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A Spatial and Multivariate Approach to Examining Effects of Urbanization on Nitrogen Sources, Organic Matter Inputs, and Trophic Structure in Streams of Cobb and Paulding Counties, Georgia.

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A Spatial and Multivariate Approach to Examining Effects of Urbanization on Nitrogen Sources, Organic Matter Inputs, and Trophic Structure in Streams of Cobb and Paulding Counties, Georgia.

Abstract

Urbanization and land use changes have a negative effect on streams often causing numerous physical changes in stream morphology, a change in nutrient concentrations, and altered ecosystems contributing to a loss of habitat, decreased biodiversity, and loss of stability for ecosystem function (Walsh et al., 2005; Meyer et al., 2005; Cardinale and Palmer, 2012). The use of stable isotope analysis for monitoring could be valuable because it accounts for temporal integration from anthropogenic wastewater inputs, characterized by a shift in the abundance of ^{15}N. This project had three main objectives: (1) to examine how nitrogen and carbon sources are altered with the use of spatial trends of $\delta^{15}N$ values in streams with varying levels of urbanization; (2) to examine how the effects of urbanization, altered nitrogen and organic matter inputs play a role in trophic structure within aquatic ecosystems by examining isotopic food webs; and (3) to see if these variables could be utilized for spatial predictability of urban impacts on streams. We found support for the notion that high levels of developed land use and agricultural land use correspond with an increase in the $\delta^{15}N$ values of macroinvertebrates. Our food webs and $\delta^{15}N$ values suggested that certain organisms changed their role in the food web consistent with a shift in the food web towards omnivory. Use of impervious surface area, land cover land use and $\delta^{15}N$ to monitor water quality could provide an early indicator for stream degradation.

Urbanization

Water is one of our greatest resources, and the majority of water and food we consume is sourced from lakes, rivers, and streams (Hauserman, 2015). Industries such as recreation (fishing and swimming), farming, and hydroelectric power also rely on clean water (Hauserman, 2015). Aquatic and terrestrial species, some of which provide us with important ecosystem services, such as flood control, play an important role in the food web and rely on healthy watersheds to survive (Hauserman, 2015).

The human population has increased drastically in the last 50 years and will continue to increase in the future. As it increases, more land is needed to house and sustain the growing population. Land consumption typically increases at twice the rate of population growth (Alberti, 2007). Landscape modifications and anthropogenic pollution impact water quality of lakes, streams, rivers, and coastal waterways in numerous ways and affects. Recognizing the ongoing threats to water bodies, the U.S. government established regulations to help mitigate the decline of water quality in streams. Despite their commercial and ecological significance, many bodies of water across the U.S. have been designated as “impaired” or “area of concern” based on clean water standards mandated by the Clean Water Act (CWA) (Hauserman, 2015).

Clean Water Act

In 1969, the United States Congress passed the National Environmental Policy Act, in response to growing concerns about acute environmental problems, such as the Cuyahoga River Fire. Soon after, the Environmental Protection Agency (EPA) was created and the Clean Water Act of 1972 was passed (Keiser et al., 2019; Hauserman, 2015). This act amended the Federal Water Pollution Control Act of 1948 and established the basic structure for pollution discharge regulations by setting wastewater standards for industry and making it unlawful to discharge any pollutant into waterways (Hauserman, 2015), regulations were then scaled back in 2001, reinstated in 2015, then withdrawn again in 2017, which removed monitoring requirements for over half of all U.S. streams and rivers (Keiser et al., 2019). The overall goal of the CWA is to maintain and restore the chemical, physical, and biological integrity by establishing a two-part reporting that requires states to identify impaired and threatened bodies of water every two years and calculate the pollution reduction levels needed to meet maximum contaminant levels for 94 different contaminants ranging from microorganisms (fecal coliform) to inorganic chemicals (i.e. cyanide, disinfectants, etc.) (Keiser et al., 2019; Hauserman, 2015).

This information is then used for the management and implementation of recovery efforts (Keiser et al., 2019; Barbour et al., 1999; Hauserman, 2015). Water quality improved after the passing of this legislation and enforced monitoring; however, 40% of water bodies in the U.S. are still below water quality standards due to urban runoff, leaky wastewater infrastructure, and other various forms of pollution (Keiser et al., 2019; Hauserman, 2015). Polluted waterways in urban areas typically have a higher presence of impervious surfaces (e.g. paved surfaces, buildings, concrete, etc.) within their watersheds which often leads to greater runoff (Booth et al., 2016; Zhang et al., 2018; Smucker et al., 2018). Up to 28% of the United States (U.S.) population receives drinking water from sources that violate CWA standards. The CWA largely ignores non-point source (NPS) pollution because it is difficult to monitor (Keiser et al., 2019).

Pollution types

Anthropogenic pollution from urban areas is classified into two main categories of pollution that degrade the quality of water (Liu et al., 2018; Adu and Kumarasamy, 2017). Point source (PS) pollution comes from a localized and identifiable source, typically from agricultural, industrial, or municipal sewer discharge, including leakage from wastewater treatment plants (WWTP), infrastructure with old sewer lines, faulty septic systems, or flooding (Keiser et al., 2019; Adu and Kumarasamy, 2017). This type of pollution is easier to identify because it often has a specific easily identifiable source. Non-point pollution (NPS) on the other hand typically comes from a non-local diffused source and is considered a leading threat to water quality (Adu and Kumarasamy, 2017). NPS pollution can be a combination of storm water runoff from urban, residential, agricultural, and even industrial land contamination. According to Liu et al. (2018), NPS pollution affects between 30 – 50% of global rivers. The effects of urban storm water runoff increase as percent impervious surface area (ISA) increases (i.e. asphalt, concrete, buildings/roofs). The type and concentration of NPS pollution also changes, causing water quality to degrade (Johnson et al., 2013; Liu et al., 2018). Most studies have found a water quality degradation threshold to be around 10% ISA and other studies have found thresholds as large as 30%. This threshold often changes based on the severity and frequency of disturbances that result from urbanization (Kim et al., 2016; Paul and Meyer, 2001; Zhou et al., 2014).

Urban Stream Syndrome

Urban populations have increased over the last few decades. For example, the human population in Atlanta, Georgia increased 97% between 1970 and 1995 (Meyer et al., 2005). Urban land use has also increased with the residential, industrial, and commercial needs of those urban dwellers (Meyer et al., 2005). As land use is modified and ISA increases there is often less or slower percolation and infiltration of storm water into the ground and an increase in runoff (Kim et al., 2016). As runoff flows from impervious surfaces into streams, it has the potential to pick up pollutants along the way. This increase in runoff combined with a decrease in riparian vegetation can lead to flashy hydrology and more frequent larger flow events (Booth et al., 2016; Meyer et al., 2005; Walsh et al., 2005). Collectively, these events often result in channel incision, scouring of substrate, and the accumulation of fine sediment (siltation), as well as decreased submerged and streambank vegetation, which can lead to decreased organic matter inputs and retention (Booth et al., 2016; Kim et al., 2016; Walsh et al., 2005).

Direct construction that modifies the stream itself, such as the building of bridges, flood control measures, and the installation of culverts result in a direct physical change, such as channel straightening and the removal of riparian vegetation. Collectively, these widespread changes are known as Urban Stream Syndrome (USS) (Meyer et al., 2005; Wallace et al., 2012; Paul and Meyer, 2001). Ultimately, USS results in a reduction in species diversity leading to altered stream function and ecosystem services (Booth et al., 2016; Meyer et al., 2005; Adu and Kumarasamy, 2017; Lin, 2019; Kim et al. 2016). Maintaining proper ecosystem services is important to the general health of the stream. It provides direct and indirect benefits to us through flood control, recreation, and promotion of biodiversity. Riparian vegetation helps with soil stabilization and biodiversity promotes healthy ecosystem function, allowing for nutrient cycling to take place (Troyer et al., 2016). The quality and abundance of riparian vegetation plays a large role in water quality and function within the stream.

Riparian Vegetation

Riparian vegetation is essential to freshwater ecosystems. This ecotone is a transition zone between aquatic and terrestrial habitats, where terrestrial plants represent an organic matter (OM) source, providing food and nutrients for various aquatic organisms (Lamberti et al., 2017; White et al., 2014; Kuglerova et al., 2018). The amount of OM inputs and how much is retained can fluctuate based on substrate, riparian features, stream morphology, and woody debris (Lamberti et al., 2017; Kuglerova et al., 2018). The decrease or loss if of riparian vegetation often leads to a decrease in wood entering the stream and an infiltration of non-native or invasive species (White et al., 2014; Alberti et al., 2007; Walsh et al., 2005).

The loss of riparian vegetation changes the dynamics of aquatic ecosystems. Riparian zone is often a source of instream food, acts as a buffer from urban impacts, and acts as temperature fluctuation control (Vannote et al., 1980; Alberti et al.,2007). This change in carbon sources can disrupt food habits for microbial colonization and invertebrates and alter the food web within the stream. (Vannote et al., 1980; Alberti et al., 2007; Kuglerova et al., 2018). Riparian zone vegetation influences organic carbon from allochthonous coarse particulate organic matter (CPOM; i.e. leaves and wood). It is a major carbon source for small stream ecosystems where canopy cover is significant. When there is a significant loss of riparian canopy cover there is also a shift from detrital allochthonous OM system to an autochthonous OM system based on primary productivity (Docile et al., 2016; Lamberti et al., 2017). Autochthonous sources are often reliant on light availability, therefore, as canopy coverage decreases the autochthonous OM increases. Concentrations of nutrients and nutrient uptake are often altered in urban streams due to the changes in OM and urban surface runoff (Walsh et al., 2005).

Organic and Inorganic Inputs

Point source, NPS discharge, and lawn fertilizers are often a source of organic and inorganic pollution in urban streams (Smucker et al., 2018). Some cities used combined sewer overflows (CSO) to divert storm-water and untreated sewage directly into streams and rivers during storm events to avoid flooding (Paul and Meyer, 2001). While CSOs are no longer being constructed, and have been replaced with newer sewer systems, some of the older CSOs still remain in use and contribute to pollution inputs (Tchobanoglous et al., 1972).

Elevated concentrations of nitrogen (N), phosphorus (P), ammonium, and a decrease in the efficiency of nutrient removal is often seen due to urban NPS runoff and a loss of biodiversity that is needed to process those inputs (Paul and Meyer, 2001). A study conducted by Adu and Kumarasamy (2018) examined various studies and found that total nitrogen and total phosphorus typically make up a large portion of the total pollution load. The biggest contributing factors of P and N are from human activities such as wastewater and fertilizer, which can become mobilized during surface disturbance events as a part of NPS pollution (Tromboni and Dodds, 2017; Adu and Kumarasamy, 2018;). Elevated N concentrations have been found in streams hundreds of km outside of urban centers (Paul and Meyer, 2001). The extent of the increase in N and P concentrations depends on the amount of fertilizer use in the area, and the quality of sewer and WWTP infrastructure. This increase in the concentrations of N and P could potentially lead to an increase in algae and a shift in consumer food sources because algae rely heavily on N for building proteins and P for energy transformations with in their cells (Chen et al., 2015; Tromboni and Dodds, 2017).

Nutrient concentrations and OM inputs play a role in microbial decomposer community composition. Aquatic hyphomycete fungi and bacteria are the main colonizers of OM. They facilitate nutrient transfer between terrestrial and freshwater ecosystems and help with the breakdown of CPOM into fine particulate organic matter (FPOM), biomass, and CO_2 (Pascoal et al, 2005; Tant et al., 2015; Tant et al., 2013). Fungi are the main colonizers of CPOM and often influence downstream food webs, due to their role in the formation of FPOM. They also boost the nutritional value and palatability of detritus for consumers by decreasing the C to nutrient ratio. Bacteria dominate fine particulate organic matter (FPOM), which gets consumed, transported downstream, or settles on the stream bed. An increased N and P concentrations from urban and agricultural land use can stimulate microbial decomposition and increase macroinvertebrate activity on leaves (Tant et al 2013; Tant et al., 2015; Pascoal et al., 2005). However, excessive nutrient concentrations resulting in eutrophication can lead to loss of macroinvertebrates, which are an important link between basal resources and higher trophic levels.

Functional Feeding Groups

As nutrient concentrations and OM inputs are altered due to urban inputs, the flow of energy through the food web also changes. The functional feeding group of an organism depends on its feeding activities and the mechanisms used to consume its food (Cummins and Klug, 1979; Ramírez and Gutiérrez-Fonseca, 2014). The input of allochthonous OM typically found in small, upstream, and rural sites becomes CPOM and gets colonized by microorganisms and shredded into FPOM by shredders (SH). Shredders eat and break down both living plant materials (herbivores) and decomposing CPOM (detritivores; Ramírez and Gutiérrez-Fonseca, 2014). However, Pascoal et al. (2005) found that the loss of shredder taxa does not always result in lower breakdown rates possibly due to an increase in microbial breakdown activity that overcompensates for the loss in shredders. As the CPOM is broken into FPOM and transported downstream it is often captured by collector filterer (CF) either directly from the water column using adaptations (i.e. mouth parts or net). As the FPOM settles on the stream bed, collector gatherers (CG) consume it using modified mouth sieve parts, often resulting in repacking and resuspension of these particles, which are potentially transported downstream. Scrapers (SC) consume epiphytic algae and biofilms that are attached to rocks and other hard substrates (i.e. matrix of algae, bacteria, and polysaccharides; Ramírez and

Gutiérrez-Fonseca, 2014). Predators are the highest trophic level for macroinvertebrates that capture and consume their prey by using modified mouth parts (i.e. labium) or specialized behaviors (i.e. injecting prey with poison; Ramírez and Gutiérrez-Fonseca, 2014). Some macroinvertebrates fall into multiple FFGs, these generalists have adapted multiple feeding habits for survival and some change feeding groups when they become adults.

Having a diverse assemblage helps stabilize the ecosystem because multiple species can perform the same function (Schaefer et al., 2012). This is important, especially in urban streams with frequent disturbances. It is important to monitor these assemblages because alterations in stream function can be an indicator of a larger problem.

Biomonitoring

The CWA requires bi-annual biological sampling in order to track water quality because community structure and species assemblage alterations also alter stream function and can be an indicator of water quality. In order to monitor water quality and stream health, local, state, and federal governments use water chemistry changes and biomonitoring. Measuring chemical and physical pollution is useful but it is also costly, time-consuming, and does not integrate conditions over time. Some studies (Karr et al., 1982; Guareschi et al., 2016; Barbour et al., 1999) suggests biomonitoring is cost-efficient and produces quick results, is scientifically valid, and easily translatable to the public and management. Biomonitoring allows resource managers to track biota variability to determine a stream's health and identify impacts (Meyer et al., 2005; Booth et al., 2016). Fish, periphyton, and benthic macroinvertebrate indices have been developed to assess anthropogenic impacts. Many aquatic biological communities are sensitive to disturbances in their habitat, which generally present as changes in abundance, diversity, or species composition, and play an important role in trophic dynamics (Rawi et al., 2014; Mangadze et al., 2016; Luo et al., 2017). Some of these organisms have been used as biomonitors, such as algae and macroinvertebrates.

Algal Communities

Algae are used in ecological and environmental indices as a way to track water quality. They are directly affected by light availability changes due to landscape alterations and nutrient changes in the water column because they are primary producers (Chen et al., 2016). Algal sampling is simple, inexpensive, requires few people, and has minimal impact on the other biota within the stream (Barbour et al., 1999). They have a rapid rate of reproduction, a short life span, and respond to disturbances often manifesting as changes in community structure or assemblages due to different species having different pollution tolerances, but also water quality changes can be detected by using biomarkers at a sub-organismal level (i.e. DNA damage, osmotic shock, and stimulation of nitrate-nitrite reductase or phosphate transporters (Bellinger, 2017; Chen et al., 2016). Algae are a basal food source, meaning that changes in algal assemblages often cause changes in higher trophic level organisms (Chen et al., 2016; Teittinen et al., 2015). Biodiversity is not always a measure of an increase or decrease in pollution, but rather an indicator of the type of pollution, especially since organic pollution highly influences algal flora (Palmer, 1969; Chen et al., 2016).

Many urbanization gradient studies have used diatoms, blue-green algae, and green algae assemblages as a way to monitor water quality because they respond to the intensity of human activities. Green algae are typically more dominant in low nutrient water, blue-green algae are more prevalent in oligotrophic waters, and diatoms are more dominant in high nutrient waters. Because high nutrient waters are also often high in organic pollution, diatoms are typically more abundant in waters with high amounts of wastewater runoff (Bellinger, 2017). Diatoms are the most common algal indices used for monitoring. Changes in diatom biodiversity is often due to eutrophication, low dissolved oxygen, and organic loading in freshwater systems, which are accompanied by a shift in the intake rate of consumers within the community (Schmidt et al., 2018; Chen et al., 2016). Because urban streams tend to have a reduction in riparian zone vegetation, an increase in light availability, and an increase in N and P concentrations there tends to be a shift towards autochthonous inputs and alterations in the food web (Docile et al., 2016). In streams with frequent high flow events, mobile diatoms tend to be more abundant and in low flow streams, stalked diatoms are more abundant (Chen et al., 2016). Conductivity, water temperature, and light availability also play a large role in diatom community structure. Urban streams typically have higher concentrations of ions, variable water temperatures, and a lot of available light due to loss of canopy cover, loss of riparian vegetation, and proximity to roadways and other impervious surfaces (Bellinger, 2017; Chen et al., 2016); this makes urban stream diatoms abundance and assemblage indicator, however, the use of macroinvertebrates as indicators is more widely studied.

Macroinvertebrates

Benthic macroinvertebrates are essential for aquatic ecosystem function and are the most widely used organisms for biotic indices in stream systems. Most aquatic invertebrates are found in benthic habitats at some point in their life cycle and have limited mobility providing a representation of their habitats in the stream ecosystem (Gal et al., 2019; Forio et al., 2013). Macroinvertebrates have complex life cycles and respond quickly to short-term environmental variations. Macroinvertebrate communities consist of a variety of functional feeding groups and trophic levels with various pollution tolerances (Barbour et al., 1999). They feed on algae, detritus, fine particulate organic matter (FPOM), suspended particulate organic matter (SPOM), and other invertebrates, as well as being a source of food for higher trophic levels, such as fish and amphibians (Gal et al., 2019). They are among the most diverse organisms in freshwater and their abundance and biomass have been used for decades to examine stream health (Hilsenhoff, 1987; Dalu et al., 2017).

