food sources differ in the TF and TI tributaries during the summer but are similar during the winter. The summer $\delta^{13}C$ values of the periphyton and leaf pack food sources were distinctly different in the TF tributary while they were very similar and closer to allochthonous source $\delta^{13}C$ values in the TI tributary. Imberger et al. (2014) found a similar dominance in $\delta^{13}C$ values from allochthonous carbon in urban streams. The $\delta^{13}C$ values of the collector-gatherers, herbivore-scrapers, predators, and shedders were closer to the $\delta^{13}C$ values of the periphyton in both tributaries. In the winter, the $\delta^{13}C$ values of the periphyton and leaf pack food sources were distinctly different in both tributaries. Not all the FFGs followed the expected food source association. The $\delta^{13}C$ values of the herbivore-scrapers were closer to the $\delta^{13}C$ of the leaf pack in the TF tributary but were closer to the periphyton in the TI tributary. This variability may be due to omnivorous feeding behavior of some herbivore-scrapers like the *Psephenus herricki* (Coleoptera, Psephenidae) that include small prey organisms in their diet (Murvosh 1971) while others ingested detrital and other organisms along with algae (McNeely et al. 2006; Allen et al. 2009; Tamura and Kagaya 2019). It is possible that detritus from allochthonous sources were intermixed with the periphyton on the rocks in the TI tributary (McNeel et al. 2006). During the summer and winter in both tributaries, the $\delta^{13}C$ values of collector-filterers were closer to the periphyton values, indicating that collector filterers were dependent on algal sources upstream in both streams. Finlay et al. (2002) found similar dependence on upstream algal food sources by collector-filterers. Predators in both streams and in both seasons had $\delta^{13}C$ values closer to the periphyton values indicating that most of their prey were dependent on algal food sources. This relationship between predators and prey that relied on algal food sources was also found by Finlay et al. (2002). These results partially support my hypothesis that the carbon sources would be altered by the increased stormwater input.

Functional feeding groups of benthic macroinvertebrates provide insight into habitat conditions and trophic dynamics. Habitat heterogeneity and stability results in benthic macroinvertebrate assemblages with higher feeding strategy specializations and less generalist or omnivorous feeding habits (Gebrehiwot et al. 2017). Omnivorous feeding behavior increases in urban streams where habitat diversity and stability are impaired by increased stormwater flashiness and fine sediment deposition (Rabeni et al. 2005; Allen et al. 2009: Gebrehiwot et al. 2017; Ntloko et al. 2021). Rabeni et al. (2005) found that the taxa richness and densities of all taxa in all FFGs except collector-gatherers. In my study, all the FFG trait richness except for the collector-gatherer were similar between tributaries. The collector-gatherer richness was significantly greater in the TF tributary.  Collector-gatherers have been found to be the most abundant benthic macroinvertebrate feeding groups in impaired urban streams like TI (Finlay et al. 2002; Rabeni et al. 2005; Gebrehiwot et al. 2017; Lundquist and Zhu 2018; Mangadze et al. 2019). These results did not support my prediction that all trophic groups would have fewer taxa in each trophic group in the TI tributary.

### 4.6.1 APPLICATION TO STREAM RESTORATIONS

Stream restoration has become a multimillion-dollar industry. Stream restoration techniques, such as natural channel designs (Doll et al. 2003), are being used by watershed managers to repair degrading urban stream channels and stream biota to a more natural state. Unfortunately, the biological uplift expected through urban stream restoration has not occurred in most cases which may be due to the general focus on stream channel stabilization and less on instream habitat restoration (Sudduth and Meyer 2006; Louhi et al. 2011; Violin et al. 2011;

Ernst et al. 2012; Stranko et al. 2012) or restoration of the stream’s natural hydrologic regime (Konrad and Booth 2005; Hayes et al. 2018; Zerega et al. 2021;  Bower et al. 2022). Any alteration in the natural hydrological regime will ultimately have a negative effect on a stream’s benthic macroinvertebrate assemblage (Mérigoux and Dolédec 2004; Buffagni et al. 2016; Erba et al. 2020; Zerega et al. 2021). This study’s results show that unmitigated stormwater will have significant negative effects on the benthic macroinvertebrate assemblages. Addressing the sources of  hydrologic alterations as part of a stream restoration plan may lead to more successful restoration of an urban stream aquatic ecosystem.

