Macroinvertebrate Assemblage Survey of Sandy Creek in Durham County, NC: Current Status, Pre-Restoration, and Post-Restoration Comparisons

by

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Executive Summary:

Stream and wetland restoration has become a widely used intervention for combatting the negative effects of urban stream syndrome and the water quality detriments that accompany it. Urban stream syndrome often occurs in watersheds with high impervious surface land cover and can lead to degradation of water quality, streambank instability, streambed incision, and disrupted sediment transport. All of these effects culminate in the loss of plant and animal biodiversity. Restoration projects such as the one being researched here aim to combat these issues. Macroinvertebrates have been used as a proxy for water quality and to compare streams of different quality. They serve as a tool for investigating the success of a restoration project on improving biodiversity and ecosystem function. This study aims to contribute to long-term monitoring of restored streams at the Duke University Wetland Center's Stream and Wetland Assessment Management Park (SWAMP) as well as draw temporal conclusions about the success of the restoration efforts.

SWAMP is composed of restored areas of stream, riparian wetlands, constructed wetlands, and retention ponds to better treat runoff into Sandy Creek in Durham County, NC. This system's drainage area is dominated by upstream urban development leading to poor water quality and extreme erosion and incision, typical of streams experiencing urban stream syndrome in the Piedmont of North Carolina. A variety of ecological indexes and parameters were measured using kick-net, D-net, and leaf litter pack collection methods conducted in the spring and fall of 2022.

Results indicate an overall improvement in North Carolina Biotic Index (NCBI) in all sites over time with the current NCBI of SWAMP being similar to that of the reference reach Mud Creek (MC). Comparing sites before restoration in 2001 to 2023 shows that the NCBI has

improved for WT1, a restored site, by nearly 30% and improved since 2008 by approximately 12%. Since 2008 MC, the reference site, has improved by approximately 17%. NCBI improvements at both sites could indicate outside factors such as water quality protection policies/practices curbing pollution in addition to the improvements resulting from the restoration have enhanced water quality. Water quality parameters investigated largely improved following restoration of WT1, with subsequent improvement in NCBI, while no notable changes occurred in MC. Both magnitude and variability of total phosphorus and total suspended solids decreased dramatically following the 2012-2013 restoration upstream of WT1. Other parameters investigated were Shannon Diversity Index and EPT percentage.

There seems to be important improvements in macroinvertebrate assemblages because of the restoration although they do not seem to be fully conclusive of the restoration being the sole reason for macroinvertebrate assemblage improvement/recovery. This study contributes to the long-term records of SWAMP macroinvertebrate assemblages, allowing for comparisons of recovery over time. The results also highlight the link between macroinvertebrate populations and water quality improvements resulting from the restoration. This restoration project and others like it aim to return degraded streams to a near-natural state and improve the water quality and ecosystem function within them. The results of this study indicate trends toward the recovery of ecosystem functions though there are other factors outside of the scope of this study that could be contributing to the changes over time.

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Introduction:

Sandy Creek is located near Durham, North Carolina within the Duke University Wetland Center's (DUWC) Stream and Wetland Assessment Management Park (SWAMP). Sandy Creek is a headwater stream in the Cape Fear River Basin and drains approximately 8.5 square miles (22 km^2) of southwest Durham, including much of Duke's campus. This portion of the stream was heavily incised and demonstrated many characteristics typical of a stream affected by the urban stream syndrome due to approximately a 79% impervious landcover according to the 2011 National Land Cover Dataset (NLCD). Restoration on the SWAMP section of Sandy Creek included five total phases with the first beginning in 2004 and the final stage being completed by 2014 (Richardson et al., 2011). Restorative actions and structures in this project include stream realignment and reconnection to floodplains/riparian wetlands, pond/dam construction, constructed wetland cells, an anabranching stream channel, and a set of triple-tiered stormwater BMP ponds.

The poor water quality of this section of Sandy Creek indicated the need for restoration on this system (Richardson et al., 2011). Figure 1 shows a water quality comparison of reference site Mud Creek (MC), a water quality monitoring site just upstream of DS1 (included in this study) that was restored in 2004/2005 (WT5), and a site with restoration performed in 2012/2013 (WT1). Both total phosphorus (UTP) and total suspended solids (TSS) values were highly

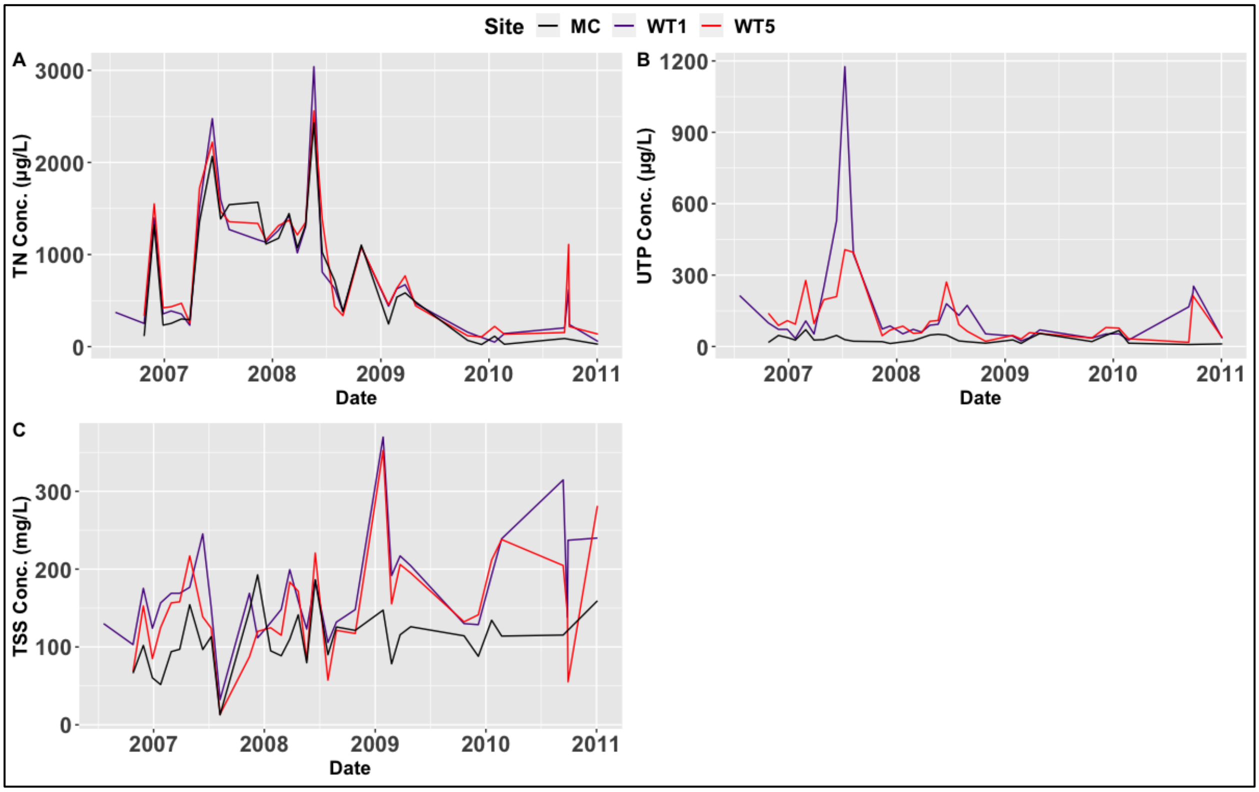

Figure 1: Water quality data (TN, UTP, & TSS) from 2007 to 2011 for MC (reference), WT5 (restored 2004/2005), and WT1 (to be restored in 2012/2013)