The community changes in relation to disturbances making them good ecological indicators of localized conditions (Forio et al., 2013). Many studies focus on the changes in Ephemeroptera, Plecoptera, and Trichoptera (EPT) communities due to their sensitivity to anthropogenic pollution, although, Coleoptera and Hemiptera are also highly sensitive and good indicators as well. In contrast, Diptera are a highly tolerant order because they are opportunistic feeders and can colonize highly polluted waters (Gal et al., 2019; Siziba et al., 2018; Docile et al., 2016). As percent ISA increases with the landscape conversion to urban areas, the chemical, biological, and physical disturbances alter the water column, therefore changing food and habitat availability, and flow regimes (Dalu et al., 2017; Johnson et al., 2013). These all can result in reduced biodiversity among macroinvertebrate species. Many studies that examine this phenomenon report reduction of species richness and an abundance of disturbance tolerant species (Gal et al., 2019).

As these macroinvertebrate communities change the energy and function within the water column is altered impacting higher trophic level organisms, such as freshwater fish and amphibians. For example one Toronto study saw the proportion of tolerant benthic macroinvertebrate species increase in association with road density and urban runoff; whereas, species richness decreased (Wallace et al., 2013). A study in China found significant variation within the macroinvertebrate community along an urbanization gradient. Macroinvertebrate diversity declined and the proportion of tolerant species increased when the percent of impervious surface crossed a threshold of 10-30% (Luo et al., 2017). Many studies have examined forested and urban streams and found an that urban-based pollution had a significant impact on macroinvertebrate communities (Gal et al.,2019; Dalu et al.,2017). Urban streams tend to show significant difference in macroinvertebrate assemblages. Because macroinvertebrates are the intermediaries between top predators (fish) and basal resources (algae and detritus), their role in the food web is important to study. Urban streams tend to shift from detrital system to systems with more primary productivity as riparian and canopy coverage decrease. This shift in a basal food source also plays a role in its consumer's diet and community structure and function (Docile et al., 2016).

Biotic indices are developed and applied globally to assess water quality and have been used for decades (Barbour et al., 1999). However, there are limitations to using macroinvertebrate and algal indices. Algae are dependent on light availability and season (late summer/early fall) and they do not integrate environmental effects over time but rather represent the ecosystem at the time they are sampled (Barbour et al., 1999). Macroinvertebrates indices require consistent sampling periods due to seasonal variability. Both of these indices are limited by high flow periods causing scouring and turbidity, which alters substrate, OM retention, and light availability, potentially resulting in skewed indices for both macroinvertebrates and periphyton (Barbour et al., 1999; Walsh et al., 2005). It is important to study stressors and their effects on community assemblages because they respond to different stressors and varying degrees of those stressors in different ways. Because urban stressors differ depending on region, climate, and intensity of land use, performing a multi-variate study in an attempt to establish indicator predictability is necessary. Combining data on organic and inorganic inputs, biotic indices, and stable isotope analysis will allow for a long term and short term representative picture of both urban and forested stream ecosystems.

Stable Isotope Ecology

Stable isotopes can be an effective way to examine aquatic ecosystems. They can be used to identify the presence of wastewater inputs and to examine food webs and trophic positioning. The two elements are commonly used to accomplish these objectives, N and C. The light isotope of N, ^{14}N, reacts more quickly due to differences in mass. So there is a slight bias in which atoms react (Fry, 2006). It also forms bonds that require less energy to break, leaving ^{15}N to slightly accumulate in a relatively predictable way. The net result is a change in the $\delta^{15}N$ value, an expression of the relative abundance of heavy to light isotopes, in the tissues of the organism (Fry, 2006). This process is called fractionation and can be used to identify trophic relationships and examine changes in food webs. Some of that N is going to be incorporated it into biomolecules. As organisms metabolize biomolecules they produce nitrogenous waste. The excretion of that nitrogenous waste favors the light isotopes, because they are easier to react with. On the other hand, the heavy isotopes form double and triple bonds and have a slower diffusion rate, which makes them harder to react with. Therefore, the waste that is excreted is going to disproportionately eliminate ^{14}N and lead to that accumulation of that the heavy isotope in the tissues of the organism.

A consumer eating a primary producer will have a $\delta^{15}N$ value of 2-4 per mille higher than primary producer, then a predator on that consumer ingests a food source that is 2-4 per mille higher than the original primary producer, leading to a cumulative increase as you move up through the food web (Fig. 1; Smucker et al., 2018; Fry, 2006). Since different food sources have different isotopic signatures, the shifts in the abundance that result from urbanization may be reflected in their contribution to the diet of consumers. (i.e. they contribute less energy to the food web).

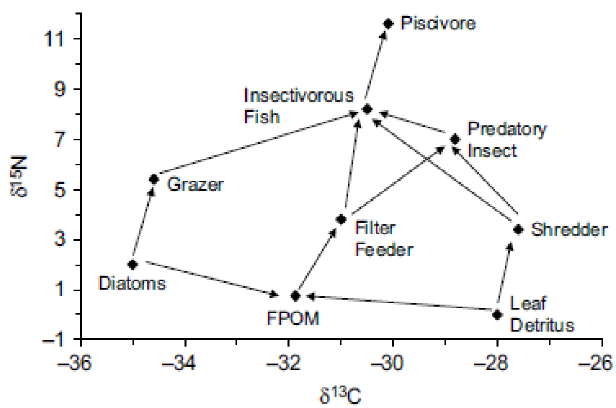


Figure 1. Hypothetical values for d13C versus d15N for various components of a typical stream food web. Arrows indicate trophic transfers based on fractionation of approximately 2 - 4 per mil for N and 0.5 per mil for C for each trophic level (Hershey et al, 2006).

Understanding food webs within an urban stream can help us understand how changes in organic matter, sources of nitrogen, and loss of biodiversity may alter food web structure and the flow of energy within the community (Costantini et al., 2014; Smucker et al., 2018). There have been many studies that use stable isotope signatures to examine trophic positioning along an urbanization gradient to see if urban land use leads to diet changes (Fry, 2006; Smucker et al., 2018). Another process that happens is mixing, which is when different ratios of isotopes are mix together. This happens when an organism has multiple food sources. Stable isotope ecology focuses on what processes are causing mixing and what processes are causing fractionation.

The ratios of heavy to light isotopes are frequently used as an indicator of wastewater or other N inputs (McClelland et al., 1997) because wastewater also becomes enriched with ^{15}N. As denitrification occurs within wastewater facilities, nitrite is converted to N_2 gas and disproportionately removes ^{14}N, as we talked about earlier, leaving behind ^{15}N. So the N left in the wastewater has a higher $\delta^{15}N$ value. This also happens in situ in sewer lines and in the environment or wherever wastewater or nitrite pools occur. Measuring stable isotope in streams could be a valuable tool to understanding the effects of human land use changes (Fry, 2006; Smucker et al., 2018).

Stable isotopes of carbon are also useful in examining food webs. Different producers may have different $\delta^{13}C$ ratios based on their metabolic pathways. C_4 plants (or algae with C concentrating mechanisms) may have higher (-10 per mille) $\delta^{13}C$ values than C_3 plants, for example (Fry, 2006). If producers have different isotopic values, then we can use that to examine the source of carbon for higher trophic levels.

If urbanization changes the abundance of those producers (with different isotope values), we, again, should be able to see that in the isotope values of the consumers and infer how urbanization changes the importance of different trophic pathways in the food web (Fry, 2006). This provides insight as to the type of basal resources available, consumed, and assimilated.

As allochthonous and autochthonous inputs become altered as a result of urbanization, the food web also becomes altered (Smucker et al., 2018). Trophic structure can be a good indicator of stream health because the nutrient inputs from urbanized land, directly and indirectly, influence species composition and tolerance guilds, which can result in community structure and food web changes (Smucker et al.,2018; Ana et al., 2013). As one prey declines, there is a proportional increase in the consumption of other prey. For example, as allochthonous

OM inputs decline with removal of riparian vegetation, then autochthonous OM (algae) becomes more consumed or there is a mix of both sources being consumed. This change could can then be detected due to mixing and fractionation of the stable isotopes, thus changing the $\delta^{15}N$ and $\delta^{13}C$ values (Costantini et al., 2014; Smucker et al., 2018; Lamberti, 2017; Fry. 2006).

The impact that urbanization has on freshwater streams is significant and often irreversible. By 2050, it is expected that 68% of the population will live in cities (Gal et al., 2019). This has large scale implications for freshwater communities. As the biodiversity decreases, the available ecosystem services they provide humans will also decrease. Ecologically friendly building and watershed management practices, as well as quick and efficient ways to monitor stream communities must be put into use in order to avoid complete loss of biodiversity and possible extinction of lower and upper trophic level organisms.

This study used an integrative approach in an attempt to develop a more comprehensive representation of how urbanization changes water quality and how that relates to stream community structure and function. This project examined: (1) how nitrogen and carbon sources are altered in urban streams; (2) how the effects of urbanization, altered nitrogen sources, and organic matter inputs play a role in trophic structure within aquatic ecosystems; and (3) to see if these variables can be utilized for spatial predictability of urban impacts on streams. We predict that as urban ISA increases: (1) wastewater inputs will alter N sources and contribute to an increase in $\delta^{15}N$; (2) organic matter inputs will become altered; and (3) macroinvertebrate communities will decrease in richness and shift towards omnivory.

Methods

To examine land use effects on streams I looked for large spatial patterns in water quality variables and their association with stable isotopes of nitrogen using a two tiered sampling approach. The first objective was to look at large scale spatial patterns in $\delta^{15}N$ of organisms present at various locations and see if they were related to any of the water quality variables that are regularly monitored by Paulding and Cobb Counties. The second objective was to take a closer look at a subset of streams across the gradient in urbanization and examine structural and functional differences that may be associated with the patterns in $\delta^{15}N$.

Broad Spatial Sampling

Three representative macroinvertebrate families representing three functional groups were collected including *Baetidae* (CG), *Heptageniidae* (SC), and *Hydropsychidae* (CF; Voshell, 2002). Sampling occurred between February and October 2019 from 22 Cobb County and 12 Paulding County (Fig. 2) sites located within the Chattahoochee and Etowah drainage basins that are a part of the CWA mandated county monitoring program. The use of multiple functional groups (FFGs) allowed for an examination of $\delta^{15}N$ values for consumers with different roles within the food web. The macroinvertebrates were collected by hand and dip nets, sorted, and identified to family using a dissecting microscope and taxonomic keys (Merritt et al., 2008; Morse et al., 2017). Individuals were dried in a drying oven at 60°C for 48 hours or until constant weight. Dried tissue was then ground using a Wig-L-Bug® grinding mill (Dentsply Rinn Corporation), weighed, and wrapped in tin-capsules (Brigham et al., 1982). Wrapped samples were sent to University of Georgia Stable Isotope Ecology Lab (SIEL) and were analyzed using an elemental analyzer coupled to an isotope ratio mass spectrometer. The analysis provided the carbon and nitrogen content of the sample tissues as well as the $\delta^{15}N$ and $\delta^{13}C$ value.

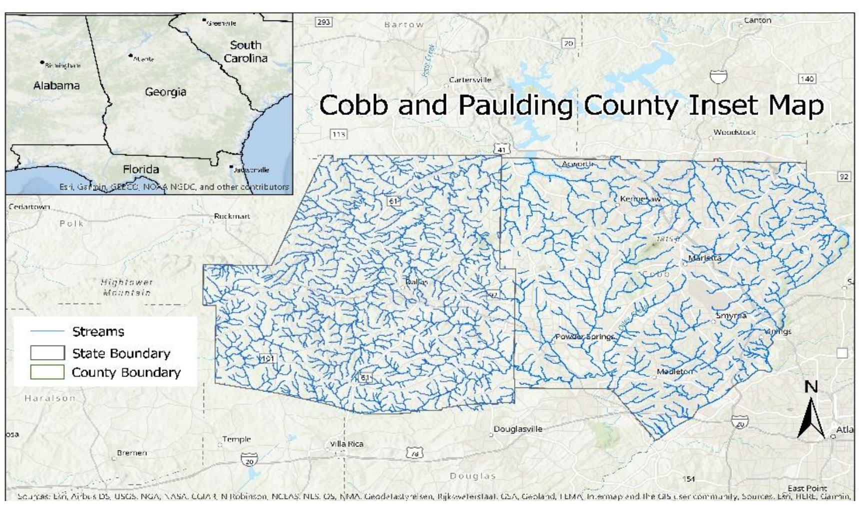


Figure 2. Cobb and Paulding Counties, Georgia, where the study was conducted and where the counties are located in Georgia.

Intensive Sampling

Intensive sampling at a six of the 35 sites was conducted from October until December 2019 to examine differences in macroinvertebrate assemblages, food web structure, along a gradient of potential N inputs as indicated by differences in $\delta^{15}N$ values. Two sites with relatively high $\delta^{15}N$ values (SP3 and P3), two with intermediate $\delta^{15}N$ values (ND1 and ND4), and two with relatively low $\delta^{15}N$ values (R2 and R3) were selected in order to examine community structure and function across a range of potential influence from urban N inputs. Sites with intermediate values and sites with low values occur on the same stream, permitting upstream and downstream comparisons.

Data collection at the six intensively sampled sites followed the EPD Macroinvertebrate Biological Assessment of Wadeable Streams in Georgia protocol within a 100-meter standardized representative stream reach making sure the reach was at least 100 m upstream or downstream from roads and bridges (Barbour et al., 1999; EPD, 2007).

Stable Isotope Analysis of Trophic Structure

Representative organisms from each site were collected to examine food web structure. Macroinvertebrates and leaves were collected and identified to family and species, respectively. Periphyton was collected from all substrates (sand/sediment, cobble, wood, pebble, etc.) encountered at the sites. Each substrate was scrubbed and rinsed into whirl bags. The periphyton slurry was centrifuged at 3000 RPM for 25 – 30 minutes until all sediment and suspended materials were concentrated with minimal water. Periphyton was placed in a drying oven at 60ºC for 48 hours or until constant weight and then processed for stable isotope analysis as described above. These samples were sent to University of California, Davis Stable Isotope Laboratory. The analysis provided the carbon and nitrogen content of the sample tissues as well as the $\delta^{15}N$ and $\delta^{13}C$ value. Trophic levels of organisms were reconstructed from $\delta^{15}N$ values using the following equation:

$$TL = 1 + (\delta^{15}N_{(organism)} - \delta^{15}N_{(primary\ producer)})/2.6$$

TL is the trophic level of the organism of interest, and $\delta^{15}N_{(organism)}$ is the $\delta^{15}N$ value of the organism of interest. $\delta^{15}N_{(primary\ producer)}$ is the $\delta^{15}N$ value of a representative primary producer sampled from the community. In all but one case, leaf tissue of the American Sycamore, *Platanus occidentalis*, was selected as the primary producer. At site R3, the primary producer was Tulip Poplar, *Liriodendron tulipifera*. The constant 2.6 represents fractionation of $\delta^{15}N$ associated with trophic transfers as estimated by (Reid et al., 2008).

Periphyton

A composite periphyton sample was collected following the EPA sampling protocol. A 100 m reach was measured out and then divided into five 20 m transects using the transect tapes. The width of the stream was measured at each transect. The stream was then divided into three sections (center, left, and right) with the center section being twice the width of the left and right sections. Using a random number table, a sample was collected from one of sections. The collecting method was dependent on the substrate at each sampling point. To remove periphyton from hard small to intermediate substrates, such as pebble, a rubber delimiter and toothbrush were used to scrape a known area of the surface and rinsed into Whirl-Pak® bags. For large substrates, such as boulder or cobble, a modified syringe sampling device was used (Stevenson and Bahls, 1999). The depressed syringe is placed directly onto the large substrate so that it forms a seal. The algae were then dislodged from the substrate using a scouring pad attached to the syringe plunger. A spatula was inserted under the syringe to remove the sample from the stream, and the sample was rinsed into whirl bags. For any loose sediment substrate, such as silt, sand or clay, an inverted PVC cap was utilized to trap sediments, then a spatula was placed under the trapped sediment to remove from water and put into container. The samples were then rinsed, collected in a labeled whirl bags, and placed on ice for transport (Barbour et al., 1999).

Macroinvertebrates

Sampling took place at 20 different locations within the standardized 100m reach, starting downstream and moving upstream. Each habitat was sampled in accordance with the GA EPD protocol. Three samples were taken from each habitat, if a habitat was not present, the three samples were allocated to top priority habitats (Table 1; EPD, 2007).

Priority	Habitat Type	Number of Samples
1	Fast Riffle	3
2	Slow Riffle	3
3	Woody debris/Snags	5
4	Undercut Banks/Rootwads	3
5	Leaf Packs	3
6	Soft Sediment/Sand	3

Table 1. EPD habitat priority list for sampling macroinvertebrates (EPD, 2007).

Following the EPD habitat priority list, 20 forceful jabs or kicks were performed into productive habitats with the D-frame net downstream. Riffles required six riffle kicks in areas of different velocities (three fast, three slow). Gently rubbing loose debris off rocks and kicking the substrate just upstream of the riffle dislodged any burrowing organisms. Small to intermediate submerged woody debris was dislodged into the D-frame net by jabbing the debris (EPD, 2007). Larger woody debris was rubbed clean into the D-frame net (ignoring any debris too large to move). Undercut banks and rootwads were sampled by jabbing the net along the substrate in areas with different flow regimes. Leaf packs were gathered by obtaining a large handful of well-conditioned matter (not newly fallen). Soft substrate was sampled by kicks or jabs with foot covering a 0.3 m area and sweeping the D-frame net through the disturbed material (EPD, 2007). Materials collected in the D-frame net were compiled into a sieve bucket (30 mesh) and large debris was carefully rinsed and inspected for organisms, then discarded. The samples were placed into labeled containers with 80% ethanol for transport.