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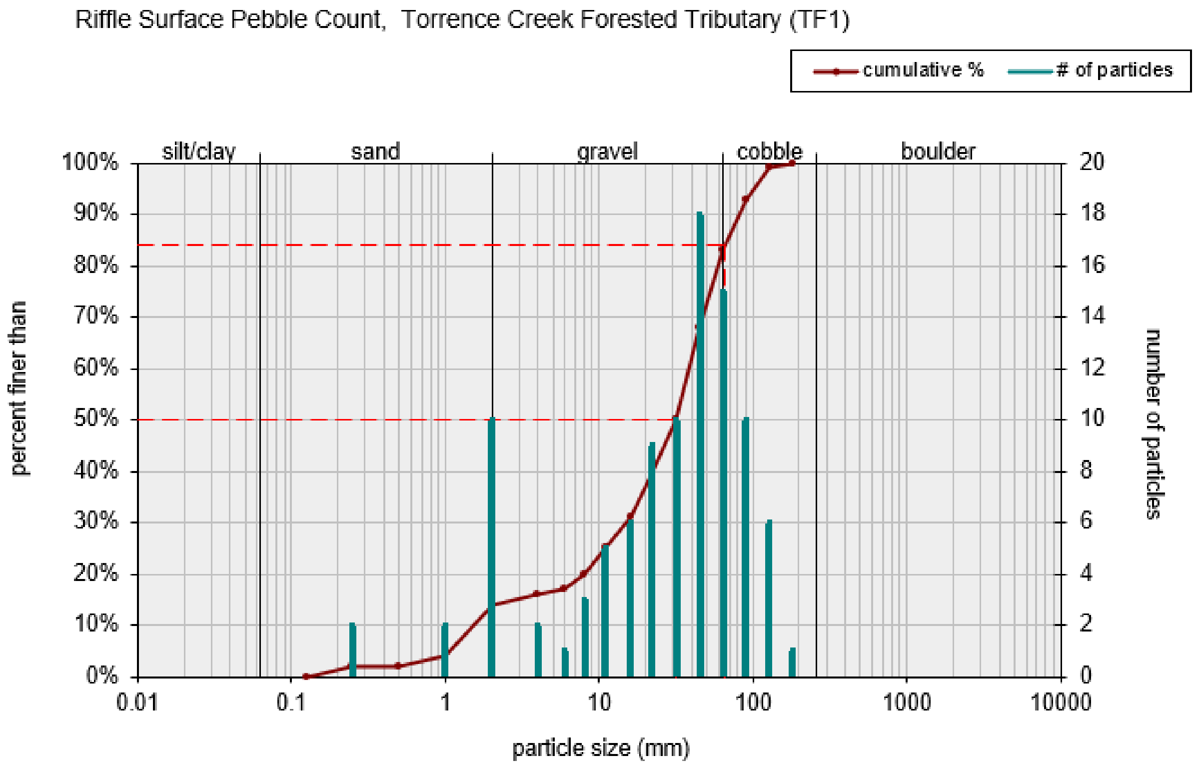
## 4.8 APPENDIX