variable from year to year and higher at WT1 and WT5 than MC in the 2007-2011, while total nitrogen (TN) tracked the reference site and showed decreasing N concentrations. Driving these water quality issues is the level of impervious surfaces in the watershed, outlined earlier. Key water quality issues were high levels of nitrogen, phosphorus, total suspended solids, and fecal coliforms (Richardson et al. 2011). The aim of stream and floodplain restoration was to address these water quality issues through natural ecosystem processing and return this stream/wetland ecosystem to a restored, near-natural state while improving habitat suitability for wildlife. Palmer et al (2010) presents the importance of improving the “most limiting factor”, water quality in the case of SWAMP, to cause a subsequent improvement in the biological structure of the ecosystem.

Urban Stream Syndrome:

Decades of urbanization has led to drastic changes in stream morphology, flood regimes, and the ecology of in-stream and riparian habitats (Walsh et al. 2005, Richardson et al. 2011). This problem is especially apparent in the Piedmont region of North Carolina where there have been issues with soil erosion for hundreds of years (Macfall et al. 2014, Doll 2016). With soils predisposed to erosion, the urbanization of areas adjacent to streams often results in bank erosion/instability, streambed incision, habitat loss, all of which result in negative effects on water quality (Lenat and Crawford 1994). Anthropogenic activities have only exacerbated this issue over time, creating more dire situations in streams across the landscape, particularly in small first order streams (e.g., Gage et al. 2004; Pond, 2010; Smith & Lamp, 2008).

Hardened infrastructure and impervious surfaces in developed areas quickly gets precipitation into these smaller first and second order streams causing flash flooding in low-lying urban areas, leading to dramatic discharge variation between baseflows and storm-level flows (Roodsari and Chandler 2017). Resulting hydrographs have large, frequent pulses of discharge rather than gradual increases and decreases in flow like that seen in typical rural/natural streams. This pattern of flow has been found to have many ecological impacts such as altering the metabolic processes of streams (Blaszczak et al., 2019). Such impacts include interfering with biofilm nutrient processing, due to disruptions in surface water and turbidity changes, as well as creating hypoxic conditions at baseflow due to increased organic matter and stagnation between scouring storm flows. Urban stream management has also changed sediment transport in impacted streams as they are deprived of upstream sediments that would typically accrete during baseflows (Doll 2016, Blaszczak et al., 2019). However, today there is little to no accretion occurring between storms and then rapid erosion during the storms leading to the widespread, dramatic incision seen in streams across the Piedmont of North Carolina.

Restoration Benefits:

Stream restoration has become a common method to combat urban stream syndrome and revert stream and riparian habitats to their pre-urbanized state. Increasing heterogeneity within the stream and riparian areas is a key tool used in restoration as it has been shown to increase biodiversity and ecosystem productivity (Frainer et al. 2018). Decreasing channelization and promoting the buildup of woody debris and rocks is another common tool used in restoration as this creates areas for organic matter to be deposited providing habitat for macroinvertebrates as well as amphibians and other organisms (Reid and Church 2015). As previously mentioned, stream morphology is greatly impacted in urbanized streams and improving stream morphology has been shown to benefit overall biodiversity of both in-stream and adjacent riparian communities (Milner et al. 2015).

Restoration of streams and wetlands also promotes natural nutrient cycling, flood control, and pollutant filtration (Richardson 1994). In addition to the ecological benefits restoration aims to provide, it is also utilized to improve or re-establish ecosystem services that once existed but have been diminished due to human actions (Meli et al. 2014). However, the effectiveness of restoration has long been questioned (Walter and Merritts 2008) leading to the need for quantitative methods of assessing a restoration project's success at improving ecological function and ecosystem services. The restoration conducted here largely used a meandering stream model while it has been found that braided, anabranching systems may have been the natural state of pre-settlement streams in the Piedmont of North Carolina (Walter and Merritts 2008).

Invertebrates as Indicators:

Utilizing macroinvertebrates as an indicator of stream and ecosystem health is a common practice as many organisms rely on them as a primary food source while they also provide vital ecosystem services such as the processing and decomposition of leaf litter and other organic matter that makes its way into the stream channel (Collier et al. 2016). Because they are at the base of the food web and provide a variety of ecosystem services macroinvertebrates are often used to predict and measure the state of stream and riparian ecosystems (King and Richardson 2003). Fish and other organisms will sometimes be used as indicators of ecosystem health, but they are often not present in headwater tributaries, furthering the need to utilize macroinvertebrates. Direct causality between stream ecosystem health and macroinvertebrate assemblages is not certain, but there certainly is a relationship between the two (Lepori et al. 2005). Invertebrates are responsible for high-level biological processes like litter organic matter breakdown and indices used in this study, namely the North Carolina Biotic Index (NCBI) and EPT (Ephemeroptera, Plecoptera, and Trichoptera) percentage, have been proven to be closely related to this level of biological function (Donatich et al. 2020). This relationship is why they are the typical method for assessing restoration success and why I will be using them to assess the success of the SWAMP restoration project.

Objectives/Hypothesis:

This study aims to investigate the impacts of restoration on the ecology of this stream and wetland system using macroinvertebrates as an indicator of ecosystem biodiversity and stream health. A variety of sampling methods and analyses will be employed to assess current macroinvertebrate assemblage biodiversity within the SWAMP, how it compares to that of a reference reach nearby, as well as how it compares temporally to historical macroinvertebrate data collected at this restored stream pre-restoration. I hypothesize that the current macroinvertebrate assemblage biodiversity will indicate improved water quality in the restored sites compared to unrestored streams but comparable to the reference reach. I also postulate that stream restoration has improved stream biodiversity, and therefore water quality, temporally since the pre-restoration sampling that was conducted in 2001.

Methods:

Study Area:

As previously mentioned, Sandy Creek is a headwater tributary of the Cape Fear River Basin in Durham County, North Carolina. It drains parts of South Durham and nearly all of Duke University's campus with much of its land cover dominated by impervious surfaces. Three of the four research sites were located within Duke University's SWAMP project (AS, DS1, and WT1). The fourth research site was the reference site located in the Durham Division of the Duke Forest along Mud Creek (MC). This area is adjacent to Duke's campus and the SWAMP sites, just across the highway running along Duke's West Campus (Fig. 2).