The composite samples were taken back to the lab. If the invertebrate abundance were less than 160 organisms, all individuals were identified to family and counted. If the abundance were greater than 160, subsampling was performed by placing the sample in a tray (30 x 36cm) with 30 marked (6 x 6 cm) squares. The samples were spread out evenly with DI water (EPD, 2007) and a random number generator was used to select at least four grids. A metal barrier was inserted around the periphery of each grid, and the materials were then pipetted onto a white tray and identified to family using a dissecting microscope. A minimum of 160 and maximum of 240 organisms were identified (EPD, 2007).

ArcGIS Pro

National Landcover Database (NLCD) 2016 impervious surface and landcover data was used to determine percent impervious surface area (ISA) and land cover/land use % (LC/LU) for each watershed. The watershed layer shapefiles for each site was obtained by using the watershed-modeling web app *Model my Watershed* (wikiwatershed.org). The shapefiles were uploaded into ArcGIS Pro 2.4 and extract by mask tool was used to calculate percent ISA and LC/LU percent for each watershed.

There are 15 land use categories used in this project. Open water category consists of water and has less than 25% cover of vegetation (https://www.mrlc.gov/). Developed open space has some constructed materials and vegetation but less than 20% of total cover is impervious surface (https://www.mrlc.gov/). Developed low intensity is a mixture of constructed materials and vegetation with impervious surface accounting for 20 – 49% impervious surface (https://www.mrlc.gov/). Developed medium intensity is constructed material and vegetation with an impervious surface cover of 50 – 79% (https://www.mrlc.gov/). Developed high intensity has an impervious surface cover above 80% with little vegetation (https://www.mrlc.gov/). Barren land is areas of bedrock, scarp, talus, gravel pits, and other earthen materials with a vegetation cover of less than 15% (https://www.mrlc.gov/). Deciduous forests are areas dominated by trees greater than 5 meters tall, vegetation cover is greater than 20%, and at least 75% of the trees respond to seasonal changes (https://www.mrlc.gov/). Evergreen forests are areas dominated by trees greater than 5 meters tall, vegetation cover is greater than 20%, and at least 75% of the trees maintain their leaves all year (https://www.mrlc.gov/). Mixed forests are areas dominated by trees greater than 5 meters tall, vegetation cover is greater than 20%, and neither deciduous nor evergreen species are greater than 75% of total tree cover (https://www.mrlc.gov/). Shrub areas are dominated by true shrubs, young trees or stunted trees and are less than 5 meters tall with shrub canopy greater than 20% of total vegetation (https://www.mrlc.gov/). Herbaceous areas is dominated by herbaceous vegetation, greater than 80% of total vegetation and are not subject to intense management (https://www.mrlc.gov/). Pasture/Hay are areas of grasses, legumes, or a grass-legume mixture for livestock grazing or production of seed or hay crops accounting for greater than 20% of total vegetation (https://www.mrlc.gov/). Cultivated crops are areas used to produce annual crops, such as corn, soybeans, vegetables, and also including orchards and vineyards, and accounting for greater than 20% of total vegetation (https://www.mrlc.gov/). Woody wetlands are areas where forest or shrubland vegetation accounts for greater than 20% of total vegetation where soil is periodically saturated or covered with water (https://www.mrlc.gov/). Emergent herbaceous wetlands are areas where perennial herbaceous vegetation account for greater than 80% of vegetative cover and are periodically saturated with or covered with water (https://www.mrlc.gov/).

Statistical Analysis

Pearson correlation coefficients were assessed at $\alpha = 0.05$ to determine significance and were calculated to examine broad-scale associations among water quality variables, habitat descriptors, land use metrics, and $\delta^{15}N$ values of the three representative families collected at all 35 sites. Historic data from 2015 - 2018 on water quality and habitat descriptors were provided by Cobb County Water System and Paulding County Water System. Values for the past sampling dates closest to our sampling dates were averaged prior to calculating correlation coefficients.

A multivariate statistical approach was used to examine the relationship between biological communities and water quality parameters. Multidimensional scaling (MDS) was used to graphically represent the relationship between water quality variables and relative abundance of biological communities found from 27 sites the statistical computer program SPSS. The variables were normalized using the z score, prior to analysis, and Euclidean distance was used to generate the distance matrix.

MDS was used to analyze 11 WQ variables including $\delta^{15}N$ and $\delta^{13}C$ Hydropsychidae values from 27 sites. In order to reduce 2D stress BOD, COD, pH, conductivity, temperature, DO, and d13C were removed one at a time and stress was checked after each removal until 13.98% 2D stress remained. The MDS for relative abundances was run initially using all 40 families for 27 sites (2D stress: 29.7%). In order to reduce MDS 2-dimensional stress families only found at a specific number of sites were removed and stress was checked after each removal. The first families removed were only found at one site and families were removed until the only families remaining were found at 16 sites or more. The stress was checked after each family removal until a 2D stress of 16.8% remained and 7 families remained (*Philopotamidae, Hydropsychidae, Leptoceridae, Chironomidae, Tipulidae, Empididae,* and *Simuliidae*). To further assess biodiversity of local benthic macroinvertebrate assemblages, Richness (S), Shannon-Wiener diversity formula was used:

$$\mathrm{H'} = \sum_{i=1}^{S} pi \cdot ln(pi)$$

And evenness was also calculated using the formula:

$$\gamma = \Sigma \mathrm{p}_{\mathrm{i}}^{2}$$

Results

Broad Spatial Sampling

Percent ISA ranged from 0.6% at R1 to 40.3% at RT4. Percent ISA was used to designate land use categories of urban, suburban, and rural based on White et al. (2014) and Smucker et al. (2018). The six largest watersheds were rural (≤ 5% ISA) and found in Paulding county. Eleven out of 14 suburban (5.1 - 20% ISA) and 12 urban (> 20.1% ISA) sites were found in Cobb County (Table 2; Fig. 3).

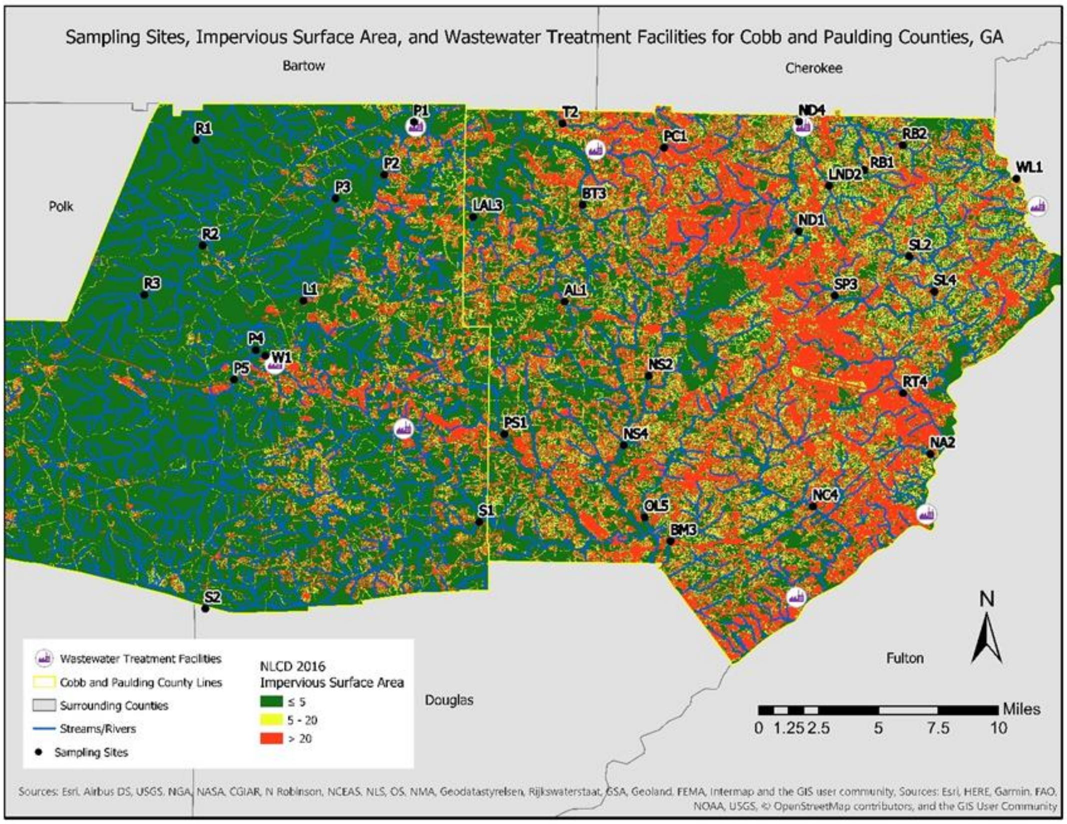


Figure 3. Cobb and Paulding WQ Sampling sites, ISA, and WWTF. Green indicates 0-5% ISA (rural land use), Yellow indicates 5-20% ISA (suburban land use), and red indicates > 20% ISA (urban land use).

The highest δ^{15}N values were from SP3 (13.88 ± 2.64‰) and P3 (10.54 ± 0.29‰; Fig. 4). The lowest values for δ^{15}N were found at the sites with the lowest ISA, R1 (4.35 ± 0.24‰), R2 (4.15 ± 0.94‰), and R3 (3.45 ± 0.67‰; Fig. 4). There is an increase in δ^{15}N macroinvertebrate values until ISA hits 5% and above δ^{15}N tended to remain relatively constant with some site to site variation. That 5% literature value corresponds to a functional transition point in our data but there is not an obvious inflection point. Hydropsychidae tended to have higher δ^{15}Ns at intermediate ISA compared to Baetids and Heptageniids (Fig. 4).

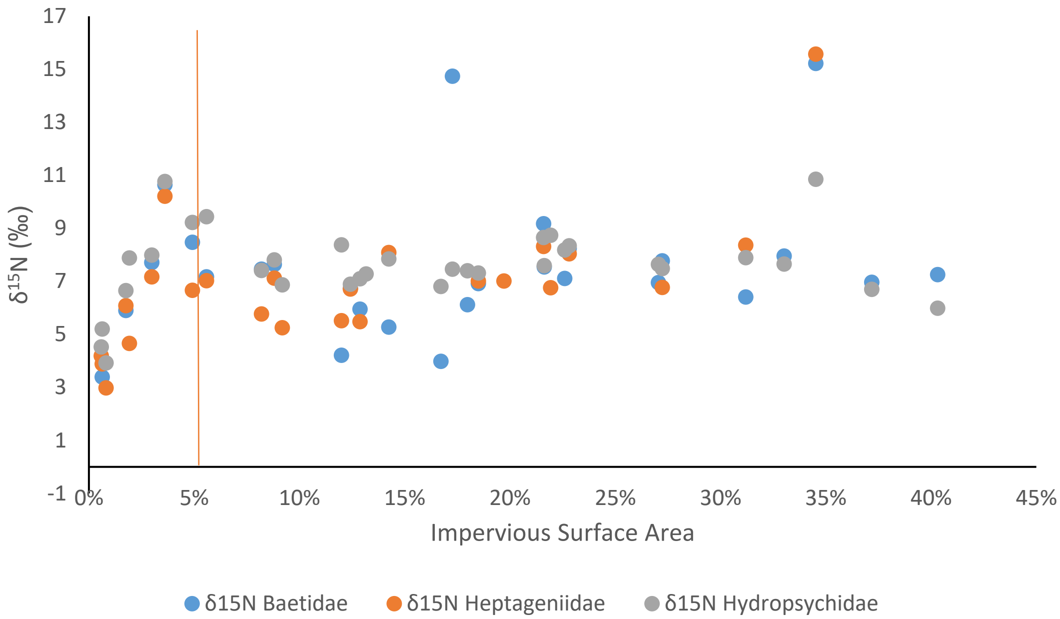


Figure 4. Percent impervious surface area and δ^{15}N values for Hydropsychidae, Heptageniidae, and Baetidae from 34 sites across Cobb and Paulding counties with a 5% ISA indicator.

Pearson Correlations

Watershed area was normalized using the natural log and was significantly positively correlated with turbidity (r= 0.49) and total phosphorus (r= 0.46). Temperature had a significant positive correlation with area (r= 0.45), TSS (r= 0.49), turbidity (r= 0.8), total phosphorus (r= 0.60), fecal (r= 0.60), and BOD (r= 0.58). Dissolved oxygen % saturation was negatively correlated with δ^{15}N *Hydropsychidae* (r= -0.42), TSS (r= -0.44), and conductivity (r= -0.44). Fecal coliform had a significant positive correlation BOD (r= 0.89) and with several erosion related WQ variables, such as TSS (r= 0.66), turbidity (r= 0.65), and total phosphorus (r= 0.89) and a negative correlation with ISA (r= -0.48; Fig. 5). There were also significant negative correlations between ISA and BOD (r= -0.53), turbidity (r= -0.41), and total phosphorus (r= -0.64) and a negative correlation with conductivity (r= 0.43; Fig. 5). There was a significant positive correlation between δ^{15}N *Hydropsychidae* and NO_x (r= 0.40) and conductivity (r= 0.64).

The $\delta^{15}N$ values for *Heptageniidae* were positively correlated with ISA (r= 0.67; Fig. 4-5) and conductivity (r= 0.52).

Several relative abundance values for macroinvertebrates found at the 34 sites also had significant correlations with WQ variables. *Chironomidae* was significantly positively correlated with $\delta^{15}N$ *Hydropsychidae* (r= 0.38) and DO (r= 0.37), and negatively correlated with temperature (r= -0.54), total phosphorus (r= -0.40), and BOD (r= -0.39). *Hydropsychidae* was positively correlated with DO (r= 0.37) and negatively correlated with area (r= -0.42) and temperature (r= -0.42). *Leptoceridae* was positively correlated with $\delta^{15}N$ *Hydropsychidae* (r= 0.45), $\delta^{15}N$ Heptageniidae (r= 0.77), and $\delta^{15}N$ *Baetidae* (r= 0.59). *Philopotamidae* was positively correlated with overall ISA (r= 0.64) and $\delta^{15}N$ *Heptageniidae* (r= 0.75). *Simuliidae* was negatively correlated with conductivity (r= -0.49).

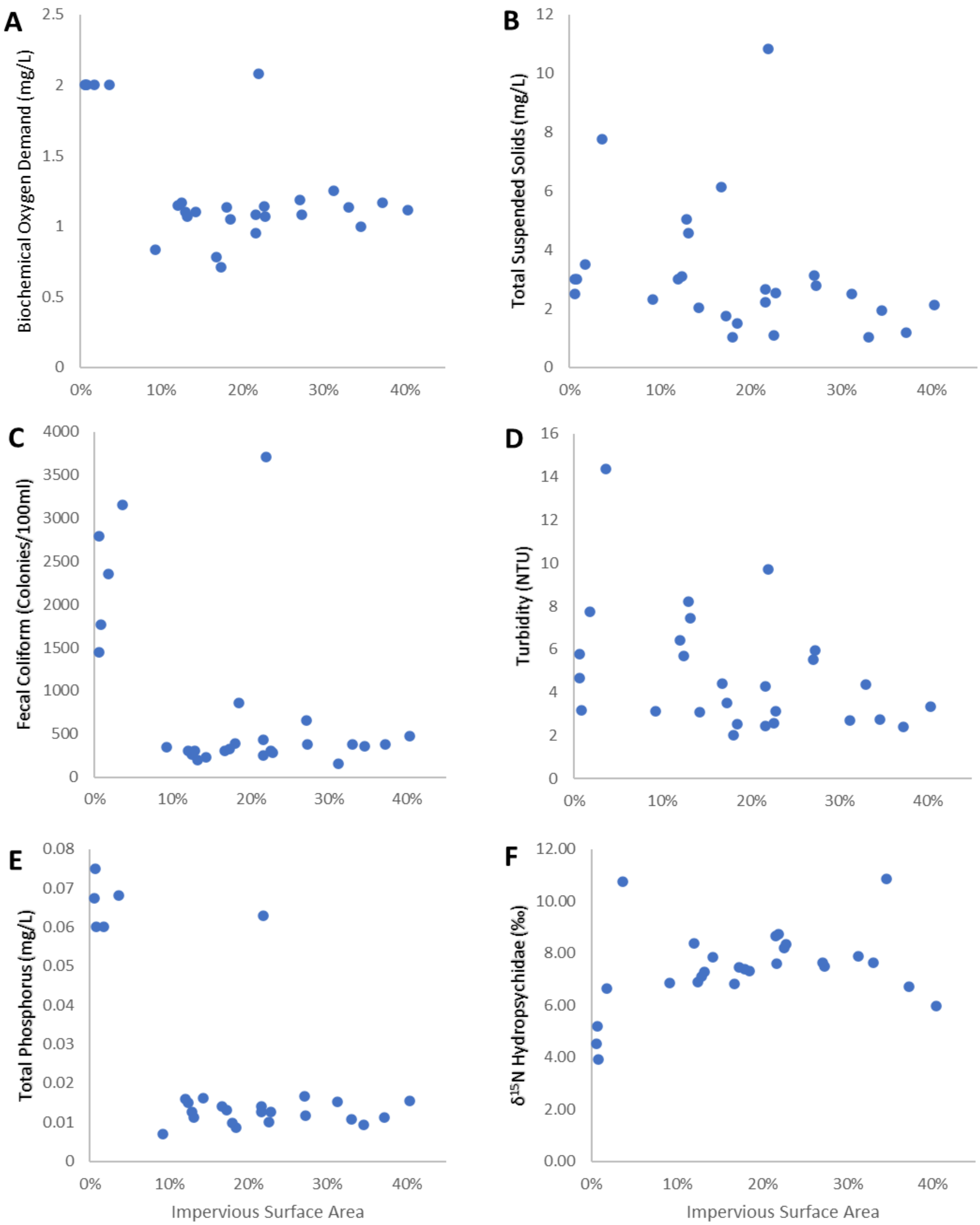


Figure 5. Percent impervious surface area and (A) Biochemical oxygen demand, (B) Total suspended solids, (C) Fecal coliform, (D) Turbidity, (E) Total phosphorus, and (F) $\delta^{15}N$ values for Hydropsychidae. Averaged Values from 27 sites across Cobb and Paulding counties along ISA gradient.