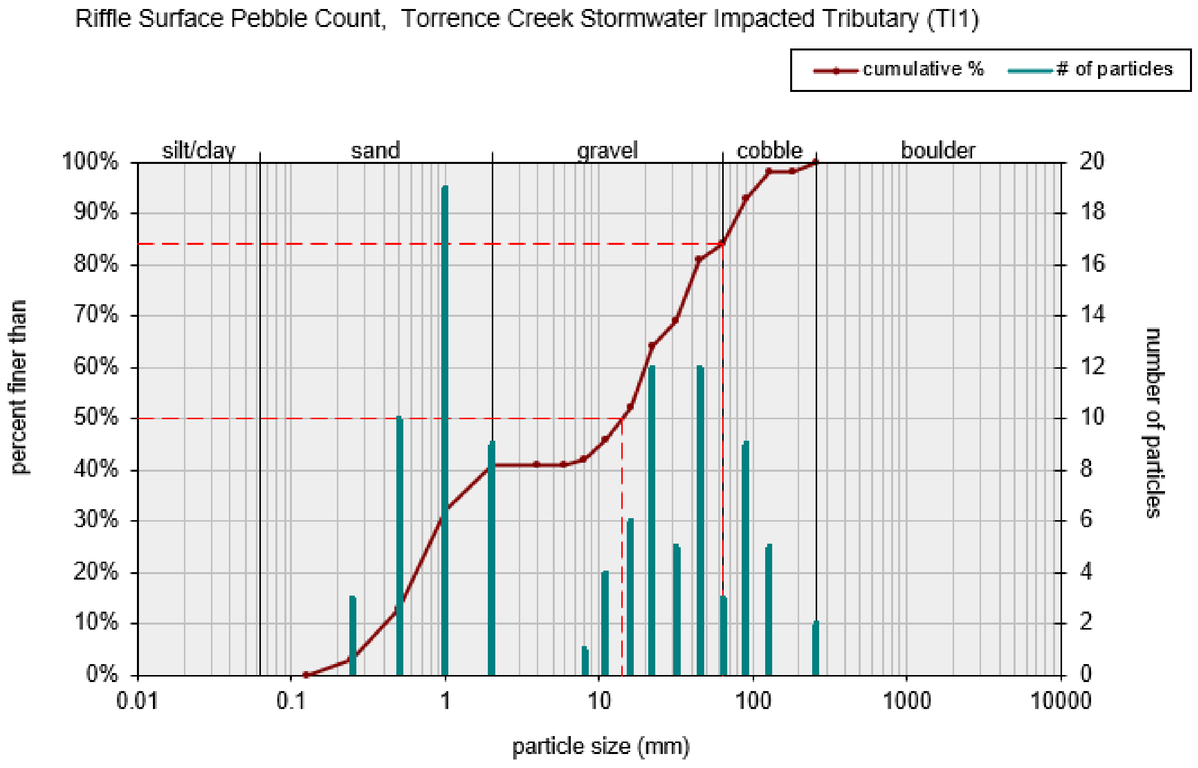


| Size (mm) | |
|---|---|
| D16 | 4 |
| D35 | 18 |
| D50 | 32 |
| D65 | 43 |
| D84 | 66 |
| D95 | 100 |

| Size Distribution | |
|---|---|
| mean | 16.2 |
| dispersion | 5.0 |
| skewness | -0.27 |

| Type | |
|---|---|
| silt/clay | 0% |
| sand | 14% |
| gravel | 69% |
| cobble | 17% |
| boulder | 0% |

**Figure A16.** 100-pebble Count Data for the Forested Tributary Conducted from the Riffle at Site TF1 on February 17, 2018.



| Size (mm) | |
|---|---|
| D16 | 0.56 |
| D35 | 1.3 |
| D50 | 14 |
| D65 | 24 |
| D84 | 64 |
| D95 | 100 |

| Size Distribution | |
|---|---|
| mean | 6.0 |
| dispersion | 14.8 |
| skewness | -0.26 |

| Type | |
|---|---|
| silt/clay | 0% |
| sand | 41% |
| gravel | 43% |
| cobble | 16% |
| boulder | 0% |

**Figure A17.** 100-pebble Count Data for the Stormwater Impacted Tributary Conducted from the Riffle at Site TI1 on February 17, 2018.

**Table A10.** Water Chemistry in the Forested and Stormwater Impacted Torrence Creek Tributaries taken from December 2016 through November 2017.

| Site | Parameter | units | 12/12/16 | 2/20/17 | 6/14/17 | 7/18/17 | 11/1/17 | Minimum |
|---|---|---|---|---|---|---|---|---|
| TC-F | NH3-N | mg/L | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 |
| TC-F | BOD | mg/L | <2 | <2 | <2 | <2 | <2 | 2 |
| TC-F | COD | mg/L | 15 | <10 | <10 | <10 | <10 | 10 |
| TC-F | ECOLI | MPN/100 ml | 350 | 910 | 723 | <100 | 108 | 10 |
| TC-F | FECAL | CFU/100 ml | 230 | 1500 | 510 | <100 | 144 | 10 |
| TC-F | NOX-N | mg/L | 1.1 | 0.8 | 0.48 | 0.88 | 0.81 | 0.05 |
| TC-F | SSC | mg/L | <3.9 | 4.3 | 4 | 4.3 | <3.9 | 3.9 |
| TC-F | TKN | mg/L | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | 0.25 |
| TC-F | TP | mg/L | 0.09 | 0.04 | 0.043 | 0.049 | 0.032 | 0.01 |
| TC-F | TSS2 | mg/L | <5 | <5 | <10 | <5 | <2.8 | 5 |
| TC-F | TURB | NTU | 3.9 | 3.1 | 6.3 | 3.1 | 2.3 | 0.5 |
| TC-I | NH3-N | mg/L | <0.1 | <0.1 | <0.1 | <0.1 | <0.1 | 0.1 |
| TC-I | BOD | mg/L | <2 | <2 | <2 | <2 | <2 | 2 |
| TC-I | COD | mg/L | 11 | <10 | <10 | <10 | <10 | 10 |
| TC-I | ECOLI | MPN/100 ml | 180 | 110 | 583 | 310 | 60 | 10 |
| TC-I | FECAL | CFU/100 ml | 210 | 220 | 883 | <100 | 213 | 10 |
| TC-I | NOX-N | mg/L | 0.35 | 0.25 | 0.41 | 0.66 | 0.3 | 0.05 |
| TC-I | SSC | mg/L | <3.8 | <3.9 | <4 | <3.8 | <4 | 3.9 |
| TC-I | TKN | mg/L | <0.25 | <0.25 | <0.25 | <0.25 | <0.25 | 0.25 |
| TC-I | TP | mg/L | 0.054 | 0.04 | 0.051 | 0.058 | 0.037 | 0.01 |
| TC-I | TSS2 | mg/L | <5 | <5 | <5 | <5 | <2.8 | 5 |
| TC-I | TURB | NTU | 2.4 | 2.1 | 4.5 | 2.7 | 3.4 | 0.5 |