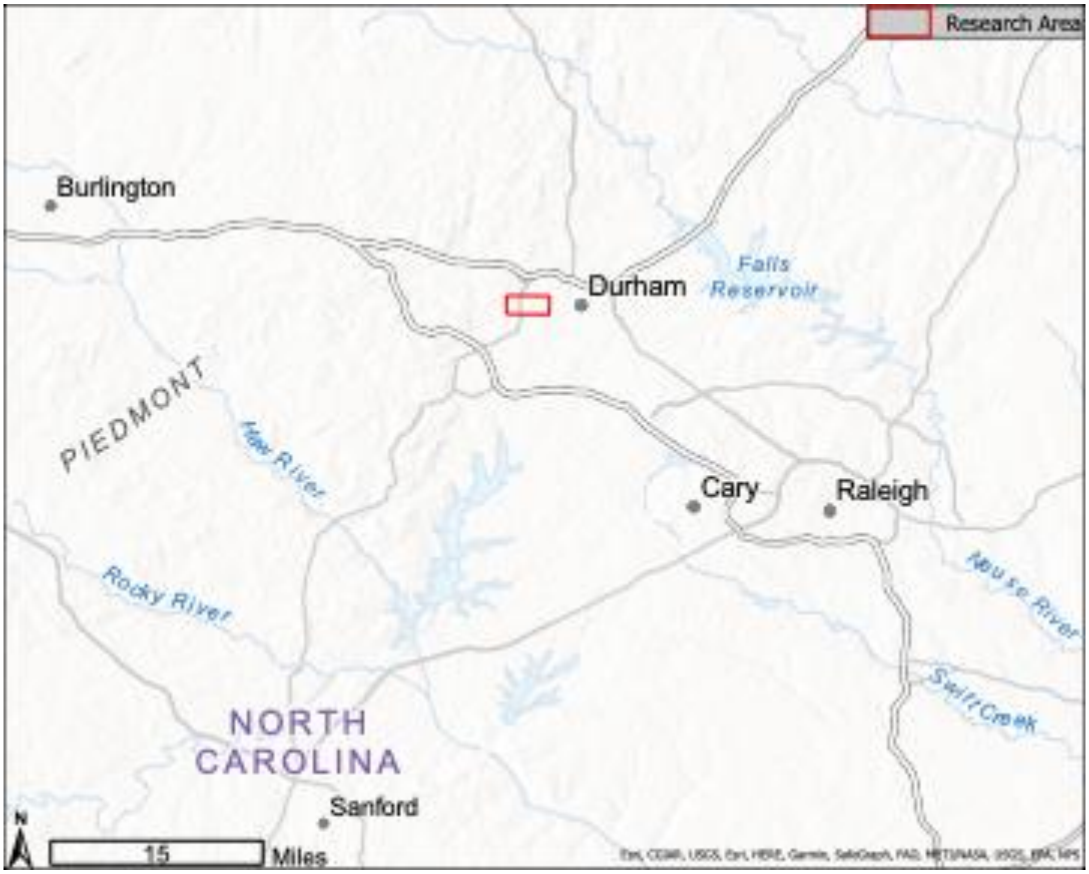

Figure 2: The study area includes reaches of Sandy Creek (research sites) and Mud Creek (reference site) and its relationship to the surrounding area of North Carolina.

The AS site is located on a sandy, typically slow-moving portion of the stream with few riffle areas and some overhanging bank vegetation (Fig. 3). This site is just east of Anderson Street, where Sandy Creek is carried under the roadway in a culvert. DS1 is located at the outlet of the SWAMP project, downstream of all restoration. It is just below an old milldam with a sediment-filled pool behind it, all of which is just downstream of the main pond and dam constructed during the project. This site is rocky with some riffle habitat, slow-moving water, and little bank vegetation as it is non-restored and deeply incised. The WT1 site has stronger flow, more riffle habitat, and some bank vegetation overhanging into the stream. This site is located just downstream of Highway 751 where Sandy Creek is carried under the roadway through a culvert (Fig. 3). This site was chosen for focused analyses in comparison to the reference site as it had a specific period (2012-2013) in which it experienced dramatic changes in its upstream reach via new stream restoration and stormwater treatment ponds, while also being in the heart of the restoration project. The AS site has little restoration work in its upstream reach as it is at the most upstream extent of the SWAMP project while DS1 is at the outlet, just outside of the restored section of stream and is just downstream from an old milldam. For these reasons WT1 is used as the main restored site for in-depth comparisons made with the reference site.

The reference site is located on a slow-moving, rocky reach of Mud Creek in the Duke Forest (Fig. 3). This site has some overhanging bank vegetation, strong bedrock riffle areas, some deeper pools, and drains approximately 2 square miles (518 ha) with nearly 50% impervious surface landcover. Much of the non-impervious cover of this sub-watershed is made up of the Duke Forest, a managed forest of over 7,000 acres (2,833 ha) that is protected and used for research and teaching by Duke University. Though this site has a substantial amount of urban development upstream, there is no restoration along this reference reach.

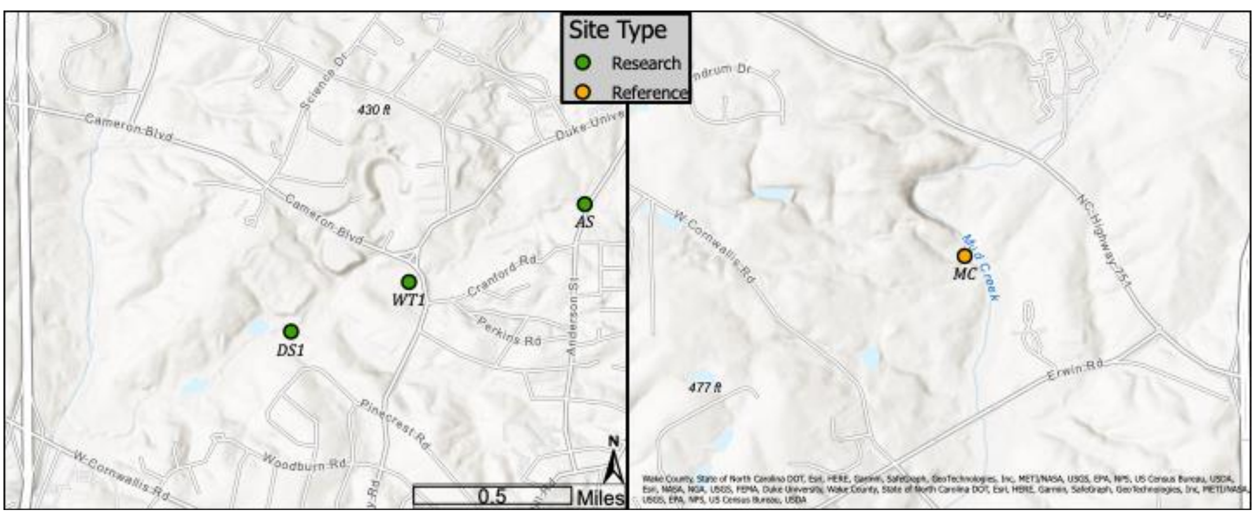

Figure 3: Research sites (left and reference sites (right), and their relative location along each stream reach.