Several significant correlations when comparing WQ variables, $\delta^{15}N$ values, and NLCD 2016 LC/LU%. There was a positive correlation found between $\delta^{15}N$ *Hydropsychidae* and developed open space (r= 0.37) and developed low intensity (r= 0.43). There was a significant positive correlation found between $\delta^{15}N$ *Heptageniidae* and developed open spaces (r= 0.45), developed low intensity (r= 0.54), developed medium intensity (r= 0.64) and developed high intensity (r= 0.70). There was positive correlations found between $\delta^{15}N$ *Hydropsychidae* and developed open space (r= 0.37), developed low intensity (r= 0.43), and cultivated crops (r= 0.45) and negative correlations with deciduous forest (r= -0.52), evergreen forest (r= -0.38) and mixed forest (r= -0.41). *Heptageniidae* $\delta^{15}N$ values were negatively correlated with deciduous forest (r= -0.60), evergreen forest (r= -0.56), and mixed forest (r= -0.60) and positive correlations found with developed open space (r= 0.45), developed low intensity (r= 0.54), developed medium intensity (r= 0.64) and developed high intensity (r= 0.70). Area was negatively correlated with developed open spaces (r= -0.37), developed low intensity (r= -0.51), and developed medium intensity (r= -0.40). Area was positively correlated with barren land (r= 0.39), deciduous forest (r= 0.47), evergreen forest (r= 0.52), mixed forest (r= 0.46), shrubs (r= 0.56), herbaceous land (r= 0.49), cultivated crops (r= 0.40), woody wetlands (r= 0.65) and emergent herbaceous wetlands (r= 0.37). BOD was negatively correlated with developed open space (r= -0.67) and developed low intensity (r= -0.60) and positively correlated with deciduous forest (r= 0.75), evergreen forest (r= 0.65), mixed forest (r= 0.69), shrubs (r= 0.73), herbaceous land (r= 0.80). Total suspended solids were positively correlated with shrubs (r= 0.53) and herbaceous land (r= 0.55). Fecal coliform was negatively correlated with developed open space (r= -0.52) and developed low intensity (r= -0.48) and positively correlated with deciduous forest (r= 0.58), evergreen forest (r= 0.56), mixed forest (r= 0.56), shrubs (r= 0.79), herbaceous land (r= 0.82). Turbidity was negatively correlated with open space (r= -0.40) and developed low intensity (r= -0.39) and positively correlated with barren land (r= 0.48), evergreen forest (r= 0.48), shrubs (r= 0.77), herbaceous (r= 0.72), hay/pasture (r= 0.45), cultivated crops (r= 0.68), woody wetlands (r= 0.56) and emergent herbaceous wetlands (r= 0.64). Total phosphorus was negatively correlated with developed open spaces (r= -0.68), developed low intensity (r= -0.68), developed medium intensity (r= -0.43) and developed high intensity (r= -0.39) and positively correlated with deciduous forest (r= 0.79), evergreen forest (r= 0.72), mixed forest (r= 0.77), shrubs (r= 0.75), herbaceous land (r= 0.80), and cultivated crops (r= 0.38). Conductivity was positively correlated with developed open spaces (r= 0.40), developed low intensity (r= 0.60), developed medium intensity (r= 0.41), and negatively correlated with deciduous forest (r= -0.49) and mixed forest (r= -0.37).

When calculating correlations for LU/LC % and relative abundances for macroinvertebrates *Philopotamidae* had the most significant correlation coefficients. *Philopotamidae* was positively correlated with developed medium intensity (r= 0.60), developed high intensity (r= 0.79), and overall ISA (r= 0.64), and negative correlations with deciduous forest (r= -0.41), evergreen forest (r= -0.48), mixed forest (r= -0.43), herbaceous land (r= -0.47) and hay/pasture (r= -0.37). *Leptoceridae* was positively correlated with developed medium intensity (r= 0.41), high intensity (r= 0.47), and ISA (r= 0.39). *Hydropsychidae* was positively correlated with developed high intensity (r= 0.41). *Simuliidae* was negatively correlated with developed medium intensity (r= -0.38) and positively correlated with open water (r= 0.58).

Multidimensional Scaling

The historical data for relative abundance was available for 28 of the 34 sites and since there was no $\delta^{15}N$ *Hydropsychidae* data for LND2 in the WQ MDS, both MDS analyses were run using 27 sites. The initial MDS for relative abundance and WQ were run with s-stress convergence of 0.001, a minimum s-stress value of 0.005, a maximum of 30 iterations; for this study we were aiming for a s-stress of around 15% or less. The initial relative abundance for WQ variables had a 2D stress of 18%. There were 30 different combinations of WQ data that were attempted in order to find the best combination to reduce s-stress; ultimately the elimination of $\delta^{13}C$ *Hydropsychidae*, pH, COD, BOD, conductivity, temperature, and DO resulted in the lowest s-stress at 13.98%.

The ordination shows a division between high (9.19 – 40.32%) and low ISA (0.59% - 3.61%) on a slight diagonal axis that stretches from the lower left quadrant up through the top quadrant (Fig. 6). The sites NS4, NS2, PS1, SL4, ND4, LAL3, WL1, BT3, T2, OL5, BM3, SL2, AL1, RT4, RB4, RB2, PC1, NA2, ND1, RB1, SP3, and NC4 were located near each other and had higher ISA (Fig. 6). The sites R3, R2, R1, P5 and P3 all had relatively high levels of fecal coliform (3158 – 1444.6 colonies/100ml) and high total phosphorus but a low ISA (0.59% - 3.61%). There was also a $\delta^{15}N$ gradient with high $\delta^{15}N$ values (8.73 – 10.77‰) falling in the lower left, intermediate $\delta^{15}N$ (6.08 – 8.70‰) values in the center and low $\delta^{15}N$ (2.98 – 6.07‰) values in the top right with the exception of P3 and W1 (Fig. 6). Sites W1 and P3 were relatively close to each the in the graph and had relatively high $\delta^{15}N$ (8.73 – 10.77‰), fecal coliform (3158 & 3710.7 col/100ml), high TSS (7.75 – 10.8 mg/L), turbidity (9.7 – 14.4 NTU), and NO_x (0.61 – 0.69 mg/L; Fig. 6). There was also a geographical gradient starting at east cobb sites in the lower left, then through west cobb sites in the center, through to the Paulding sites in the top right.

The initial macroinvertebrate family's relative abundance MDS had a 2-dimensional (2D) stress of 29.67%. Seven trials were run on relative abundance until 7 families were left and the s-stress was 16.8%. The biological relative abundance MDS shows a majority of the sites fall within the left quadrants due to *Chironomidae* abundance values which were being separated on the x axis because their x values were different (Fig. 7). Within those sites on the left, there were further separations due to *Hydropsychidae* abundance on the y axis due to their y values. There was also a few sites in the lower right quadrant with medium to low *Chironomidae* and high abundance of *Philopotamidae. Chironomidae* abundance appeared to be the primary factor for distinguishing sites on the x-axis and relative abundance of *Philopotamidae* and *Hydropsychidae* were primary contributors to differences in sites along the y-axis (Fig. 7).

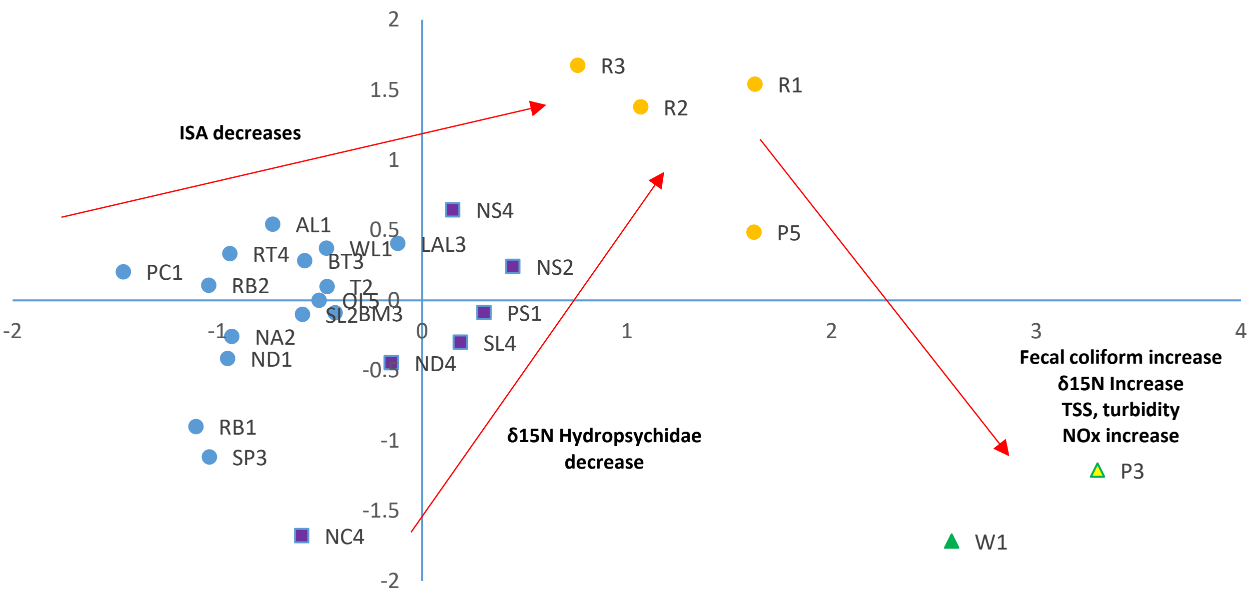


Figure 6. Multidimensional Scaling output for WQ variables at 27 Cobb and Paulding county sites. Arrows indicate a general trend of increasing ISA as you move from right to left on the graph. These arrows are for interpretive purposes and are not statist

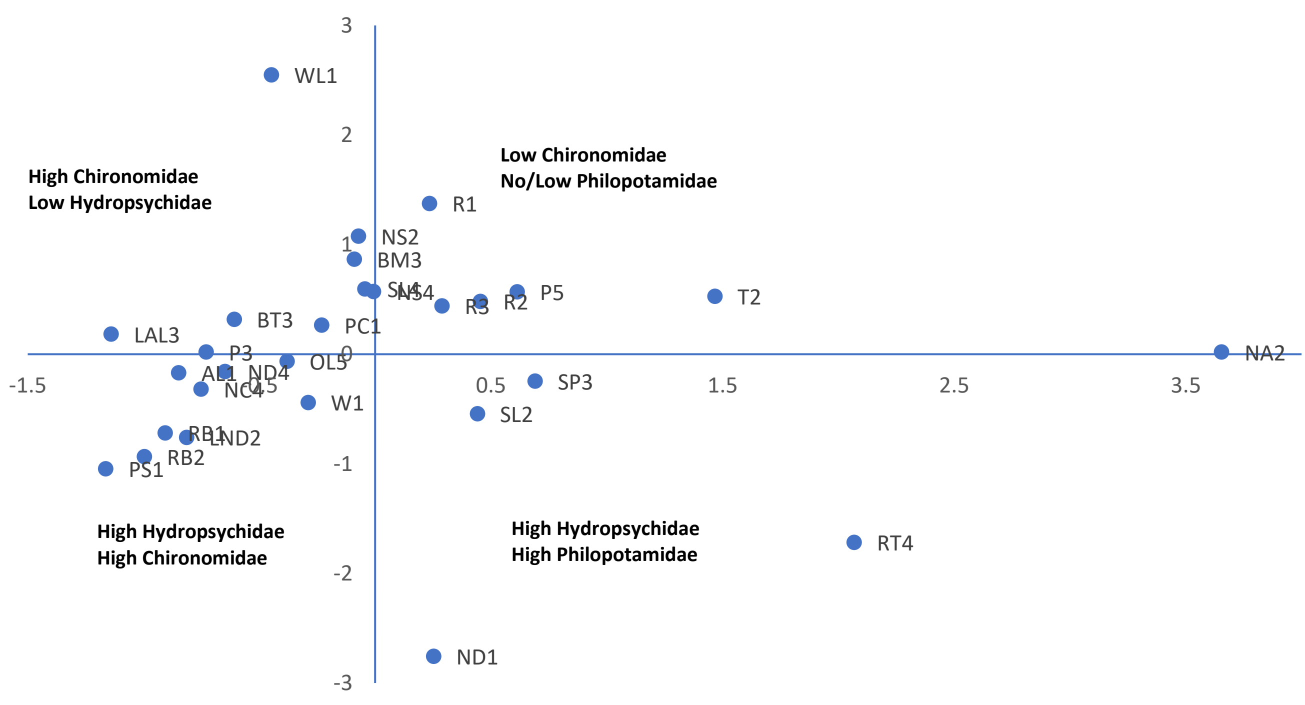


Figure 7. Multidimensional scaling results for relative abundance from 27 sites using resent and historical data from Cobb and Paulding Counties.

We also noticed similar groupings of sites in both graphs suggesting the change that was happening in ISA and d15N was associated with a change in biology. (P3 and W1; R1, R2, R3 and P5; NS2, NS4, and SL4; OL5, ND4, AL1, and BT3; Fig. 6 & 7).

Community level effects of urbanization

Six sites were chosen and sampled more intensively to examine community-level patterns in $\delta^{15}N$ values of FFG and detect potential differences in trophic dynamics between rural and urban sites. The three Paulding sites were considered rural (R3, R2, P3; ISA ≤ 5%) based on ISA (Smucker et al., 2018), and the three Cobb county sites were urban (ND1, ND4, SP3; ISA > 20%). The macroinvertebrate count for all 6 sites produced 34 families total from 12 different orders. Collector filterers had the highest relative abundance in urban sites and collector gatherers had the highest relative abundance in rural sites.

FFG	Urban	Rural
CF	0.09 ± 0.12	0.03 ± 0.04
CG	0.04 ± 0.09	0.06 ± 0.13
Gen	0.01 ± 0.01	0.02 ± 0.03
PR	0.005 ± 0.02	0.01 ± 0.01
SC	0.04 ± 0.07	0.02 ± 0.03
SH	0.01 ± 0.01	0.03 ± 0.05

Table 2. Average Relative abundance (± standard deviation) of invertebrates by functional feeding group from the 6 community level sampling sites.

Rural sites had a family richness of 23.7 +/- 2.1, compared to 16.3 +/- 1.2 for urban sites (t = 0.011; $p < 0.05$). Average $\delta^{15}N$ for the two rural Raccoon creek sites is relatively low (2.44 ±2.59) compared to the third rural site, P3 (11.43 ± 3.96) and all three urban sites (6.30 ±3.34; Table 3). Diptera was found with high frequency throughout all 6 sites (Fig. 8). *Hydropsychidae* was abundant at ND4 and SP3 (Fig. 8), and *Cyrenidae*, represented by *Corbicula fluminea,* was also abundance at ND4 (Fig .8).

Site	Family Evenness	Family Richness
ND1	0.14	17
ND4	0.22	15
SP3	0.23	17
P3	0.31	22
R2	0.16	23
R3	0.14	26

Table 3. Family richness (number of families in sample) and Simpson's Index of evenness for all macroinvertebrates collected during the EPD protocol sampling.

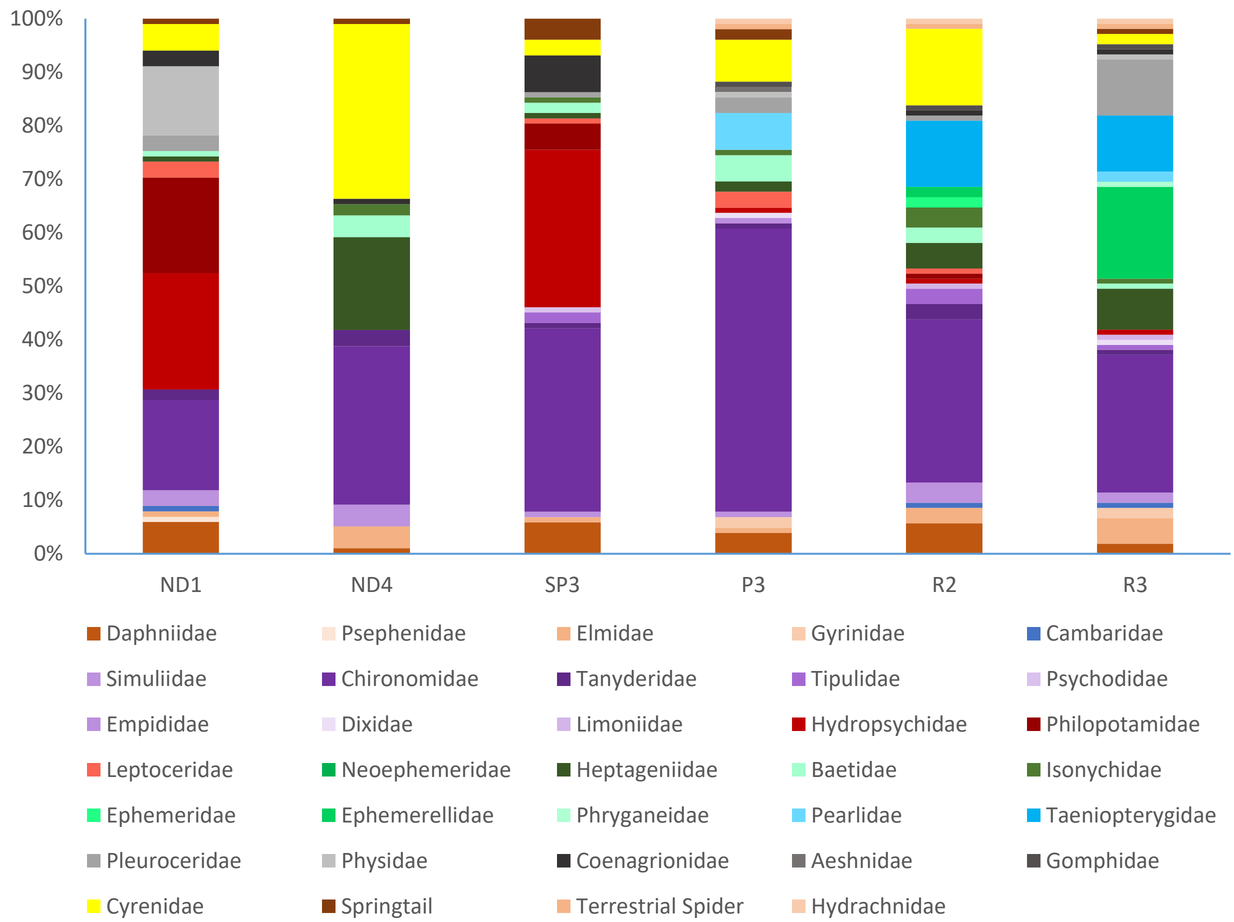


Figure 8. Relative abundance for EPD invertebrate count from the 6 sites sampled for community level examination.