**Table A11.** Chemistry Tests, Bottles and Preservatives for Water Sample Analyses

| Parameter | EPA Test | Bottle Size (ml) | Preservative |
|---|---|---|---|
| Fecal Coliform Bacteria | SM9222D-11 | 100 | $Na_2S_2O_3$ |
| E-Coli Bacteria | SM 9223B | 100 | $Na_2S_2O_3$ |
| Ammonia Nitrogen | SM4500NH3-H-11 | 500 | $H_2SO_4$ |
| Nutrients:  Nitrate/Nitrite | EPA 353.2-93 | 500 | $H_2SO_4$ |
| Nutrients:  Total Kjeldahl Nitrogen | EPA 351.2-93 | 500 | H2SO4 |
| Nutrients:  Total Phosphorus | SM4500P-H-11 | 500 | H2SO4 |
| BOD | EPA Method 405.1 | 1000 | None |
| COD | EPA Method 410.4 | 250 | $H_2SO_4$ |
| DOC |  | 250 | None |
| TOC |  | 250 | None |
| Suspended Solids (TSS) | SM2540D-11 | 1000 | None |
| Suspended Sediments (SSC) | ASTM D3977-11 | 250 | None |
| Turbidity | SM2130B-11 | 1000 | None |

**Table A12.** List of taxa collected from the Torrence Creek tributaries during the study period

| BugClass | BugOrder | Family | Genus Species | Tolerance |
|---|---|---|---|---|
| Insecta | Coleoptera | Dryopidae | *Helichus* spp. | 4.1 |
| Insecta | Coleoptera | Dytiscidae | *Hydroporus* spp. | 7 |
| Insecta | Coleoptera | Dytiscidae | *Neoporus* spp. | 5 |
| Insecta | Coleoptera | Elmidae | *Ancyronyx variegatus* | 6.8 |
| Insecta | Coleoptera | Elmidae | *Dubiraphia vittata* | 5 |
| Insecta | Coleoptera | Elmidae | *Stenelmis* spp. | 5.6 |
| Insecta | Coleoptera | Psephenidae | *Ectopria nervosa* | 4.3 |
| Insecta | Coleoptera | Psephenidae | *Psephenus herricki* | 2.3 |
| Insecta | Diptera | Ceratopogonidae | *Atrichopogon* spp. | 6.1 |
| Insecta | Diptera | Ceratopogonidae | *Palpomyia complex* | 5.7 |
| Insecta | Diptera | Chironomidae | *Ablabesmyia mallochi* | 7.4 |
| Insecta | Diptera | Chironomidae | *Brillia* spp. | 5.7 |
| Insecta | Diptera | Chironomidae | *Chironomus* spp. | 9.3 |
| Insecta | Diptera | Chironomidae | *Cladotanytarsus* spp. | 4 |
| Insecta | Diptera | Chironomidae | *Corynoneura* spp. | 5.7 |
| Insecta | Diptera | Chironomidae | *Cricotopus annulator (complex)* | 8.4 |