Collection:

The macroinvertebrate collection procedures for this study align with previous studies, which follow much of the guidance provided by North Carolina Department of Environmental Quality, Division of Water Resources standard operating procedures (NCDEQ 2016). Three sampling methods were employed for this specific study including artificial leaf pack installation and collection, D-net sweep sampling, and kick-net sampling (NCDEQ 2016). Using these three methods, sampling was performed in a variety of habitats within the stream to better represent leaf pack colonization, edge habitat, and benthic habitat.

Artificial leaf packs were constructed using plastic mesh to control for leaf pack size and leaf type. Primarily oak leaves, dropped from the previous fall, were used in the packs. Whole leaves were gathered and air dried for one week in the lab prior to being packed into each leaf pack. Three packs were placed at each of the four sites per round of sampling, and each pack contained 12 (± 0.2) grams of leaves. Packs were then staked to the streambed at each research site, left out to be colonized, and then retrieved one pack at a time weekly for three weeks. The staggered retrieval was used to allow shorter and longer colonization periods to ensure a representative sample was obtained. The waiting period prior to beginning the retrieval process was 5 weeks in the spring collection period and 3 weeks in the fall collection period. The shorter waiting period in the fall was attributed largely to quickly cooling weather resulting in less ideal collection conditions. However, there was still overlap in the length of colonization for the two collection periods. After collection, packs were stored in a cold room at 4° C, until being sorted through and macroinvertebrates removed and placed in 70% ethanol.

To collect edge habitat macroinvertebrates a D-net with 500-micron mesh was used to sweep these sections of stream at each research site. Again, three repetitions (depending on habitat availability), each consisting of 20 jabs/sweeps, were employed to gather a representative sample of the assemblages in this section of stream habitat. The contents of the net from each repetition were then sorted on a tray in the field and macroinvertebrates were placed in tubes of 70% ethanol. Two rounds of D-net collection were performed in the spring sampling period and one round was performed in the fall sampling period.

Like that of the D-net collection, three repetitions of kick-net collections were performed at each site with two rounds being conducted in the spring sampling period and one round in the fall sampling period. A one meter by one meter kick-net with 500-micron mesh was placed on the stream bed at an angle and one m^2 just upstream was disturbed to kick up benthic macroinvertebrates to be caught by the downstream kick-net. A chain along the bottom of the net kept it flush to the streambed, preventing invertebrates from slipping under and past the net. Occasionally sites would have low water flow limiting the number of repetitions performed (see Table 1). After disturbing the streambed, the net was kept in position until the water running through was clear. The net was then carefully transferred onto white plastic bags for easier sorting and to prevent loss of invertebrates that may fit through the net openings. Macroinvertebrates were then picked off the net with forceps and placed in tubes with 70% ethanol for preservation and later identification.

Table 1: Table of sampling methods and dates performed. Leaf pack collections are numbered based on sampling period and individual pack collection sequence. Site exceptions indicate the following: (*) two repetitions instead of three due to lack of habitat/low flows, (**) leaf pack missing due to washing out during storm, (***) leaf pack collected on 10/1/2022 due to Duke Forest Closure

Date	Sample Method	Site Exceptions
3/16/2022	D-Net	DS1*
3/30/2022	Kick-Net	DS1*
4/12/2022	Leaf Pack 1.1	
4/14/2022	Kick-Net	DS1*
4/19/2022	Leaf Pack 1.2	
4/25/2022	D-Net	DS1*
4/26/2022	Leaf Pack 1.3	DS1**, MC**
9/16/2022	D-Net	DS1*
9/30/2022	Leaf Pack 2.1	MC***
10/7/2022	Leaf Pack 2.2	
10/14/2022	Leaf Pack 2.3	
10/14/2022	Kick-Net	AS*, DS1*, MC*

Analysis:

Macroinvertebrates preserved in 70% ethanol were kept in the lab until identification was performed. Due to some limitations, and previous studies' precedent, samples were identified to family level to better allow comparison across the temporal gradient since restoration began. The identification of samples was performed using personal knowledge in conjunction with Maryland DNR's *Family-Level Key to the Stream Invertebrates of Maryland and Surrounding Areas.* Any vertebrates that were collected during sampling were returned to the stream and not included in this study. Also, any Oligochaetes collected were not identified further and were compared directly to the insect families collected as if they were taxonomically equivalent. A complete list of macroinvertebrate families collected, abundances, and collection information is presented in Appendix A.

A variety of analyses were performed to compare restored sites to the unrestored site with a selection of these being used to draw comparisons along the temporal gradient previously mentioned. The analyses performed were sourced from previous studies for direct comparisons and included order abundances, EPT percentage, Family-Level Shannon Diversity Index, and the NCBI. The NCBI was performed using Microsoft Excel, while all other analyses were performed using R statistical software. Comparisons between fall and spring sampling periods were not made as it is to be expected that there will be seasonal differences in assemblage makeup. Additionally, the variety of sampling techniques used in this study were to provide a more representative sample of the macroinvertebrate assemblages at each site and not for comparison of one method with another.

Order abundances within each sampling period and collection method were compared to prevent these differences from confounding the results. These comparisons were then graphed to compare each of the restored sites with the unrestored site. The response figures allowed for a visual comparison of order, diversity, and the relative abundances across research sites. No direct abundance comparisons were made as there was variability in the number of repetitions across collection methods and sites due to water level habitat availability.

Percent EPT uses the fraction of these three orders to the total abundance at each site as a predictor of habitat suitability. These three orders represent mayflies, stoneflies, and caddisflies and are typically more sensitive to poor water quality and habitat. A higher percentage indicates more dominance by these orders and can be used as a predictor of desirable stream habitat and better water quality. Percent EPT was calculated for each site and sampling method and graphed to compare across sites.

The Shannon Diversity Index is used to measure categorical data, typically that of species data, while taking into account evenness and abundance to compare diversity across sites/treatments. The index is calculated as follows:

Equation 1:

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

n_i =The number of individuals in species i; the abundance of species i.

S = The number of species.

N = The total number of all individuals

p_i = The relative abundance of each species, calculated as the proportion of individuals of a given species to the total number of individuals in the community: $\frac{n_i}{N}$

(Shannon 1948)

Though the Shannon Diversity Index is typically applied to species data (Eqn. 1), here it is used to calculate Family-Level Shannon Diversity Index, using families as the categorical variable rather than species. This Family-Level Shannon Diversity Index was calculated to account for abundance and evenness of macroinvertebrate families found at each research site for each of the sampling methods. The key comparisons here were made between the reference site, MC, and the research site in the middle of SWAMP, WT1.