When calculating trophic level using $\delta^{15}N$ values leaf tissue of the American Sycamore, *Platanus occidentalis*, was used as the primary producer for 5 of the 6 sites. At site R3, the primary producer was Tulip Poplar, *Liriodendron tulipifera*. Calculated trophic levels for all FFGs (except OM) are higher than trophic levels associated with feeding modality reported in the literature for all sites except R2. (Table 4). Predators at R2, P3 had lower $\delta^{15}N$ values than consumers. Primary producers had higher $\delta^{15}N$ values than all consumers at ND4 (2.8) and SP3 (4) and PP (4.1) had higher values than PR (3.66) at P3 (Table 4). Average calculated trophic level for OM remained lower than literature trophic level except for ND1 where calculated trophic level (1.17) is slightly above literature trophic level (1). P3 has the highest calculated trophic levels across all FFG when compared to the other 5 sites except for CPOM.

Site	TL	PR	CF	Gen	CG	SC	SH	PP	CPOM
ND1	**Calculated**	3.81	3.41	3.7				3.2	1.17
	Literature	3	2.5	2.25				1	1
ND4	**Calculated**	2.77	2.66	1.89	2.57	2.71		2.84	0.3
	Literature	3	2.5	2.33	2	2		1	1
SP3	**Calculated**	3.54	3.37		3.17			3.99	0.59
	Literature	3	2.5	2.25	2	2	2	1	1
R2	**Calculated**	1.33	1.39	0.77	1.3	0.91		1.24	0.73
	Literature	3	2.5	2.44	2	2	2	1	1
R3	**Calculated**	3.56	3.49	2.91		2.93	2.03	2.61	0.85
	Literature	3	2.5	2.2	2	2	2	1	1
P3	**Calculated**	3.66	3.66	4.25	4.18	4.38	4.59	4.08	0.23
	Literature	3	2.5	2.5	2	2	2	1	1

Table 4. Calculated and Literature trophic levels for all FFG across all 6 community level sampling sites. Leaves of Sycamore (ND1, ND4, P3, R2, SP3) and Tulip poplar (R3) collected from within the streams were used as trophic level 1 for calculated trophic level (Ramírez & Gutiérrez-Fonse, 2014).

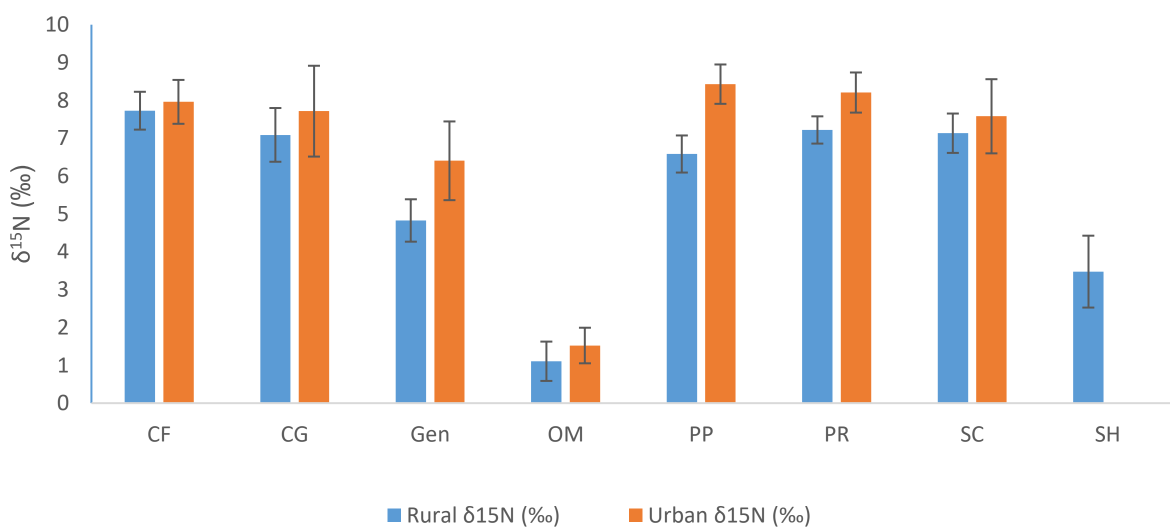


Figure 9. Average Urban $\delta^{15}N$ versus average rural $\delta^{15}N$ for all collected invertebrate, periphyton, and plant FFG across all 6 community level sampling sites.

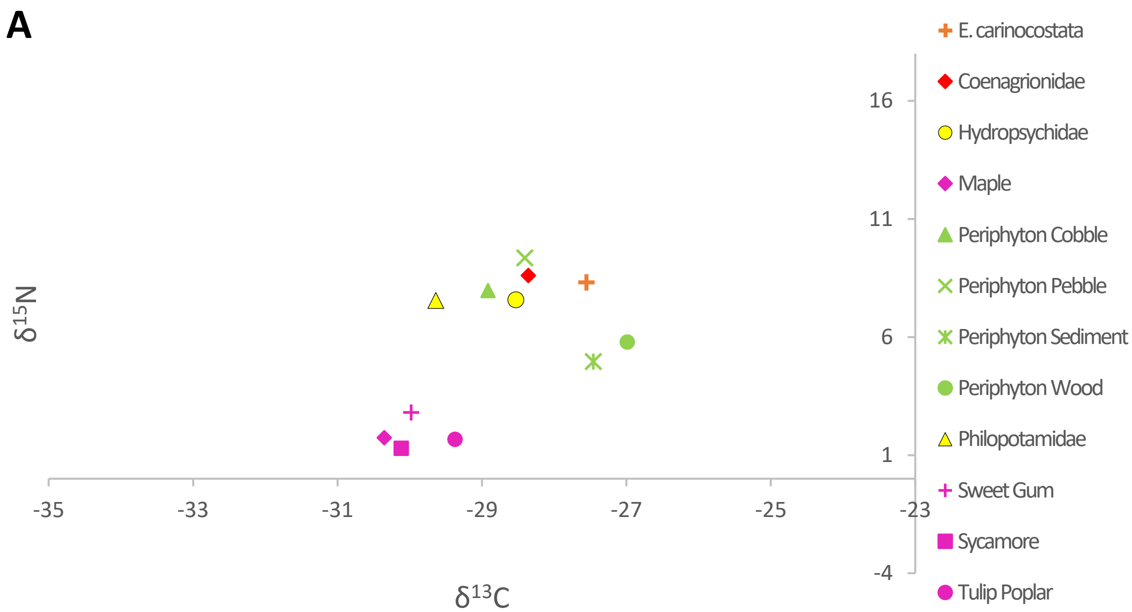
Averaged urban $\delta^{15}N$ is higher across all FFGs. Average $\delta^{15}N$ was higher at urban sites for CF (3% higher), CG (8%), generalists (25%), OM (27%), PP (22%), PR (12%), and SC (6%)

than rural sites (Fig. 9). When comparing $\delta^{15}N$ from R3 and R2, which have the lowest ISA (0.81% and 0.63%, respectively), to the other 4 sites that were sampled, consumers tended to have lower averaged $\delta^{15}N$ values (R2= 0.75 – 4.70‰; R3= 0.75 – 4.70‰). The consumers in P3 had greatly elevated $\delta^{15}N$ values (11.95 – 14.38‰), despite P3 having low ISA (3.61%), and all 3 urban sites (6.88 – 8.51‰) have elevated $\delta^{15}N$ values (Fig. 9).

When examining isotope biplots, periphyton on cobble and pebble had higher $\delta^{15}N$ values than periphyton on wood and sediments in all sites except P3. Periphyton on cobble and pebble had higher trophic levels than consumers and predators at ND4 and SP3. At ND1, ND4, SP3, and P3 the OM tends to fall multiple trophic levels below most consumers with the exception of Tipulidae at ND4 (Fig. 10A- B; Fig. 11A- B; Fig. 12A-B).

When examining trophic relationships based on fractionation of C (0.5) and N (2.6). In P3 Cambaridae (Gen) fall in the trophic position to be consuming periphyton from sediment (PP) and multiple predators consuming Cambaridae (Gen) and Corbiculidae (CF; Fig. 11B). In R2 Perlidae (PR) could potenially be consuming Philopotamidae (CF) and Ephemeridae (CG) and Gyrinidae, Corydalidae, and Gomphidae (PR) could potentially be comsuming Tipulidae (Gen). It also looks as though Corbiculidae (CF) and Ephemeridae (CG) are consuming periphyton from sediment (PP), tulip poplar (OM), or preiphyton from wood (PP; Fig. 12A). In R3 Tipulidae and Peltoperidae look as though they are consuming Sweet gum (OM) or White Oak (OM) and Various predators are consuming Pteronarcyidae (SH), Tipulidae (Gen), and Peltoperlidae (SH). There seem to be no clear trophic relationships found in ND1, ND4, or SP3 (Fig. 12B).

In general PR tend to be at the same trophic level as some consumers. For example, ND1 PR were even with CF and Gen, ND4 PR tend to fall in the same trophic level as SC, CF, and Gen, SP3 tends to have PR at the same trophic level as GG and GF, P3 tends to have PR at the same trophic level as SH, CG, SC, GF, and Gen, R2 tends to have PR tropic levels even with some SC, Gen, CF, and CG, and at R3 tends to have a few PR even with some Gen and CF (Fig. 12A- B).



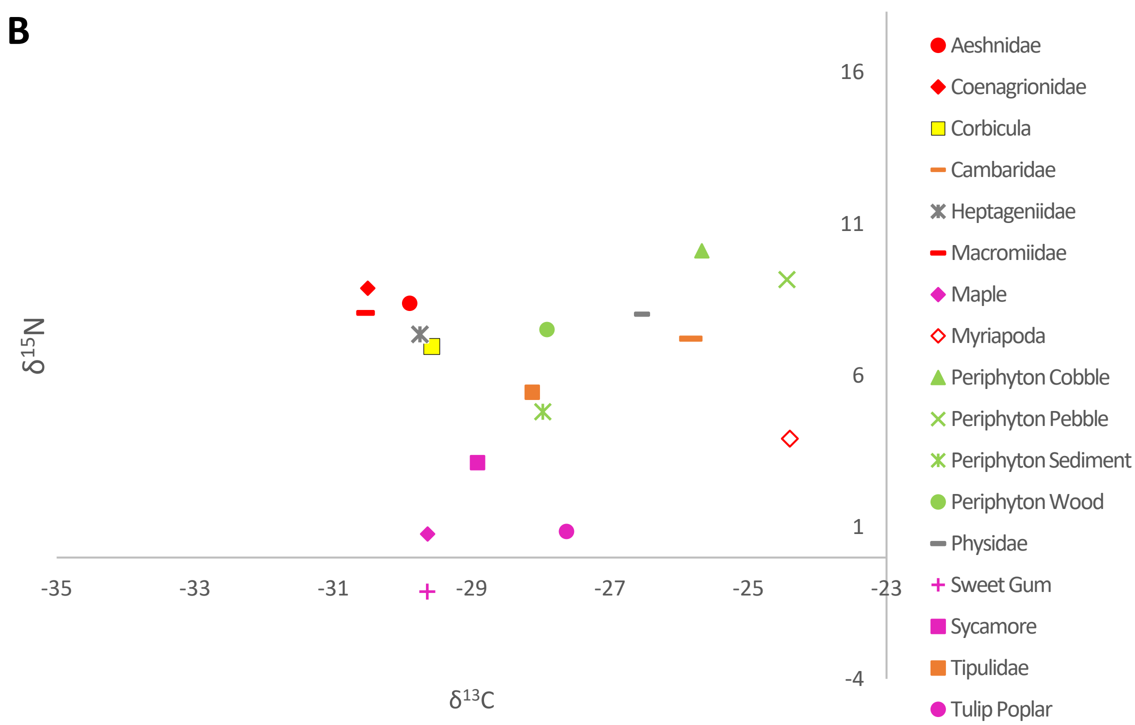


Figure 10. δ15N and δ13C values for food web isotopic bioplot color coded by functional feeding group. A. Upper Noonday creek (ND1); B. Lower Noonday creek (ND4). Predators are red, generalists are orange, collector filterers are yellow, collector gatherers are blue, scrapers are grey, shredders are black, organic matter (CPOM) is fuchsia, primary producers (periphyton) is green.

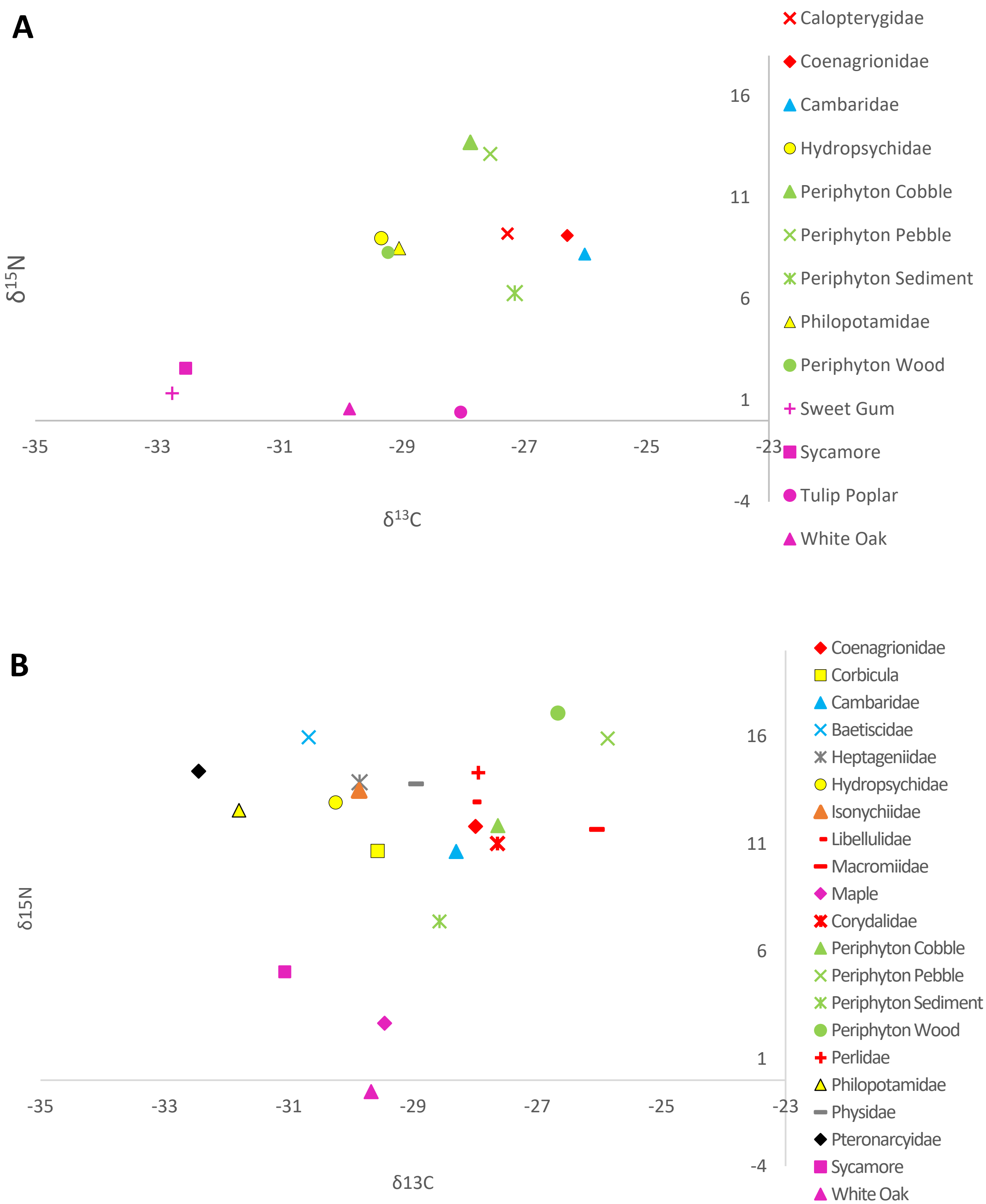


Figure 11. δ15N and δ13C values for food web isotopic bioplot color coded by functional feeding group. A. Sope creek (SP3); B. Middle Pumpkinvine creek (P3). Predators are red, generalists are orange, collector filterers are yellow, collector gatherers are blue, scrapers are grey, shredders are black, organic matter (CPOM) is fuchsia, primary producers (periphyton) is green.