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| Insecta | Diptera | Chironomidae | *Cricotopus bicinctus* | 8.7 |
| Insecta | Diptera | Chironomidae | *Cryptochironomus* spp. | 6.4 |
| Insecta | Diptera | Chironomidae | *Diamesa* spp. | 6.6 |
| Insecta | Diptera | Chironomidae | *Dicrotendipes neomodestus* | 7.9 |
| Insecta | Diptera | Chironomidae | *Diplocladius cultriger* | 8 |
| Insecta | Diptera | Chironomidae | *Goeldichironomus* spp. | 10 |
| Insecta | Diptera | Chironomidae | *Labrundinia pilosella* | 6.2 |
| Insecta | Diptera | Chironomidae | *Microtendipes* spp. | 4.6 |
| Insecta | Diptera | Chironomidae | *Nanocladius* spp. | 7.4 |
| Insecta | Diptera | Chironomidae | *Natarsia* spp. | 9.6 |
| Insecta | Diptera | Chironomidae | *Nilotanypus* spp. | 4.1 |
| Insecta | Diptera | Chironomidae | *Orthocladius* spp. | 4.4 |
| Insecta | Diptera | Chironomidae | *Paracladopelma* spp. | 6.3 |
| Insecta | Diptera | Chironomidae | *Parakiefferiella* spp. | 4.8 |
| Insecta | Diptera | Chironomidae | *Paramerina* spp. | 4.1 |
| Insecta | Diptera | Chironomidae | *Parametriocnemus* spp. | 3.9 |
| Insecta | Diptera | Chironomidae | *Paratanytarsus* spp. | 8 |
| Insecta | Diptera | Chironomidae | *Paratendipes* spp. | 5.6 |
| Insecta | Diptera | Chironomidae | *Phaenopsectra* spp. | 6.5 |
| Insecta | Diptera | Chironomidae | *Polypedilum fallax* | 6.5 |
| Insecta | Diptera | Chironomidae | *Polypedilum flavum* | 5.7 |
| Insecta | Diptera | Chironomidae | *Polypedilum halterale* | 7.4 |
| Insecta | Diptera | Chironomidae | *Polypedilum illinoense* | 8.7 |
| Insecta | Diptera | Chironomidae | *Polypedilum scalaenum* | 8.5 |
| Insecta | Diptera | Chironomidae | *Potthastia longimanus* | 8.4 |
| Insecta | Diptera | Chironomidae | *Procladius* spp. | 8.8 |
| Insecta | Diptera | Chironomidae | *Rheocricotopus robacki* | 7.9 |
| Insecta | Diptera | Chironomidae | *Rheotanytarsus* spp. | 6.5 |
| Insecta | Diptera | Chironomidae | *Stenochironomus* spp. | 6.3 |
| Insecta | Diptera | Chironomidae | *Stictochironomus* spp. | 5.4 |
| Insecta | Diptera | Chironomidae | *Tanytarsus* spp. | 6.6 |

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| Insecta | Diptera | Chironomidae | *Thienemanniella* spp. | 6.4 |
| Insecta | Diptera | Chironomidae | *Thienemannimyia group* | 8.4 |
| Insecta | Diptera | Chironomidae | *Tribelos jucundum* | 5.7 |
| Insecta | Diptera | Chironomidae | *Tvetenia bavarica (group)* | 3.6 |
| Insecta | Diptera | Chironomidae | *Xylotopus par* | 6.1 |
| Insecta | Diptera | Chironomidae | *Zavrelimyia* spp. | 8.6 |
| Insecta | Diptera | Empididae | *Hemerodromia* spp. | 7.6 |
| Insecta | Diptera | Psychodidae | *Psychoda* spp. | 9.6 |
| Insecta | Diptera | Simuliidae | *Simulium* spp. | 4.9 |
| Insecta | Diptera | Tipulidae | *Antocha* spp. | 4.4 |
| Insecta | Diptera | Tipulidae | *Dicranota* spp. | 0 |
| Insecta | Diptera | Tipulidae | *Hexatoma* spp. | 3.5 |
| Insecta | Diptera | Tipulidae | *Limonia* spp. | 9.3 |
| Insecta | Diptera | Tipulidae | *Pilaria* spp. | 7 |
| Insecta | Diptera | Tipulidae | *Tipula* spp. | 7.5 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella nadineae* | 1.9 |
| Insecta | Ephemeroptera | Baetidae | *Acentrella parvula* | 4.8 |
| Insecta | Ephemeroptera | Baetidae | *Baetis intercalaris* | 5 |
| Insecta | Ephemeroptera | Baetidae | *Baetis pluto* | 3.4 |
| Insecta | Ephemeroptera | Baetidae | *Centroptilum* spp. | 3.8 |
| Insecta | Ephemeroptera | Baetidae | *Labiobaetis propinquus* | 5.8 |
| Insecta | Ephemeroptera | Caenidae | *Caenis* spp. | 6.8 |
| Insecta | Ephemeroptera | Ephemerellidae | *Eurylophella bicolor* | 4.8 |
| Insecta | Ephemeroptera | Heptageniidae | *Stenonema modestum* | 5.7 |
| Insecta | Ephemeroptera | Heptageniidae | *Stenacron interpunctatum* | 6.4 |
| Insecta | Ephemeroptera | Leptophlebiidae | *Paraleptophlebia* spp. | 1.2 |
| Insecta | Heteroptera | Corixidae | *Sigara* spp. | 8.7 |
| Insecta | Megaloptera | Corydalidae | *Nigronia fasciatus* | 6.1 |
| Insecta | Megaloptera | Sialidae | *Sialis* spp. | 7 |
| Insecta | Odonata | Aeshnidae | *Aeschna* spp. | 5 |
| Insecta | Odonata | Aeshnidae | *Boyeria vinosa* | 5.8 |