The NCBI was used to determine a weighted average tolerance value at any given site that can function as a proxy for water quality. The tolerance values used in this index range from 0 to 10, 0 being very sensitive and requiring the best water quality and 10 being very insensitive and having the ability to survive in highly polluted waters. The index is calculated as follows:

Equation 2: $$\text{Biotic Index (BI)} = \frac{\text{Sum(TVi)(ni)}}{\text{N}}$$

TVi = ith taxa's tolerance value

ni = ith taxa's abundance value (1, 3, or 10)

N = sum of all abundance values

(NCDWR 2016)

The NCBI provides a value between 0 and 10 to be compared across research sites (Eqn. 2). The abundance values used in the equation are assigned based on the abundance of each family found at each site. If 1-2 individuals are collected, an abundance value of 1 is assigned. If 3-9 individuals are collected, an abundance value of 3 is assigned. Finally, if 10 or more individuals are collected, an abundance value of 10 is assigned (NCDWR 2016).

Water quality data was provided by the DUWC from monthly monitoring sampling conducted at the MC and WT1 sites. Water quality data parameters included Total Nitrogen (TN; μg/L), Unfiltered Total Phosphorus (UTP; μg/L), and Total Suspended Solids (TSS; mg/L). The data includes monthly data (with some gaps) for WT1 for the years 2006-2020 while MC includes data for the years 2006-2011. Substantial work including both stream restoration and stormwater BMP ponds were installed upstream of WT1 between 2012 and 2013. Differences between pre- and post-restoration water quality was compared for this research site. On the other hand, there was no substantial change in the upstream reach of MC since the beginning of the monitoring, allowing a meaningful comparison to still be made between the two sites even with the disparity in the water quality sampling period.

All the forementioned analyses were performed in this study to compare the restored sites in SWAMP to the unrestored site in the Duke Forest, the first research question outlined previously. Comparisons of these analyses to that of previous studies provided by the DUWC aims to answer the second research question posed for this study while also incorporating water quality at one of the restored sites.

Results:

Current Status:

Foundational analysis of current assemblages focused largely on abundance and order/family distribution values among reference and research sites. Figure 4 shows the total

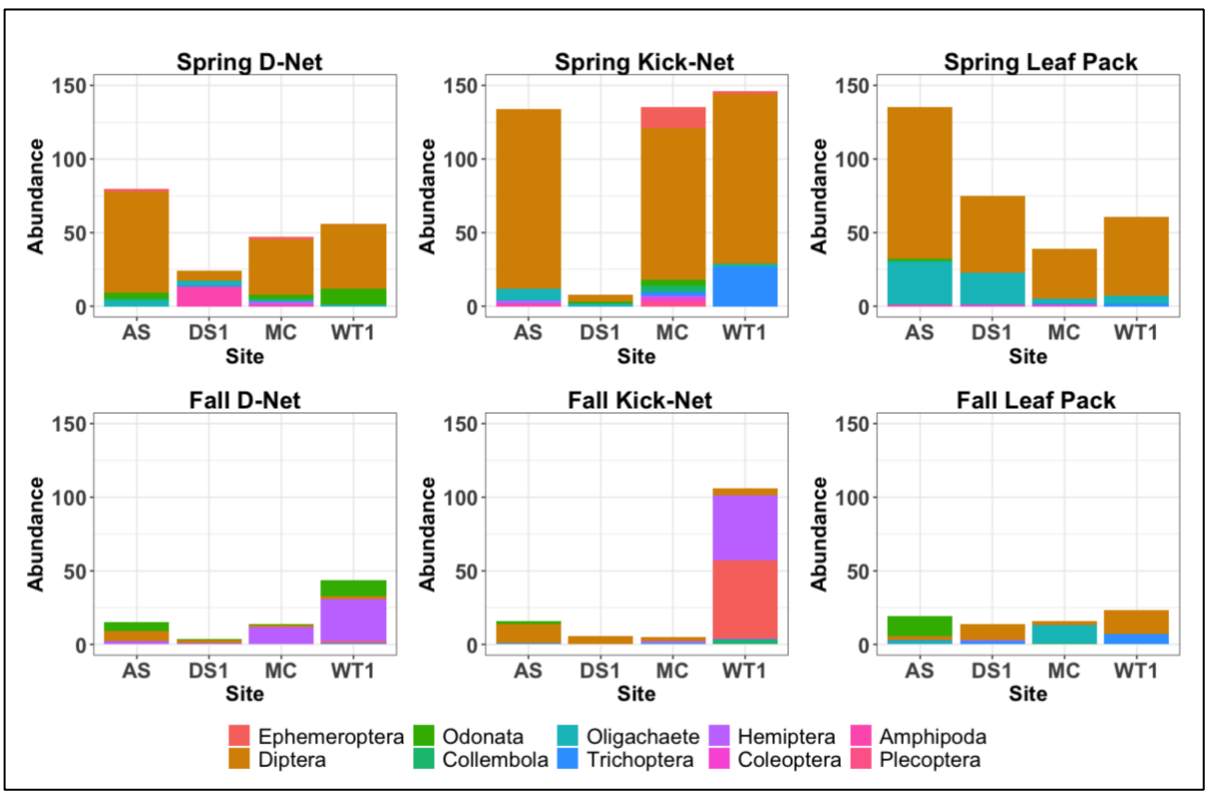

Figure 4: Abundances of each order collected for all collection methods in the spring and fall 2022 sampling periods.

abundance values at each site for both spring and fall collections of 2022 along with the breakdown of these abundances into order type. The spring season featured far greater overall abundances across all sampling methods and all sites. In most cases unrestored research site DS1 had the lowest overall abundances (typically only having two repetitions rather than three). The most common orders that were sampled were Diptera, Hemiptera, and Odonata. The subclass Olighachaete was also sampled often, particularly in leaf pack samples, possibly attributable to them occasionally getting buried in sediment from scouring stormflows. In most cases the research site WT1 showed the highest overall abundances compared to all other sites while the reference site MC was either the lowest, or second lowest, ahead of DS1. Total abundances across all sites and sampling methods ranged between 144 (Spring Kick-Net, WT1) and 14 (Fall Kick-Net, MC). Overall, abundances are quite variable across sites, seasons, and sampling methods with varying degrees of order dominance in each.