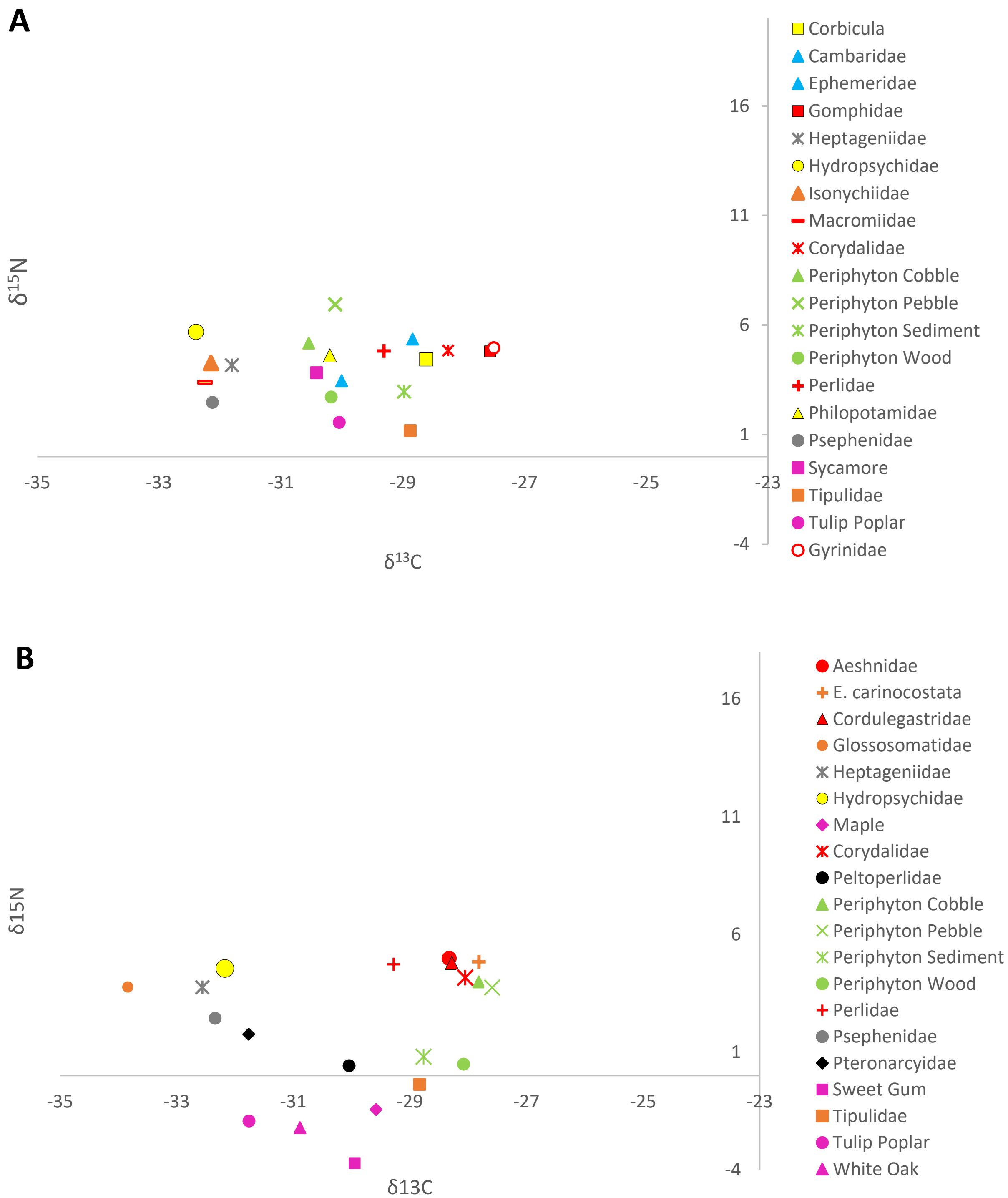


Figure 12. $\delta^{15}N$ and $\delta^{13}C$ values for food web isotopic bioplot color coded by functional feeding group. A. Middle Raccoon creek (R2); B. Upper Raccoon creek (R3). Predators are red, generalists are orange, collector filterers are yellow, collector gatherers are blue, scrapers are grey, shredders are black, organic matter (CPOM) is fuchsia, primary producers (periphyton) is green.

Discussion

Broad Spatial Sampling

Urban and agricultural land use is often associated with elevated $\delta^{15}N$ values for stream communities due to a shift in biological, chemical, and physical characteristics of the stream (Smucker et el., 2018). Alteration of the stream and the surrounding areas directly and indirectly influence water quality that typically leads to a decrease in riparian vegetation, flashy hydrology, channel incision, scouring of substrate, siltation, organic matter changes, and nutrient concentration fluctuations, ultimately resulting in a decrease in biodiversity and changes to the food web (Booth et al., 2016; Meyer et al., 2005; Walsh et al., 2005; Kim et al., 2016). Our findings support the evidence that an increase in urbanization, as estimated by ISA, influences stream health and food web structure.

Various water quality variables were negatively correlated with ISA and LC/LU often illustrating linkages between landscape processes and stream conditions. These variables (BOD, turbidity, TSS, total phosphorus) are likely related to erosion, since the combination of flash hydrology, loss of riparian vegetation, and land use change (deforestation) often destabilize stream banks and exposes soil that has accumulated NPS pollutants (Wang et al., 2020; Collier et al., 2005). Despite many studies suggesting that most WQ variable values increase due to an increase in ISA, with the exception of DO which typically increases in less impacted streams (Ferreira et al., 2016; Liu et al., 2018; Kim et al., 2016), we only found a significant positive correlation with ISA and conductivity. Instead our study found significant negative correlations with ISA and fecal coliform, turbidity, total phosphorus, and BOD (Fig. 5), which could be a result of agricultural land use, low density residential land use, active land use changes (construction), or road and bridge NPS runoff.

Fecal coliform was positively correlated with erosion-associated variables (BOD, TSS, turbidity, and total phosphorus) suggesting that the NPS runoff contained fecal coliform. A high presence of E. coli or other fecal coliforms is often associated with NPS discharge of untreated sewage associated with urban or agricultural sources such as, wildlife, livestock, sewer or septic systems, and faulty WWTPs (de Oliveira et al., 2017; Waite et al., 2019; Collier et al., 2005). Our study found a negative correlation between fecal coliform and ISA, which could be a result of an increase in wildlife, livestock waste, or an increasing use of septic systems in rural areas, which have the potential for soil contamination and sewage backups.

There were only a few significant correlations between ISA and WQ, but there were stronger correlations when comparing LC/LU and WQ variables. Impervious surface area is a useful metric of urbanization that attempts to capture the net effect of all the changes occurring during urbanization, but it does not fully capture the effects that LC/LU plays on streams (Higgisson et al., 2019; Collier et al., 2015; Tufekeioglu et al., 2020). Land cover land use percentages provide multiple categories of land use and quantifies vegetation, water, natural surfaces, and anthropogenic features, and when used over time it can show how land changes as a result of interactions between humans and the physical environment (mrlc.gov).

Various erosion-related WQ variables (BOD, Fecal coliform, turbidity, and total phosphorus) were negatively correlated with developed land and positively correlated with forested land cover and cultivated crops. The negative correlation between BOD and developed open space and developed low intensity could suggest a decrease in organic matter inputs and retention in streams in developed areas.

Forested areas were positively correlated with total phosphorus, turbidity, fecal coliform, and BOD, which contradicts findings by previous studies (Collier et al., 2015; Ferreira et al., 2016; de Oliveira et al., 2017), which found an increase in BOD, nitrogen, phosphorus, elevated turbidity, TSS, and fecal coliform in developed land use urban streams. The negative correlation with developed LC/LU and BOD, fecal coliform, and TSS WQ variables could be due to more efficient sewer systems or more efficient WWTP practices. Turbidity was positively correlated with barren land and hay/pasture suggesting that the presence of grazing livestock could influence soil exposure and soil destabilization, and potential lack of riparian vegetation. Unfortunately, not all water quality parameters adequately characterize the conditions of the stream over the long term. For example, instantaneous quarterly measurements of NO_x do not accurately reflect the day to day supply of NO_x in the stream over longer periods and TSS, turbidity and fecal coliform tend to increase during rain events (Chalise and Kumar, 2020; Shishaye et al., 2020).

When comparing our WQ variables to our $\delta^{15}N$ values from the three representative macroinvertebrate families, we found no significant correlations with $\delta^{15}N$ values of *Baetidae*; however, there were significant positive and negative correlations with $\delta^{15}N$ values of *Hydropsychidae* and *Heptageniidae*, respectively. *Hydropsychidae* $\delta^{15}N$ values had significant positive correlations with NO_x and Conductivity, which were linked to an increase in urban runoff. Dissolved oxygen was negatively correlated with $\delta^{15}N$ values of *Hydropsychidae*; this could possibly be related nutrient loading and bacterial activity driving down O, or it could be related to the habitat. *Hydropsychidae* are likely to inhabit riffle areas with hard stable structures available for them to attach to, which also typically have higher amounts of DO (Liu et al., 2020). We expected collector filterers to have been affected by urbanization and nitrogen inputs the most because the collector filterers essentially consume particles that float downstream and take up bacterial laden materials that take up dissolved WW effluent. Some of that effluent is going to have particles in it already, so there may be high $\delta^{15}N$ values in their food and in the resuspended sediments where in situ denitrification may already be happening.

Hydropsychidae $\delta^{15}N$ values also had significant positive correlations with developed open space and developed low intensity and a significant negative correlation with forested land use. *Heptageniidae* $\delta^{15}N$ values were significantly positively correlated with ISA, developed LC/LU, and negatively correlated with forested LC/LU. Heptageniidae are classified as scrappers and typically consume periphyton from hard, large, stable substrates (i.e. cobble, pebble, wood; Ramírez and Gutiérrez-Fonseca, 2014). Our cobble, pebble, and wood periphyton $\delta^{15}N$ values were elevated at many of our sites, suggesting the presence of enriched N sources in the streams that ultimately become incorporated into invertebrate tissues.

These correlations suggest that LC/LU influences $\delta^{15}N$ values possibly due to a shift in the food web or a shift in the way the food web is functioning. An increase in $\delta^{15}N$ enriched algal or periphyton consumption and fractionation by macroinvertebrate consumers causes an uptake of higher $\delta^{15}N$ dissolved nutrient sources from the water column associated with wastewater or agricultural inputs. Another possibility is that the microbial food web is cycling differently, so that there is an increase in net $\delta^{15}N$ as recycling within the periphyton community is happening. The food web of microbes consumes each other, which leads to trophic fractionation, and leads to an increase in the net $\delta^{15}N$ value. These increases in $\delta^{15}N$ values in urban streams were potentially an indirect result of loss of riparian cover and organic matter retention (Razali et al., 2018; Pascoal et al., 2005). Correlations with developed open space and low intensity land use could be the result of an increase in lawn grasses and residential homes, which makes these streams more susceptible to NPS runoff and erosion due to a decrease in riparian vegetation.

The WQ MDS indicated that ISA was an important variable for distinguishing sites in ordination space. The ordination shows a division between high and low ISA on a diagonal axis that stretches from high quadrant down through the low quadrant; this separates Cobb county sites and Paulding county sites, except for W1 near downtown Dallas, GA, which has a similar ISA to the Cobb county sites. The sites located in the top right quadrant (R1, R2, R3) have low ISA and also have relatively lower $\delta^{15}N$ *Hydropsychidae* values (< 5.5 ‰), which separates them even further from the rest of the sites. The sites with higher ISA and relatively intermediate $\delta^{15}N$ values (P5, NS4, NS2, PS1, SL4, ND4, SL2, BT3, AL1, RT4, RB2, PC1) are located higher on the y-axis than the sites with high ISA and high $\delta^{15}N$ (NA2, ND1, BM3, OL5, T2, RB1, SP3, NC4, WL1, LAL3; Fig. 6). Sites P3 and W1 were in the bottom right quadrant and were potentially falling out differently due to both having a higher turbidity, TSS, and fecal coliform when compared to the rest of the sites, which could be the result of high levels of soil instability, lack of riparian buffer, or an input of untreated sewage in both, potentially from the urban land use surrounding W1 and the highly agricultural watershed of the P3 site. Cobb and Paulding County sites being separate from each other on the MDS suggests that ISA and wastewater infrastructure play a significant role in stream characteristics.

The Paulding sites generally had lower ISA and a range of elevated and low $\delta^{15}N$ values; however, they also had high fecal coliform counts, which contradicted results found by Collier et al. (2015) and de Oliveira et al. (2017), in which elevated fecal coliform concentrations were associated with rivers impacted by anthropogenic inputs and high developed LC/LU. The observed pattern could be the result of rural areas having larger abundance of septic systems, livestock, or wildlife waste inputs, which can contribute waste products into NPS runoff and get carried into streams during rain events.

The biological relative abundance MDS shows a majority of the sites fall within the left quadrants due to *Chironomidae* abundance (Fig. 7). Located at the top of the grouped high *Chironomidae* sites are sites that also had high *Hydropsychidae* abundances (R1, R3, R2, P5, NS4, NS2, SL4, BM3, SP3). There are a few loosely associated sites in the lower right quadrant where the sites had medium to low *Chironomidae* and high abundance of *Philopotamidae* (SL2, SP3, RT4, NA2, ND1). *Chironomidae* abundance appeared to be the primary factor for distinguishing sites on the y-axis and relative abundance of *Philopotamidae* and *Hydropsychidae* are primary contributors to differences in sites along the x-axis. The Paulding sites that were separated in the WQ MDS, P3 and W1, tend to be more biologically similar to many of the Cobb sites due to their high *Chironomidae* abundance.

Pollution tolerances can change depending on species, pollution type, and geographic location, however, the family *Chironomidae* is listed as tolerant across many pollution tolerance indices and has relatively high abundances across all but 2 sites (Merritt et al., 2008; Lenat et al., 1993; and Rios-Touma et al., 2013). Unfortunately, *Hydropsychidae*, *Leptoceridae*, and *Philopotamidae* pollution tolerances vary depending on the species and type of pollution (Merritt et al., 2008; Lenat et al., 1993; and Rios-Touma et al., 2013) but our study suggests that all three are relatively tolerant to sediment loading and wastewater. There were no shredders in our MDS analysis. Although some species of *Tipulidae* are known to be shredders, however, we did not identify to species and therefore identified the family as generalists (Ramírez and Gutiérrez-Fonseca, 2014). The majority of the shredders were from the nutrient and habitat sensitive order Plecoptera and none of those families were present in any of the Cobb county sites. *Hydropsychidae* and *Chironomidae* typically respond to stream degradation with sublethal morphological deformities. *Chironomidae* exhibits mouth part deformations when exposed to sedimentation and high levels of heavy metals (Thani and Prommi, 2017). *Hydropsychidae* also exhibits gill and tracheal deformations when high levels of organic compound pollution are present in the stream. Prommi and Thamsenanupap (2013) suggest that filter feeders like *Hydropsychidae* are more exposed to pollutants in seston, flowing water, and in the organic matter accumulated in riffle microhabitats, which could also explain why *Philopotamidae* also exhibit the same pattern in the MDS, unfortunately, not much information is available on *Philopotamidae* responses to water quality variables (Prommi and Thamsenanupap, 2013). *Simuliidae* abundance is dependent on hydrological conditions, riparian cover, and streambed structures, however their preferences are very species dependent, some species prefer shaded streams, some prefer stable gravel substrate or woody debris, and some prefer turbulent flow patterns, therefore, finer taxonomic resolution is needed to determine what factors played a role in their abundance in these streams (Lautenschlager and Keil, 2005).

There were similarities when comparing both biological MDS and WQ MDS. For example there were four groups of sites that were in close proximity to each other in both graphs (P3, & W1; R1, R2, R3 & P5; NS2, NS4, & SL4; and OL5, ND4, AL1, & BT3; Fig. 6 & 7). This suggests that the change that was happening in ISA and $\delta^{15}N$ was associated with a change in biology. The proximity of those sites to each other indicate that the same WQ processes and biological communities are present at both sites.

Another similarity is that both graphs form a slight geographical gradient as well. However, the WQ MDS has more of a gradient, starting with the east Cobb sites and high ISA sites (RT4, RB1, RB2, NA2, ND1, ND4, SL2, SL4, NC4) on the lower left side of the graph that transitions into the west Cobb sites and intermediate ISA sites (AL1, BT3, T2, OL5, BM3, LAL3, NS4, NS2, PS1) in the center and the far west Paulding county and low ISA sites (R3, R2, R1, P5, P3, W1) on the right side of the graph. The biological graph has less of a gradient and has more outliers but was still following the same basic geographical transition. Some of the east cobb sites (ND4, NC4, RB2, RB1, LND2) are at the bottom left portion of the graph, which then transitions into the majority of the west Cobb sites (PC1, AL1, BT3, OL5, BM3, LAL3, NS4, NS2) located in the center left part of the graph. The gradient then transitions into the far west Paulding sites (R3, R2, R1, P5) to the right top of the graph. There are a few exceptions, for example Paulding sites, P3 and W1, both fall directly in the center of the east and west cobb sites. The east Cobb site, WL1, is located at the top of the graph, and east Cobb sites, SL2, SP3, RT4, ND1, NA2, are all located in the bottom right quadrant of the graph. The similarities suggest that a transition in WQ and biological relative abundance is happening as urban sprawl happens. On the other hand, the differences suggest a decoupling between water quality and community structure, subacute effects of water quality changes, a failure to capture important stream characteristics that influence community structure, or it could be simply that the biology has not responded to a change in WQ yet. Inclusion of physical stream characteristics and finer-scale examination of stream taxa could help clarify associations at these sites. Assessing stream health with invertebrate indicators, requires an observable biological response to WQ degradation, such as a decline in abundance. However, the MDS analyses show that WQ variables and the invertebrate response to degradation do not always manifest in the same way or at the same time, making it a challenge to identify causative associations. The biological patterns that occur in parallel to the water quality changes, suggest that these WQ variables are driving the changes in biology.

Our MDS and the positive correlations between developed land use and $\delta^{15}N$ of *Hydropsychidae* and *Heptageniidae*, the negative correlation with forested areas, and the patterns seen in the WQ MDS support the notion that high levels of developed land use and agricultural land use increase and correspond with an increase in the $\delta^{15}N$ values of macroinvertebrates. The negative correlations between developed land and BOD, fecal coliform, turbidity, and total phosphorus and the positive correlations with forested land suggest other factors may also be influencing water quality, particularly in less urban sites. Positive correlation between pasture and turbidity, as well as positive correlations with cultivated crop land use and TSS, fecal coliform, turbidity and total phosphorus suggest that agricultural land use plays a large role in water quality. Both an increase in ISA and agricultural land use can result in elevated $\delta^{15}N$ values either by means of human or animal driven waste contributing to NPS runoff getting carried into streams altering nutrient concentrations and often results in a negative effect on streams and biodiversity (Mullin et al, 2008; Bogdal et al., 2019; Smucker et al., 2018). As the primary producers become enriched with these elevated concentrations and get consumed, fractionation increases $\delta^{15}N$ values as they move up the food web into consumers and to predators. This combined with the change in hydrology and an increase in erosion associated with a decrease in riparian vegetation and soil destabilization, sensitive species may decrease in abundance and tolerant species become more prevalent, which often alters the food web and diet sources further (Rawi et al., 2014; Adu and Kumarasamy, 2018).