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| Insecta | Odonata | Calopterygidae | *Calopteryx* spp. | 7.5 |
| Insecta | Odonata | Coenagrionidae | *Argia* spp. | 8.3 |
| Insecta | Odonata | Cordulegastridae | *Cordulegaster* spp. | 5.7 |
| Insecta | Odonata | Gomphidae | *Gomphus* spp. | 5.9 |
| Insecta | Odonata | Gomphidae | *Ophiogomphus* spp. | 5.9 |
| Insecta | Odonata | Gomphidae | *Progomphus obscurus* | 8.2 |
| Insecta | Odonata | Gomphidae | *Stylogomphus albistylus* | 5 |
| Insecta | Plecoptera | Capniidae | *Allocapnia* spp. | 3.3 |
| Insecta | Plecoptera | Leuctridae | *Leuctra* spp. | 1.5 |
| Insecta | Plecoptera | Perlidae | *Eccoptura xanthenes* | 4.7 |
| Insecta | Plecoptera | Perlidae | *Perlesta* spp. | 2.9 |
| Insecta | Trichoptera | Hydropsychidae | *Cheumatopsyche* spp. | 6.6 |
| Insecta | Trichoptera | Hydropsychidae | *Diplectrona modesta* | 2.3 |
| Insecta | Trichoptera | Hydropsychidae | *Hydropsyche betteni* | 7.9 |
| Insecta | Trichoptera | Lepidostomatidae | *Lepidostoma* spp. | 1 |
| Insecta | Trichoptera | Leptoceridae | *Triaenodes ignitus* | 4.8 |
| Insecta | Trichoptera | Limnephilidae | *Pycnopsyche* spp. | 2.5 |
| Insecta | Trichoptera | Philopotamidae | *Chimarra* spp. | 3.3 |
| Insecta | Trichoptera | Psychomyiidae | *Lype diversa* | 3.9 |
| Insecta | Trichoptera | Rhycophilidae | *Rhyacophila nigrita* | 0 |
| Insecta | Trichoptera | Rhycophilidae | *Rhyacophila torva* | 1.5 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax atlanta* | 1.6 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax consimilis* | 0.3 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax oligius* | 2.4 |
| Insecta | Trichoptera | Thremmatidae | *Neophylax ornatus* | 1.3 |

**Table A13**. ANOVA and Tukey Multiple Comparisons of Means Results When Comparing Taxa and Trait Richness and Diversity in the Forested and Stormwater Impacted Torrence Creek Tributaries. The Kruskal-Wallis Rank Sum Test and Dunn Test for Multiple Comparison of Means were used with the Trait Richness data set which did not have a normal distribution. The results show no significant difference between sites within the same stream leading to the decision to use the 3 sites in each tributary as pseudo-replicates.