Similarly, EPT percentage showed a great deal of variability both in magnitude as well as between sites, seasons, and sampling methods (Fig. 5). Kick-Net samples showed the highest

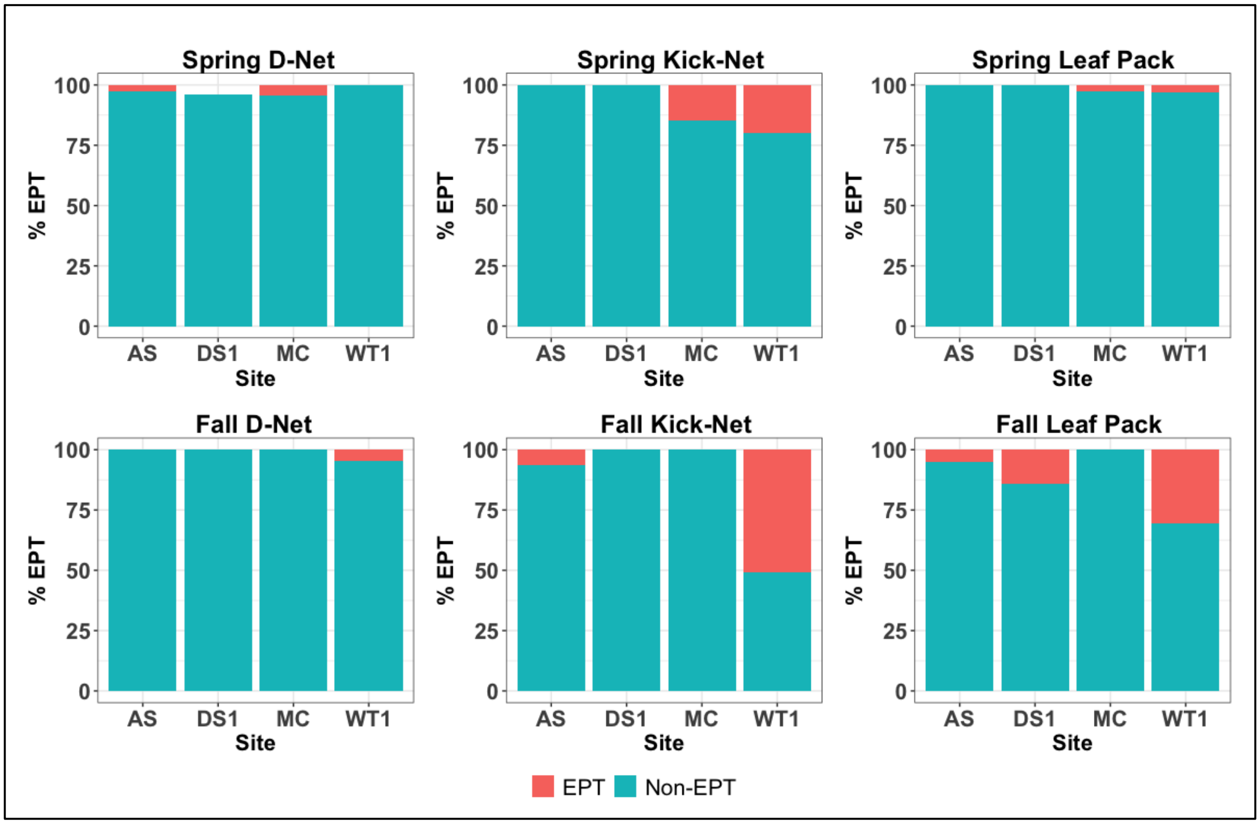

Figure 5: Percentage of each site and collection of EPT (red) and non-EPT (blue) individuals (2022)

EPT percentages, especially for the WT1 site (20%-50%), while the reference site (MC) also showed approximately 20% EPT. The reference (MC) site had an EPT percentage greater than 0% in half of the collections (all Spring collections) while WT1 had an EPT percentage greater than 0% in five of the six collections (all but Spring D-Net; Fig. 5). However, most individuals collected did belong to orders comprising EPT orders.

The Shannon Diversity Index from the spring collections using different collection techniques ranged from approximately 2.1 to 1.6 for the MC site while the WT1 site's value ranged from approximately 1.6 to 1.3. (Fig. 6). The diversity values for WT1 for all collection

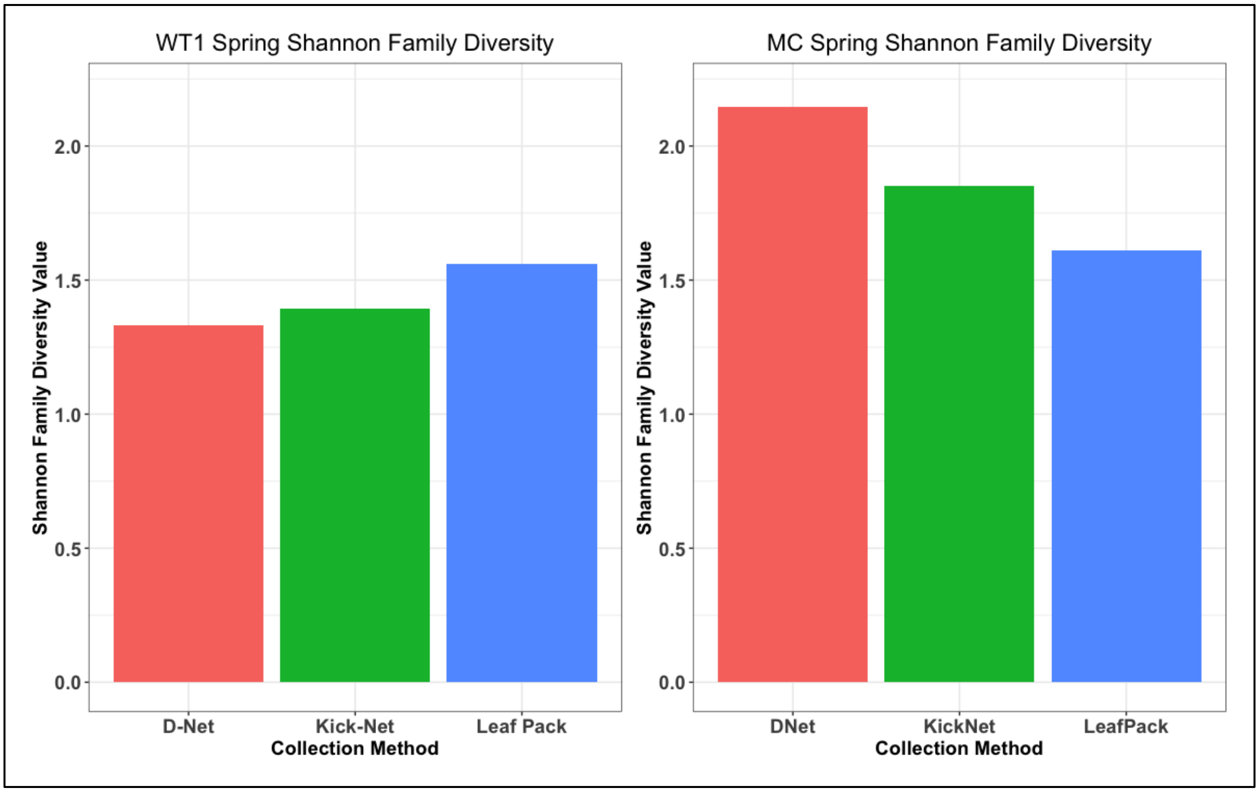

Figure 6: WT1 (A) and MC (B) Spring Shannon Diversity for each collection method in 2022.

methods were lower than the lowest diversity value from MC (leaf pack method). There was no discernable trend by collection methods on the Shannon Diversity values.