Community level sampling

Urbanization often leads to a change in resource and habitat availability with the potential to change energy flow throughout the food web. Changes in the community structure were apparent in our intensively sampled sites. Urban streams had lower family richness, suggesting urbanization is responsible for loss of richness and biodiversity (White et al., 2014). Agricultural land use also appeared to play a large role in biodiversity. For example, P3 has low ISA and a relatively high family richness when compared to the three more urban sampling sites (ND1, ND4, and SP3), but it also has elevated periphyton and macroinvertebrate $\delta^{15}N$ values. The land use surrounding P3 is predominantly forested and has relatively low pasture percent (5.10%); however, there is an abundant amount of livestock in this watershed, including roughly 225,059 chickens, 281 cows, 50 horses, and 56 sheep (wikiwatershed.org). Livestock waste and microbial processing could be causing elevated $\delta^{15}N$ values at P3, even though the watershed is associated with low ISA, low pasture land use, and relatively high family richness. Although family richness remained relatively high at P3, agricultural land use may be contributing to an increase in pollution tolerant families. The invertebrate community at P3 had a higher abundance of the order Diptera (a widely studied pollution-tolerant order) when compared to the other rural sites (R2 and R3). The mechanisms that link land use and changes in WQ variables to loss of biodiversity and changes in relative abundance are not fully known, but some studies have found that an increase BOD, total nitrogen, and total phosphorus are associated with loss of macroinvertebrate biodiversity. Our study could not corroborate this association, possibly due to infrequent, quarterly sampling of the WQ variables (Lee at al., 2020). However, our results are consistent with other studies that have documented higher biodiversity and community stability in rural, non-agricultural streams, which tend to have fewer disturbances and less physical alterations (Walsh et al., 2005; White et al., 2014; Liu et al., 2018).

Food web structure also differed between urban and rural sites. There was a complete absence of shredders at the urban sites during our sampling. The apparent loss of shredders could result from a change in the riparian vegetation. Higher lignin:N content in leaves can hinder microbial colonization and make it harder for invertebrates to process (Sena et al., 2020). If changes in riparian vegetation lower nutritional quality of leaf litter inputs, shredders may experience nutritional deficiencies that threaten persistence. However, changes in other physical, biological, and water quality characteristics of urban streams could also cause shredder populations to decline.

Isotopically derived trophic level calculations of FFGs did not always match expected trophic levels from the literature suggesting that the leaves that we used were not the food source. The actual food source is possibly another leaf with a higher $\delta^{15}N$ value. The average of all the leaf litter from the site should be used to reduce the chance of selecting an outlier. When the same species of tree was used as the base of the food web, trophic level calculations for similar consumers were lower at R2, R3, and ND4 and elevated a ND1, P3, and SP3. Known predators commonly were assigned to similar trophic levels as periphyton, filterers, and other lower-level consumers. This result could indicate omnivory by predators, elevated values in periphyton from internal microbial cycling of N and consumption of that periphyton matrix by scrapers. Generalists changed in a consistent way across sites due to opportunistic feeding habits. However, if different species from the same family were collected at different sites, the observed differences in FFGs could be a result of species differences as opposed to changes in feeding behaviors. Many sites were completely missing trophic level 2 possibly due to a missing food source that we did not sample.

The CPOM trophic level values did not vary greatly between sites, but $\delta^{15}N$-based trophic levels were elevated for many consumers at some sites. The variation in trophic levels of consumers despite unchanging values of CPOM indicates elevated ^{15}N values were driven by aquatic processing of N rather than terrestrial processes affecting riparian vegetation and allochthonous organic matter sources. Minor variations (0-0.5) in calculated trophic levels for OM from literature trophic levels could be due to natural variation in the relative availability of ^{15}N and ^{14}N in the field, but the larger changes (>0.5) in consumer and predator calculated trophic levels also indicate that there could be a change in diet.

The $\delta^{15}N$ values were lower at two of the rural sites (R2 & R3) when compared to streams surrounded by urban (ND1, ND4, & SP3) and agricultural land use (P3). Upper Raccoon has low ISA and low agriculture impacts, and the isotope biplots depict a standard pattern of trophic fractionation for consumers in the food web. The $\delta^{15}N$ values ranged from -3 to 6‰, and placement of the consumers depicts OM consumption for *Peltoperlidae* and *Tipulidae* organisms, which is supported by the calculated trophic levels for these organisms and the average SH calculated trophic level. However, potential food sources for *Pteronarcyidae, Heptageniidae, Psephenidae, Hydropsychidae,* and *Glossosomatidae* food sources were not sampled given the more negative $\delta^{13}C$ values of these organisms than the OM we sampled (Fig. 12B).

Trophic positioning starts becoming slightly altered at R2 (downstream of R3) even though it still has low ISA and low $\delta^{15}N$ values. There was a slight change in the terrestrial isotope values of organic matter, and the positioning of consumers does not follow expected patterns based on standard trophic fractionation. Perhaps the presence of livestock in the surrounding landscape could be influencing the available food sources by altering the riparian vegetation via grazing or by conversion of forest land into pasture. However, some elements of the stream food web did not change. The $\delta^{15}N$ values of primary consumers were 2.6 – 3.4‰ units above the OM, and predators were 2.6 – 3.4‰ above the primary consumers. *Tipulidae*, *Ephemeroptera*, and *Perlidae* were in similar positions as was observed at R2 (Fig. 12A). The shift towards negative values on the C axis indicated that an important food source was missed in our sampling, such as *Podostemum* or *Cladophora*, which typically provide a stable habitat for invertebrate communities in high quality streams and are more negative in $\delta^{13}C$ (Tinsley, 2012).

The Noonday Creek sites (ND1 and ND4) have elevated OM $\delta^{15}N$ values relative to Raccoon creek. The consumers' $\delta^{13}C$ values were shifted 2 - 5‰ to the right for *Tipulidae*, *Cambaridae*, and *Hydropsychidae*, which shows a change in carbon sources. This change in carbon sources could potentially reflect inputs from C_4 plants (> -19‰) such as Bermuda grass, a very popular turf grass. There is also a shift from -4 – 6‰ to 4 – 11‰ on the y axis for all periphyton, consumers, and predators, indicating either an enriched source of N inputs from wastewater or enhanced in situ denitrification (Fig. 10A – B). There is also a lack of low $\delta^{13}C$

values, and consumers and predators having similar calculated trophic levels suggest a food source was not sampled. No Plecoptera were found in any of the Cobb sites. *Hydropsychidae* had $\delta^{13}C$ values that were 4.5‰ higher in ND1 when compared to R2 and R3 and were completely missing from ND4.

The Sope Creek (SP3) and Pumpkinvine Creek (P3) sites both seems to exhibit similar trophic changes even though SP3 is urban and P3 is rural. Both had elevated $\delta^{15}N$ values ranging from -1 - 17‰. The $\delta^{15}N$ for periphyton from cobble and pebble substrates were also elevated, which could be driven by the microbial food web, in which recycling of N within the periphyton community could cause elevated $\delta^{15}N$ values in the periphyton which then get transmitted up through the food web. None of the other organisms track the change in periphyton on cobble and pebble suggesting this is not an important food source for the sampled consumers. Instead, consumers seem to be responding to periphyton in sediment possibly suggesting that it is a more importance source of energy for higher trophic levels. The consumer calculated trophic levels are all elevated in both SP3 and P3 with predators having the same trophic level or below consumers, suggesting a missing food source for the predators. There is also a loss of abundance and a decrease in diversity, as was also observed at the Noonday sites. The typical trophic relationships are not as clearly defined which suggests a shift in food sources and, perhaps, a shift towards opportunistic feeding and omnivory (Price et al., 2018). Changes to the trophic dynamics of stream food webs may be potentially an important consequence of human impacts in streams that could influence the ability of species to persist. If an organism is not able to shift towards omnivory, then the likelihood of its continued survival could potentially decrease. A disruption of energy flow in the systems could possibly be contributing to species loss associated with urbanization, but the mechanism for such changes is still unclear (Fig. 11A- B).

The sites with high ISA, ND1, ND4, and SP3, are very similar when comparing LC/LU. They have similar percentages of developed land use and forested land use with a relatively minimal percentage of shrubs, herbaceous land, and hay/pasture. They all have elevated $\delta^{15}N$ values and decreased family richness but the SP3 $\delta^{15}N$ values are higher than ND1 and ND4. Urban NPS pollution, leaky sewer lines, or WWTP effluent probably contributed to the elevated values. When examining the Raccoon creek sites, they have similar percentages of developed land use as well, however R3 has higher forested land use and R2 has higher percentages of shrubs, herbaceous, hay/pasture land use. The P3 site has a slightly higher developed LC/LU than R2 and R3 and relatively similar forest LC/LU to R2, but P3 also has the highest percentage of shrubs, herbaceous, hay/pasture LC/LU out of all 6 sites. However, P3 has an average $\delta^{15}N$ value 3.73 times higher than R2 and R3 and almost twice as high as ND1, ND4, and SP3.

A study conducted by Price et al. (2019) suggests that higher $\delta^{15}N$ for all trophic groups means a shift in resources occurred along with a move towards omnivorous feeding habits, which they define as similar $\delta^{15}N$ values across macroinvertebrate predators and primary consumers. Our results indicate that PR, primary consumers (CF, CG, SC, SH), and PP have similar $\delta^{15}N$ values at ND1, ND4, and P3. Predators at SP3, R2, P3 have similar $\delta^{15}N$ values to consumers. Primary producers have slightly higher $\delta^{15}N$ values than some consumers at ND4, SP3, R3, and P3. This altered food web positioning shows that urbanization and agriculture cause elevated nutrient concentration and these inputs have a significant effect on stream communities and stream health often causing loss of biodiversity and a shift towards more omnivorous feeding habits.

Conclusion

There is evidence that N sources vary depending on land use and this plays an important role in stream ecosystems. Family richness declined in sites with higher ISA and community structure was altered in streams with high levels of agriculture. The streams with altered community structure and low family richness all had high $\delta^{15}N$ values. These patterns support our prediction that altered nitrogen sources and OM lead to altered diets and potentially a shift towards omnivory. In addition, $\delta^{15}N$ could be a useful indicator of stream health degradation.

The use of ISA and LC/LU in combination with $\delta^{15}N$ to monitor water quality could provide an early indicator of stream degradation from NPS wastewater and agriculture before stream function is altered greatly and could provide management with a more efficient way to monitor stream health. Biological monitoring relies on tangible impacts that already observable, and recovery efforts at that late point could be costly and ineffective. The use of $\delta^{15}N$ and $\delta^{13}C$ provides an integrative picture of N and C sources in the stream when compared to episodic sampling on a quarterly basis.

Further research into habitat availability and erosion could help explain some of the community structure alterations. The intensity and frequency of known sewage/septic system leaks in the surrounding areas could also help in order to explain or mitigate further stream degradation. Examining biodiversity at a finer taxonomic resolution could yield a clearer picture of how community structure and biodiversity change as a result of urbanization and agricultural inputs. Potentially having $\delta^{15}N$ values for organisms from all FFGs at all 34 sites, as well as other potential food sources, such as *Podostemum, Cladophora*, and SPOM could also lead to a better understanding of how urban and agricultural inputs change trophic positioning and could also help determine the degree of omnivory and food web alterations. The use of $\delta^{15}N$ and ISA as an early indicator of declining stream health due to N inputs and could provide a way to monitor streams more efficiently before water quality variables indicate a problem.

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A Spatial and Multivariate Approach to Examining Effects of Urbanization on Nitrogen Sources, Organic Matter Inputs, and Trophic Structure in Streams of Cobb and Paulding Counties, Georgia.

Site Code	Creek Name	County	Latitude	Longitude	Impervious Surface Area %	LN (Area (km ^{2}))	Temperature C	Dissolved Oxygen (mg/L)	Dissolved Oxygen O % Sat	pH (S.U.)	Biochemical Oxygen Demand (mg/L)	Chemical Oxygen Demand (mg/L)	Total Suspended Solids (mg/L)	Fecal Coliform (col/100ml)	Conductivity (µmho/cm)	Turbidity (NTU)	Total Phosphorus (mg/L)	NO_x (mg/L)
AL1	Allatoona Creek	Cobb	33.9628	-84.6783	9.19%	1.39	15.6	9.3	93.3	7.1	0.83	8	2.3	343	102	3.1	0.01	0.32
BM3	Buttermilk Creek	Cobb	33.8183	-84.6144	21.63%	2.64	17.9	8.7	91.3	7.0	0.95	9	2.7	250	66	4.3	0.01	0.44
BT3	Butler Creek	Cobb	34.0213	-84.6675	18.49%	2.71	16.7	9.4	96.8	7.2	1.05	10	1.5	857	110	2.5	0.01	0.33
L1	Lawrence Creek	Paulding	33.9633	-84.8359	8.19%	3.47	19.8	8.4	92.0	7.1	2.03	9	2.7	2181	144	7.5	0.07	0.40
LAL3	Little Allatoona Creek	Cobb	34.0140	-84.7335	11.99%	2.40	17.9	8.5	89.6	7.0	1.15	11	3.0	307	105	6.4	0.02	0.13
LND2	Little Noonday Creek	Cobb	34.0328	-84.5190	19.71%	2.48	11.8	10.0	92.5	7.2	1.12	10	1.7	192	112	2.9	0.01	0.91
NA2	Nancy Creek	Cobb	33.8709	-84.4578	31.20%	1.79	21.7	8.3	93.9	7.1	1.25	12	2.5	158	66	2.7	0.02	0.42
NC4	Nickajack Creek	Cobb	33.8393	-84.5285	21.59%	3.99	19.1	9.1	98.3	7.3	1.08	10	2.2	433	105	2.4	0.01	1.10
ND1	Noonday Creek (Upper)	Cobb	34.0053	-84.5371	33.02%	1.95	16.6	8.9	91.5	7.2	1.13	11	1.0	383	129	4.4	0.01	0.56
ND4	Noonday Creek (Lower)	Cobb	34.0715	-84.5370	27.24%	4.44	17.4	9.0	93.5	7.3	1.09	10	2.8	379	121	5.9	0.01	0.55
NS2	Noses Creek (Upper)	Cobb	33.9180	-84.6277	12.88%	3.18	17.2	8.3	86.1	7.1	1.10	13	5.1	300	97	8.2	0.01	0.24
NS4	Noses Creek (Lower)	Cobb	33.8762	-84.6427	12.42%	4.62	17.6	8.6	90.3	7.2	1.17	12	3.1	267	93	5.7	0.02	0.24
OL5	Olley Creek	Cobb	33.8327	-84.6301	22.81%	3.56	19.2	8.0	86.4	7.1	1.07	12	2.5	283	98	3.1	0.01	0.29
P1	Pumpkinvine Creek (Lower)	Paulding	34.0713	-84.7693	4.92%	5.79	20.2	8.8	97.1	7.1	2.00	9	8.5	2976	142	13.8	0.09	1.25
P2	Pumpkinvine Creek (Little)	Paulding	34.0395	-84.7872	8.81%	3.97	19.8	9.4	102.9	7.2	2.00	9	3.0	3396	96	12.2	0.06	0.42
P3	Pumpkinvine Creek (Middle)	Paulding	34.0252	-84.8166	3.61%	5.38	20.3	8.6	95.6	7.2	2.00	9	7.8	3158	115	14.4	0.07	0.61
P4	Pumpkinvine Creek (Middle)	Paulding	33.9337	-84.8646	2.99%	4.90	20.1	8.7	96.4	7.2	2.00	9	5.5	2753	83	9.8	0.08	0.43
P5	Pumpkinvine Creek (Upper)	Paulding	33.9158	-84.8778	1.76%	4.71	20.4	8.6	95.1	7.2	2.00	9	3.5	2358	74	7.7	0.06	0.45
PC1	Proctor Creek	Cobb	34.0558	-84.6184	37.18%	1.10	11.9	10.3	95.5	6.9	1.17	7	1.2	375	78	2.4	0.01	0.29
PS1	Powder Springs Creek	Cobb	33.8829	-84.7147	13.16%	3.66	16.6	8.4	85.9	7.0	1.07	10	4.6	200	91	7.5	0.01	0.41
R1	Raccoon Creek (Lower)	Paulding	34.0605	-84.9008	0.59%	4.64	21.9	8.9	101.5	7.2	2.00	9	3.0	2790	56	5.8	0.07	0.21
R2	Raccoon Creek (Middle)	Paulding	33.9968	-84.8967	0.63%	3.93	18.9	9.0	97.0	7.1	2.00	9	2.5	1445	59	4.7	0.08	0.29
R3	Raccoon Creek (Upper)	Paulding	33.9670	-84.9319	0.81%	3.14	18.9	9.4	100.8	7.4	2.00	9	3.0	1763	47	3.2	0.06	0.27
RB1	Rubes Creek (Upper)	Cobb	34.0424	-84.4977	22.60%	1.79	15.9	9.5	95.6	7.1	1.14	11	1.1	307	118	2.6	0.01	0.89
RB2	Rubes Creek (Upper)	Cobb	34.0573	-84.4745	17.98%	1.39	14.7	9.8	97.1	7.2	1.13	10	1.0	389	105	2.0	0.01	0.49
RT4	Rottenwood Creek	Cobb	33.9076	-84.4742	40.32%	3.58	19.4	9.2	100.3	7.3	1.12	11	2.1	475	90	3.4	0.02	0.36
S1	Sweetwater Creek (Lower)	Paulding	33.8299	-84.7298	5.59%	5.54	24.0	6.9	82.3	7.1	2.37	10	13.7	2729	90	14.5	0.06	0.57
S2	Sweetwater Creek (Upper)	Paulding	33.7776	-84.8951	1.93%	3.61	23.7	5.5	65.2	7.2	2.08	9	2.8	3500	86	17.8	0.08	0.75
SL2	Sewell Mill Creek (Upper)	Cobb	33.9901	-84.4705	17.27%	2.30	16.5	8.5	87.1	6.9	0.71	5	1.8	325	77	3.5	0.01	0.59
SL4	Sewell Mill Creek (Lower)	Cobb	33.9690	-84.4555	16.72%	3.61	17.1	8.6	89.0	6.9	0.78	1	6.1	308	78	4.4	0.01	0.55
SP3	Sope Creek	Cobb	33.9665	-84.5154	34.52%	2.83	19.0	8.3	89.1	7.0	1.00	10	2.0	358	117	2.8	0.01	0.42
T2	Tanyard Creek	Cobb	34.0704	-84.6796	27.05%	1.79	17.5	7.8	81.5	7.0	1.19	12	3.1	658	136	5.5	0.02	0.17
W1	Weaver Creek	Paulding	33.9302	-84.8589	21.93%	1.79	22.9	7.0	81.4	7.2	2.08	9	10.8	3711	119	9.7	0.06	0.69
WL1	Willeo Creek	Cobb	34.0371	-84.4059	14.24%	2.94	20.0	8.7	95.4	7.0	1.10	12	2.0	225	60	3.1	0.02	0.24

Appendix A. Data from Cobb County (2015 – 2018) and Paulding County (2018) datasets. Averaged data for turbidity, TSS, COD, BOD, Fecal coliform, Temperature, Conductivity, total phosphorus, pH, DO, and NOx.