| **Test** | **ANOVA Model** | **Tukey Multiple Comparison of Means** |
|---|---|---|
| **Taxa Richness** | | replace p = 0.05 with p value |
| Forest:Impacted Trib Sites | **p = 1.96e-05** | |
| F1:F2 | | p =0.999 |
| F1:F3 | | p = 0.978 |
| F2:F3 | | p = 0.998 |
| I1:I2 | | p =0.906 |
| I1:I3 | | p = 0.613 |
| I2:I3 | | p = 0.993 |
| **Trait Richness** | **Kruskal-Wallis Rank Sum Test** | **Dunn Test for Multiple Comparison of Means** |
| Forest:Impacted Trib Sites | **p = 0.00189** | |
| F1:F2 | | p = 0.610 |
| F1:F3 | | p = 0.447 |
| F2:F3 | | p = 0.165 |
| I1:I2 | | p = 0.108 |
| I1:I3 | | **p<0.0123** |
| I2:I3 | | p = 0.490 |
| **Taxa Diversity** | | |

|  |  |  |
|---|---|---|
| Forest:Impacted Trib Sites | **p = 0.0169** |  |
| F1:F2 |  | p =1.000 |
| F1:F3 |  | p = 0.999 |
| F2:F3 |  | p = 0.999 |
| I1:I2 |  | p =1.000 |
| I1:I3 |  | p = 0.0741 |
| I2:I3 |  | p = 0.0728 |
| **Trait Diversity** |  |  |
| Forest:Impacted Trib Sites | p = 0.0589 |  |
| F1:F2 |  | p = 0.999 |
| F1:F3 |  | p = 0.996 |
| F2:F3 |  | p = 0.952 |
| I1:I2 |  | p = 0.999 |
| I1:I3 |  | p = 0.105 |
| I2:I3 |  | **p = 0.047** |

# CHAPTER 5: CONCLUSION

## 5.1 TYING THE THREE STUDIES TOGETHER

The Urban Stream Syndrome (Walsh et al. 2005) lists several predictable negative impacts to stream ecosystems caused by the urbanization process, one of which is the Alteration of the Natural Hydrologic Regime. My results support the conclusions that one of the more significant negative impacts of urbanization on stream ecosystems is the alteration of the natural hydrologic regime (Russell et al., 2020; Anim and Banahene 2021; Zerega et al., 2021; Hawley 2022). My first study, "*Impact of land use changes over a period of 26 years on benthic macroinvertebrate diversity and function in Piedmont streams in North Carolina"*, demonstrates that the alterations of the natural hydrologic regime, as measured by several parameters presented by Richter et al. (1996), Poff and Zimmerman (2010) and Baker et al. (2004) is directly related to declines in EPT taxa and trait richness and diversity. The R-B Flashiness Index has been shown to be significantly correlated with the negative impacts to the benthic macroinvertebrate assemblages in streams degraded by urbanization. The increased stormwater runoff volume and intensity is directly related to increases in shear stress which stimulates stream bank erosion and streambed incision which negatively impacts stream habitat stability and reduces stream habitat diversity. **T**hreshold **I**ndicator **T**axa **AN**alysis (TITAN; Baker et al. (2004)) analysis identified distinct percent IC thresholds and stream habitat conditions (MHAP scores) for sensitive EPT taxa between 5 to 10% IC and MHAP scores of 120 to 140, while tolerant EPT taxa increased in abundance beginning around 30% IC. TITAN analysis identified similar thresholds for traits associated with sensitive EPT taxa between 5 to 10% IC and 120 MHAP scores while traits associated with tolerant EPT taxa increased in abundance starting at 30% IC and declined again at below MHAP scores of 80.

My second study, “*Evaluation of the relationship between stream habitat quality and taxa traits in Piedmont streams in North Carolina”*, illustrated the importance of a stream having a heterogeneous habitat to support a highly diverse benthic macroinvertebrate assemblage. I was able to show that taxa and trait richness and diversity were correlated with stream habitat condition (as measured with MHAP scores). Trait richness and diversity did not decline at the same rate as taxa richness and diversity due to trait redundancy. I showed that microhabitats such as riffles, leaf pack, large wood, and runs were important for taxa richness while undercut banks were important for taxa diversity, and habitat diversity and small wood were important for trait richness. This study identifies important habitats that are often overlooked when stream restorations are designed.