The NCBI was averaged across collection methods and was similar among both reference and research sites as well as between spring and fall seasons (Fig. 7). The mean NCBI for the reference site ranged between ~5.7 and 7.7 while the mean NCBI for WT1, the primary research site after upstream restoration ranged between ~5.8 and 6.5. The full range of all SWAMP sites was between ~5.8 and 6.9. There seemed to be a trend of lower NCBI values in the spring collection compared to the fall collection among all sites except DS1, though not a significant

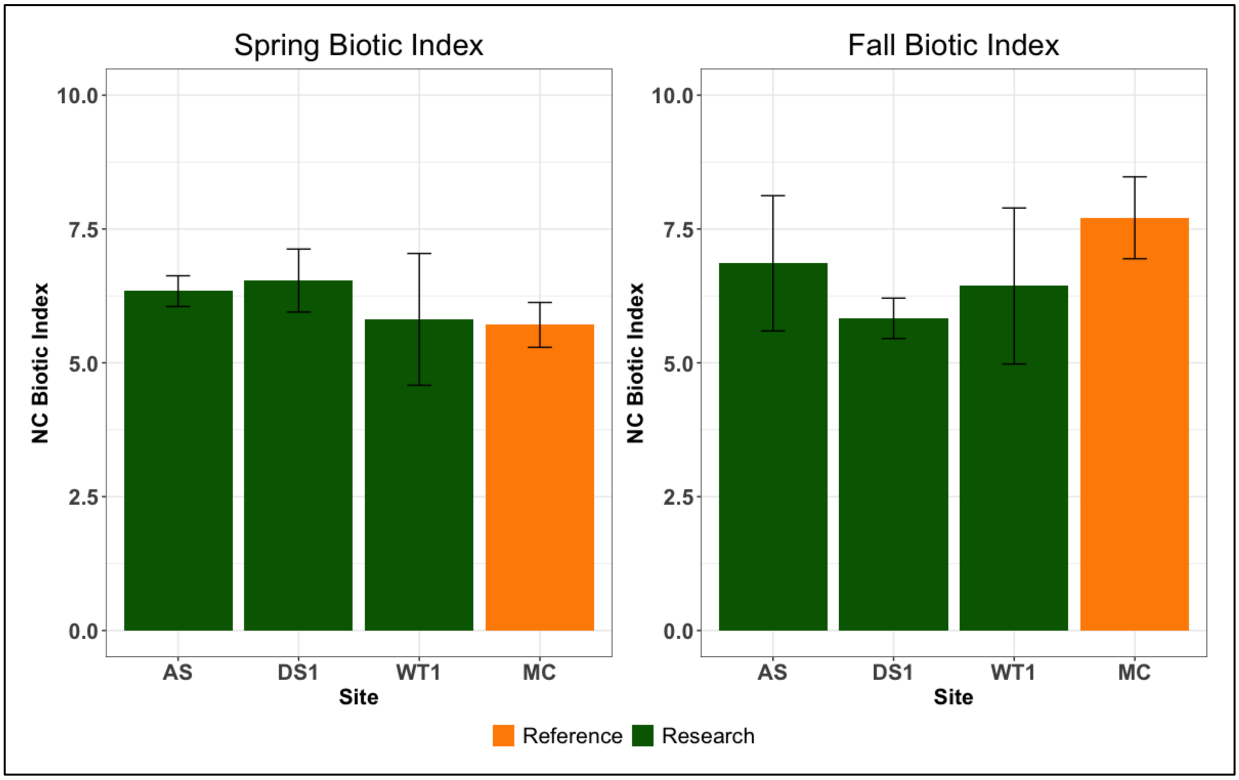

Figure 7: Spring (A) and Fall (B) Biotic Index for all sites at restored and reference sites on Sandy Creek in Durham, NC in 2022.

trend. The worst (highest) average NCBI was at the reference site in the fall sampling while the best (lowest) NCBI, though by a small margin, was also the reference site in the spring collection.

Temporal Comparisons:

The diversity values were also compared for MC and WT1 sites over time (Fig. 8). A Kruskal-Wallis Test was performed on the Shannon Diversity Values for WT1 and MC (Fig. 7) with no significant difference between sites. Samples from all three methods were then combined