Site Code	$\delta^{15}N$ Baetidae (‰)	$\delta^{15}N$ Heptageniidae (‰)	$\delta^{15}N$ Hydropsychidae (‰)	$\delta^{13}C$ Baetidae (‰)	$\delta^{13}C$ Heptageniidae (‰)	$\delta^{13}C$ Hydropsychidae (‰)
AL1	-	5.24	6.86	-	-30.13	-32.04
BM3	7.54	-	7.58	-35.64	-	-29.18
BT3	6.91	7.01	7.31	-27.52	-27.60	-28.54
L1	7.46	5.77	7.40	-34.39	-36.36	-32.48
LAL3	4.21	5.51	8.37	-31.41	-31.49	-29.56
LND2	-	7.01	-	-	-31.30	-
NA2	6.40	8.35	7.89	-27.70	-26.92	-28.80
NC4	9.17	8.31	8.65	-30.98	-28.85	-29.57
ND1	7.94	-	7.64	-35.53	-	-31.07
ND4	7.77	6.77	7.48	-33.45	-32.07	-33.08
NS2	5.95	5.48	7.09	-33.27	-31.65	-31.19
NS4	6.73	6.70	6.89	-32.81	-33.31	-29.33
OL5	8.23	8.03	8.34	-34.62	-32.76	-28.61
P1	8.47	6.65	9.22	-30.07	-32.19	-28.83
P2	7.64	7.11	7.81	-26.94	-26.60	-26.00
P3	10.64	10.21	10.77	-28.39	-26.92	-27.01
P4	7.71	7.17	7.99	-35.02	-32.20	-28.74
P5	5.90	6.07	6.64	-34.99	-33.66	-31.89
PC1	6.96	-	6.70	-29.04	-	-31.51
PS1	-	-	7.27	-	-	-31.77
R1	-	4.18	4.52	-	-27.32	-26.27
R2	3.38	3.88	5.20	-29.88	-29.10	-29.51
R3	-	2.98	3.92	-	-28.08	-28.16
RB1	7.11	-	8.18	-32.39	-	-32.41
RB2	6.12	-	7.40	-29.34	-	-28.27
RT4	7.25	-	5.98	-29.49	-	-28.31
S1	7.17	7.02	9.43	-31.91	-37.84	-33.05
S2	-	4.65	7.88	-	-37.49	-35.29
SL2	14.74	-	7.45	-34.35	-	-27.74
SL4	3.98	-	6.80	-37.39	-	-30.43
SP3	15.22	15.57	10.84	-33.69	-30.46	-28.23
T2	6.94	-	7.64	-33.99	-	-31.47
W1	-	6.76	8.73	-	-32.81	-31.22
WL1	5.27	8.08	7.84	-32.01	-29.87	-30.88

Appendix B. Stable Isotope values for $\delta^{15}N$ and $\delta^{13}C$ values for Hydropsychidae, Heptageniidae, and Baetidae macroinvertebrate families for sampling done March 2019 through October 2019. Averaged if multiple individuals from the same family were found and blank spaces indicate no individuals were found.

Site Code	Open water	Developed open space	Developed low intensity	Developed medium intensity	Developed high intensity	Barren land	Deciduous forest	Evergreen forest	Mixed forest	Shrubs & scrubs	Herbaceous	Hay & pasture	Cultivated Crops	Woody wetlands	Emergent Herbaceous Wetlands	ISA %
AL1	0.00	0.20	0.18	0.03	0.00	0.00	0.25	0.16	0.07	0.00	0.01	0.11	0.00	0.00	0.00	0.09
BM3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.22
BT3	0.00	0.34	0.31	0.06	0.02	0.00	0.10	0.11	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.18
L1	0.01	0.16	0.12	0.04	0.01	0.00	0.29	0.19	0.10	0.01	0.02	0.06	0.00	0.00	0.00	0.08
LAL3	0.01	0.24	0.23	0.03	0.00	0.00	0.15	0.19	0.08	0.00	0.01	0.04	0.00	0.00	0.00	0.12
LND2	0.01	0.38	0.26	0.08	0.04	0.00	0.07	0.11	0.06	0.00	0.00	0.01	0.00	0.00	0.00	0.20
NA2	0.01	0.31	0.26	0.20	0.08	0.00	0.09	0.02	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.31
NC4	0.01	0.33	0.31	0.09	0.04	0.00	0.10	0.07	0.05	0.00	0.00	0.01	0.00	0.01	0.00	0.22
ND1	0.00	0.25	0.24	0.19	0.12	0.00	0.07	0.07	0.05	0.00	0.00	0.01	0.00	0.00	0.00	0.33
ND4	0.01	0.27	0.25	0.14	0.08	0.01	0.09	0.08	0.04	0.00	0.01	0.02	0.00	0.00	0.00	0.27
NS2	0.01	0.20	0.16	0.06	0.03	0.00	0.28	0.14	0.07	0.00	0.01	0.05	0.00	0.01	0.00	0.13
NS4	0.01	0.25	0.20	0.04	0.01	0.00	0.19	0.14	0.06	0.00	0.01	0.06	0.00	0.02	0.00	0.12
OL5	0.00	0.30	0.29	0.11	0.04	0.00	0.09	0.08	0.05	0.00	0.01	0.02	0.00	0.01	0.00	0.23
P1	0.01	0.11	0.08	0.02	0.00	0.00	0.34	0.21	0.10	0.04	0.03	0.05	0.00	0.01	0.00	0.05
P2	0.00	0.19	0.16	0.03	0.00	0.00	0.28	0.16	0.07	0.03	0.02	0.04	0.00	0.00	0.00	0.09
P3	0.01	0.08	0.05	0.02	0.00	0.01	0.37	0.23	0.10	0.04	0.03	0.05	0.00	0.01	0.00	0.04
P4	0.01	0.06	0.04	0.01	0.00	0.01	0.42	0.23	0.10	0.03	0.03	0.05	0.00	0.01	0.00	0.03
P5	0.01	0.05	0.03	0.01	0.00	0.01	0.45	0.24	0.09	0.04	0.03	0.05	0.00	0.01	0.00	0.02
PC1	0.00	0.20	0.33	0.22	0.11	0.00	0.03	0.09	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.37
PS1	0.00	0.20	0.17	0.05	0.03	0.00	0.20	0.13	0.06	0.01	0.01	0.11	0.00	0.01	0.00	0.13
R1	0.00	0.04	0.01	0.00	0.00	0.00	0.49	0.25	0.13	0.01	0.03	0.04	0.00	0.00	0.00	0.01
R2	0.00	0.03	0.01	0.00	0.00	0.00	0.50	0.26	0.15	0.01	0.02	0.02	0.00	0.00	0.00	0.01
R3	0.00	0.02	0.02	0.00	0.00	0.00	0.62	0.18	0.14	0.00	0.01	0.01	0.00	0.00	0.00	0.01
RB1	0.00	0.35	0.29	0.10	0.04	0.00	0.07	0.08	0.04	0.00	0.00	0.01	0.00	0.00	0.00	0.23
RB2	0.00	0.34	0.24	0.05	0.05	0.00	0.18	0.07	0.05	0.00	0.00	0.01	0.00	0.00	0.00	0.18
RT4	0.00	0.24	0.25	0.21	0.17	0.00	0.04	0.05	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.40
S1	0.01	0.13	0.10	0.02	0.01	0.00	0.28	0.16	0.05	0.01	0.02	0.15	0.00	0.05	0.00	0.06
S2	0.01	0.09	0.04	0.00	0.00	0.00	0.40	0.20	0.07	0.02	0.03	0.11	0.00	0.04	0.00	0.02
SL2	0.00	0.41	0.27	0.05	0.02	0.00	0.09	0.09	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.17
SL4	0.01	0.42	0.25	0.05	0.02	0.00	0.09	0.09	0.06	0.00	0.00	0.01	0.00	0.00	0.00	0.17
SP3	0.00	0.26	0.31	0.18	0.11	0.00	0.05	0.04	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.35
T2	0.00	0.26	0.39	0.15	0.02	0.00	0.04	0.08	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.27
W1	0.00	0.25	0.26	0.12	0.04	0.00	0.12	0.08	0.06	0.02	0.02	0.02	0.00	0.00	0.00	0.22
WL1	0.02	0.36	0.22	0.05	0.01	0.00	0.15	0.10	0.07	0.00	0.01	0.01	0.00	0.00	0.00	0.14

Appendix C. Percent Land Cover Land Use calculated using NLCD 2016 for all land use categories found for all 34 sites sampled across Cobb and Paulding Counties, GA.

Water Quality Parameters	**LN(Area)**	**δ15N Hydropsychidae**	**δ13C Hydropsychidae**	**Temperature C**	**Dissolved Oxygen**	**pH**	**BOD**	**COD**	**TSS**	**Fecal Coliform**	**Conductivity**	**Turbidity**	**Total Phosphorus**	**NOx**
δ15N Hydropsychidae (‰)	-0.08	1.00	-0.03	0.08	-0.42*	-0.30	-0.25	0.16	0.23	-0.06	0.64*	0.27	-0.28	0.40*
LN(Area)	1.00	-0.08	0.26	0.45*	0.25	0.33	0.38*	-0.01	0.27	0.35	-0.21	0.49*	0.46*	0.02
Dissolved Oxygen (mg/L)	0.25	-0.42*	0.35	0.03	1.00	0.53*	0.28	0.00	-0.44*	0.12	-0.44*	-0.30	0.26	0.08
Biochemical Oxygen Demand (mg/L)	0.38*	-0.25	0.21	0.58*	0.28	0.49*	1.00	0.08	0.46*	0.89*	-0.25	0.53*	0.95*	-0.05
Chemical Oxygen Demand (mg/L)	-0.01	0.16	-0.08	0.19	0.00	0.31	0.08	1.00	-0.22	-0.14	0.28	0.03	-0.13	-0.18
Total Suspended Solids (mg/L)	0.27	0.23	-0.05	0.49*	-0.44*	0.07	0.46*	-0.22	1.00	0.66*	0.12	0.79*	0.49*	0.13
Fecal Coliform (col/100ml)	0.35	-0.06	0.24	0.60*	0.12	0.43*	0.89*	-0.14	0.66*	1.00	-0.07	0.65*	0.89*	0.09
Conductivity (µmho/cm)	-0.21	0.64*	-0.32	-0.20	-0.44*	0.06	-0.25	0.28	0.12	-0.07	1.00	0.20	-0.35	0.32
Turbidity (NTU)	0.49*	0.27	-0.03	0.38*	-0.30	0.08	0.53*	0.03	0.80*	0.65*	0.20	1.00	0.54*	0.00
Total Phosphorus (mg/L)	0.46*	-0.28	0.29	0.60*	0.26	0.39*	0.95*	-0.13	0.50*	0.89*	-0.35	0.54*	1.00	-0.05
NOx (mg/L)	0.02	0.4	-0.14	0.04	0.08	0.18	-0.05	-0.18	0.13	0.08	0.32	0.00	-0.05	1.00
Family														
Chironomidae	-0.11	0.38*	-0.17	-0.54*	0.37*	-0.14	-0.39*	-0.11	-0.06	-0.26	0.34	-0.02	-0.40	0.29
Empididae	-0.18	0.04	0.01	0.27	-0.23	-0.11	-0.01	0.27	-0.07	-0.17	-0.12	-0.11	-0.10	-0.09
Simuliidae	0.22	-0.17	0.02	0.16	-0.06	-0.23	-0.05	0.01	-0.02	-0.09	-0.49*	-0.04	0.01	-0.32
Tipulidae	-0.19	0.05	-0.16	-0.05	-0.38*	-0.31	-0.16	0.09	0.02	-0.13	0.30	0.01	-0.16	-0.24
Hydropsychidae	-0.42*	-0.01	-0.28	-0.42*	0.37*	0.14	0.02	0.11	-0.25	-0.04	0.31	-0.18	-0.11	0.24
Leptoceridae	0.00	0.45*	0.27	0.23	-0.25	-0.13	-0.13	0.09	0.03	-0.09	0.12	-0.01	-0.13	0.02
Philopotamidae	-0.13	0.05	0.21	0.00	0.06	0.05	-0.34	-0.04	-0.25	-0.30	0.07	-0.31	-0.31	0.16

Appendix D. Pearson correlations for $\delta^{15}N$ and $\delta^{13}C$ Hydropsychidae values, water quality variables, and macroinvertebrate relative abundance (indicated significance at α= 0.05). Water Quality Data from Cobb County (2015 – 2018) and Paulding county (2018) datasets. Averaged 2015 – 2018 data for turbidity, TSS, COD, BOD, Fecal coliform, Temperature, Conductivity, total phosphorus, pH, DO, and NOx. Cobb county relative abundance data from 2015 – 2018 and relative abundance data Paulding county from 2019 – 2020.*

Water Quality Parameters	Open water	Developed Open Space	Developed Low Intensity	Developed Medium Intensity	Developed High Intensity	Barren land	Deciduous forest	Evergreen forest	Mixed forest	Shrubs & Scrubs	Herbaceous	Hay & Pasture	Cultivated Crops	Woody Wetlands	Emergent Herbaceous Wetlands	Impervious Surface Area
δ15N Hydropsychidae (‰)	0.21	0.37*	0.43*	0.29	0.16	0.11	-0.52*	-0.38*	-0.41*	0.14	-0.04	-0.06	0.45	0.25	0.15	0.36
LN(Area)	0.36	-0.37*	-0.51*	-0.40*	-0.24	0.39*	0.47*	0.52*	0.46*	0.56*	0.49*	0.25	0.40	0.65*	0.37*	-0.47*
Dissolved Oxygen (mg/L)	-0.04	-0.29	-0.38*	-0.16	0.08	0.02	0.42*	0.29	0.29	0.12	0.06	-0.17	0.11	-0.22	-0.43	-0.20
Biochemical Oxygen Demand (mg/L)	-0.15	-0.67*	-0.60*	-0.30	-0.28	0.36	0.75*	0.65*	0.69*	0.73*	0.80*	0.08	0.35	0.14	0.18	-0.53*
Chemical Oxygen Demand (mg/L)	0.21	-0.09	0.09	0.22	0.20	0.08	-0.06	-0.11	-0.12	-0.10	0.01	0.08	-0.05	0.20	0.26	0.17
Total Suspended Solids (mg/L)	0.02	-0.16	-0.19	-0.18	-0.28	0.32	0.18	0.21	0.24	0.53*	0.55*	0.28	0.42	0.37*	0.60*	-0.27
Fecal Coliform (col/100ml)	-0.20	-0.52*	-0.48*	-0.31	-0.31	0.36	0.58*	0.56*	0.56*	0.79*	0.82*	0.10	0.46	0.17	0.23	-0.48*
Conductivity (µmho/cm)	-0.13	0.40*	0.60*	0.41*	0.31	0.14	-0.49*	-0.31	-0.37*	-0.05	-0.12	0.03	0.18	0.19	0.23	0.43*
Turbidity (NTU)	0.16	-0.40*	-0.39*	-0.29	-0.30	0.48*	0.34	0.48*	0.36	0.77*	0.72*	0.45*	0.68	0.56*	0.64*	-0.41*
Total Phosphorus (mg/L)	-0.14	-0.68*	-0.68*	-0.43*	-0.40*	0.28	0.79*	0.72*	0.77*	0.75*	0.80*	0.09	0.38	0.11	0.12	-0.64*
NOx (mg/L)	-0.05	0.31	0.20	0.13	0.13	0.15	-0.25	-0.31	-0.21	0.11	-0.13	-0.23	0.17	0.04	0.03	0.19
Family																
Chironomidae	0.10	0.32	0.26	-0.13	-0.12	-0.03	-0.28	-0.07	-0.18	-0.13	-0.17	0.23	0.20	0.23	0.09	0.01
Empididae	0.17	0.10	0.14	0.32	0.11	0.19	-0.12	-0.25	-0.22	-0.09	0.01	-0.09	-0.05	-0.07	-0.06	0.21
Simuliidae	0.58*	-0.05	-0.29	-0.38*	-0.34	-0.26	0.13	0.10	0.16	-0.10	0.08	-0.03	-0.14	-0.05	0.04	-0.29
Tipulidae	-0.03	0.10	0.34	0.17	-0.09	-0.21	-0.25	-0.20	-0.21	-0.15	-0.12	-0.15	-0.08	-0.13	-0.11	0.18
Hydropsychidae	-0.33	0.10	0.18	0.36	0.41*	0.04	-0.16	-0.18	-0.16	-0.13	-0.16	-0.23	-0.09	-0.21	-0.11	0.33
Leptoceridae	-0.10	0.07	0.15	0.41	0.47*	-0.01	-0.20	-0.30	-0.27	-0.04	-0.18	-0.16	0.10	-0.08	0.03	0.39*
Philopotamidae	-0.18	0.28	0.27	0.60*	0.79*	-0.12	-0.41*	-0.48*	-0.43*	-0.27	-0.47*	-0.37*	-0.13	-0.23	-0.29	0.64*

Appendix E. Pearson correlations for Land cover land use, Water quality parameters, and macroinvertebrate relative abundance (indicated significance at α= 0.05). Land cover land use calculated from NLCD 2016 . Relative abundance data from Cobb County (2015 – 2018) and Paulding county (2018) datasets. Water Quality Data from Cobb County (2015 – 2018) and Paulding county (2018) datasets. Averaged data for turbidity, TSS, COD, BOD, Fecal coliform, Temperature, Conductivity, total phosphorus, pH, DO, and NOx.*