My third study, “*Evaluation of the Impact of Stormwater on Benthic Macroinvertebrate Diversity and Stream Ecosystem Function in a Piedmont Stream in North Carolina*", demonstrates the direct impact that unmitigated stormwater has on benthic macroinvertebrate assemblages. I was able to show through the FLOWSED-POWERSED model, run in the Rivermorph software, that shear stress was predicted to be significantly higher in a small headwater tributary (TI) receiving stormwater runoff from an adjacent subdivision via stormwater infrastructure than in a nearby forested tributary (TF) that receives stormwater runoff through more natural overland and subsurface processes. The differences in shear stress in the 2 headwater tributaries resulted in significantly different habitat conditions which in turn resulted in significantly lower benthic macroinvertebrate taxa and trait richness and taxa diversity in the TI tributary. The food webs of each tributary had different food sources in the summer. The collector-gatherer richness was significantly greater in the TF tributary. Collector-gatherers were found to be the most abundant benthic macroinvertebrate feeding groups in impaired urban streams like TI. The results of this study demonstrate that unmitigated stormwater can lead to both stream channel and habitat degradation, which in turn negatively impacts the benthic macroinvertebrate community.

## 5.2 IMPLICATIONS FOR STREAM RESTORATIONS

The watershed managers' response to urban stream degradation is to restore the streams to a stable channel that resembles a more natural stream using various stream restoration techniques such as the Natural Channel Design (NCD) method (Doll et al. 2003). Several early studies of stream restoration impact on stream biota and ecosystem function have shown limited improvements to stream biota (Paul and Meyer 2001; Cuffney et al. 2010; Violin et al. 2011; Coles et al. 2012). Violin et al. (2011) compared the physical and biological structure of 4 urban degraded, 4 urban restored, and 4 forest reference streams. They found that the restored urban streams were indistinguishable from the degraded unrestored urban streams while the forested reference streams were significantly different from both types of urban streams. They also showed that the benthic macroinvertebrate communities in the restored and unrestored urban streams were compositionally similar but were significantly dissimilar to the benthic macroinvertebrate communities found in the forested reference streams. Arango et al. (2015) examined the short-term response of the benthic macroinvertebrate community to the restoration of an urban stream in the Pacific Northwest. The benthic macroinvertebrate and fish communities did not improve immediately after restoration, but rapidly returned to pre-restoration community compositions after construction.

A significant limitation of the success of stream restorations in improving the aquatic biota in degraded urban streams has been the limited scope of the restoration projects (Shields et al. 2003; Bernhardt et al. 2005; Palmer et al. 2005; Suren and McMurtrie 2005; Bernhardt and

Palmer 2007; Palmer et al. 2007). Restoring relatively short segments of a degraded urban stream (reach scale) without addressing upstream reaches provides minimal chances for the complete recovery of the stream's aquatic biodiversity and ecosystem function (Violin et al. 2011). Unmitigated stormwater entering the restored segment will continue to negatively impact the stream microhabitats, reducing the diversity of stream habitats within the restored segment. Several recent studies stressed that a truly successful restoration of an urban stream cannot be done without restoring the stream's natural hydrologic regime (Russell et al., 2020; Anim and Banahene 2021; Zerega et al., 2021; Hawley 2022).

Improving restoration design is necessary to restore the benthic macroinvertebrate community in urban streams. This study's results show that unmitigated stormwater will have significant negative effects on the benthic macroinvertebrate assemblages. Addressing the sources of  hydrologic alterations as part of a stream restoration plan may lead to more successful restoration of an urban stream aquatic ecosystem. Another improvement to stream restoration design to improve the benthic macroinvertebrate community would be to expand the habitat improvement designs that currently focus primarily on the larval aquatic insect stage to include habitats required by the adult aquatic insects (Merten et al., 2014; Jordt and Taylor 2021). The riparian zones alongside streams are generally planted with native vegetation that may or may not be suitable food sources for benthic macroinvertebrates as well as be suitable refugia for adult macroinvertebrates. More research is needed regarding the suitability of the plants selected for restoration projects that not only stabilize soil and attract wildlife but can also enhance benthic macroinvertebrate larvae instream and adult insects in the terrestrial habitat.

Geomorphological stability of restored streams is easily measured. However, the degree to which ecological uplift is obtained by restoration is rarely assessed. This may be due to a lack of a good definition of ecological uplift. Once a definition is in place, future research can be directed on how to measure ecological uplift. Since most urban stream restorations take place greater than 5 km from a good population source for recolonization, another means of reintroducing benthic macroinvertebrates to the restored stream is needed. Reintroduction of benthic macroinvertebrates from streams with a high-quality community have been explored by several researchers (Dumeier et al., 2018; Jourdan et al., 2019; Dumeier et al., 2020; Clinton et al., 2022). More research is needed to identify successful reintroduction techniques.

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