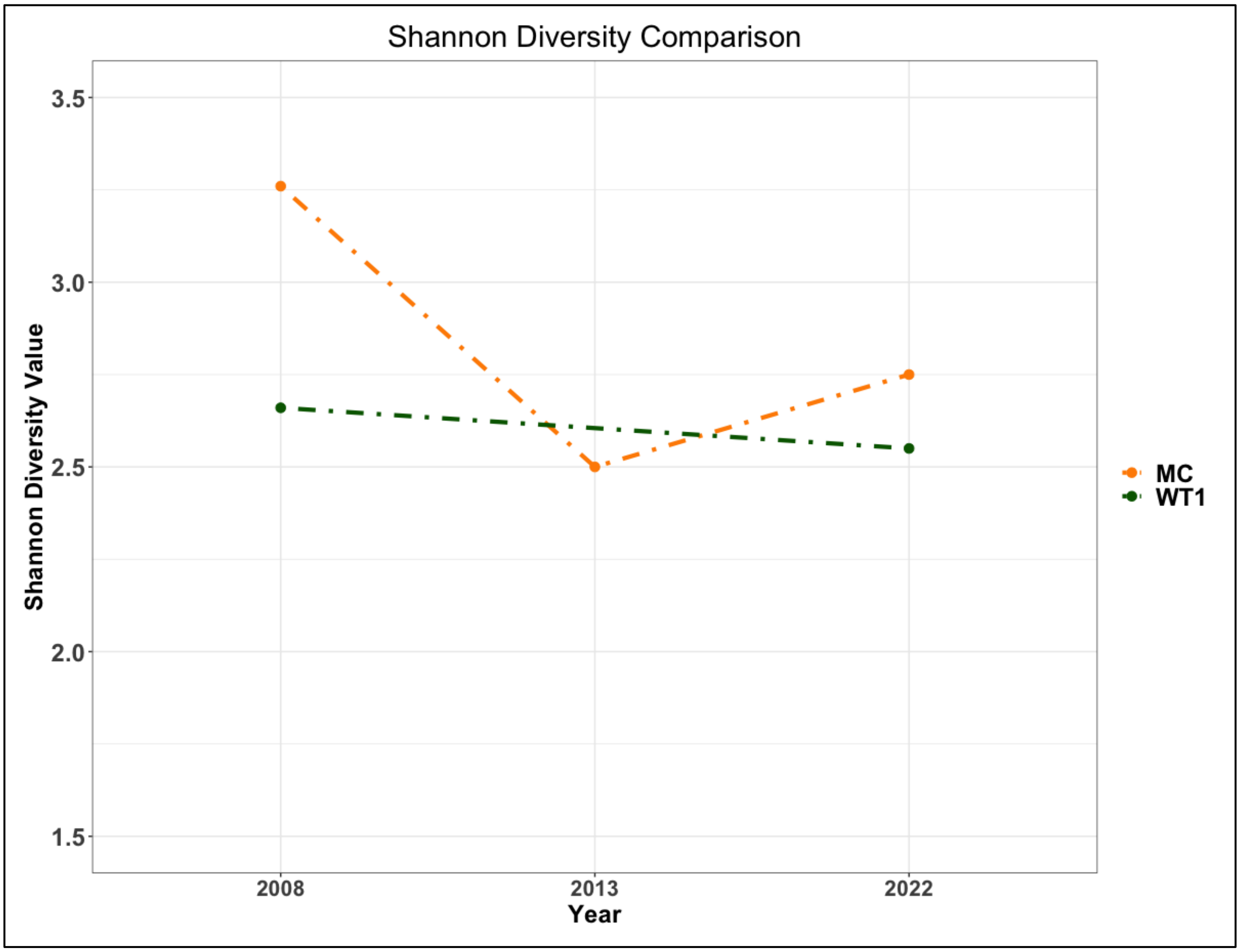

Figure 8: Temporal Shannon Diversity Index comparisons for MC, SWAMP average, and WT1 from 2008-2022. (Supplementary data: Still 2009 & Howington 2014)

and the Shannon Diversity Index was recalculated to get a value for each site, compared to previous studies here. The highest diversity value, approximately 3.3, was at MC in 2008, 20% higher than found at the unrestored WT1 site. The diversity values at MC dropped by nearly 17% while the values for WT1 dropped by around 4% over the span of 2008 – 2022. The reference site showed a 24% drop from 2008 to 2013 prior to rebounding to the current value of 2.75. By 2022 diversity at both MC and WT1 were similar compared to their 2008 values.

A temporal comparison of NCBI was also performed to investigate trends among the WT1 and MC sites over time (Fig. 9). Both WT1 and MC have shown decreasing trends over the

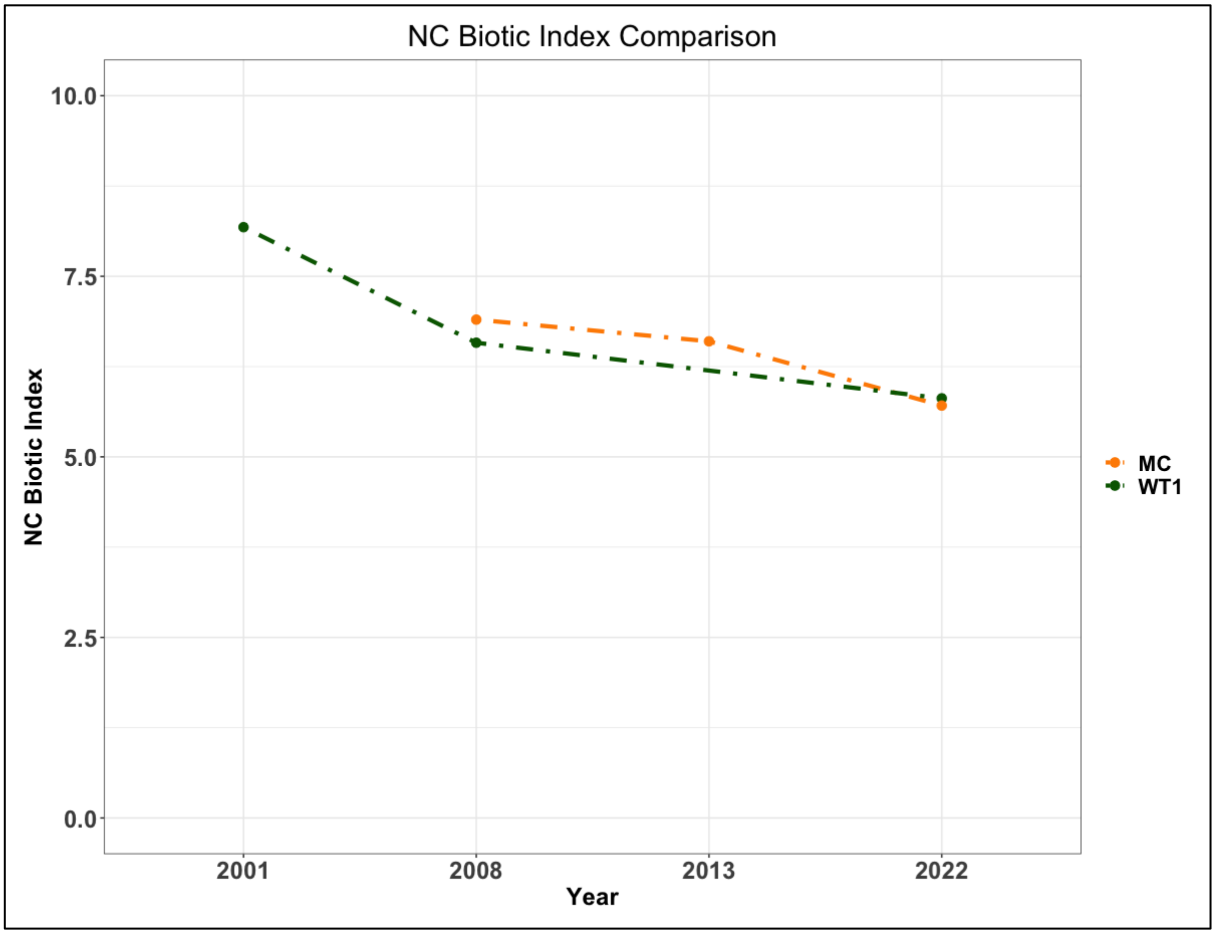

Figure 9: Temporal comparison of NCBI for MC, SWAMP average, and WT1 from 2001-2022. (Supplementary data: King 2001, Still 2009, & Howington 2014)

past two decades. MC was just under 7 in 2008 and dropped to approximately 5.7 in 2022 (~17% decrease) while WT1 was around 8.2 in 2001, dropping to just over 5.8 in 2022 (~29% decrease). The decrease in NCBI shown here is often used as a proxy for water quality as a decreasing NCBI indicates higher presence of taxa that is more sensitive to poor water quality. Because of this common relationship, several parameters of water quality were compared to the

NCBI values over time to investigate patterns that may be present or drivers of these decreasing changes.

A yearly boxplot series of each water quality parameter shows the yearly trends of each throughout the period of monitoring for WT1 as well as variability within each year (Fig. 10). In

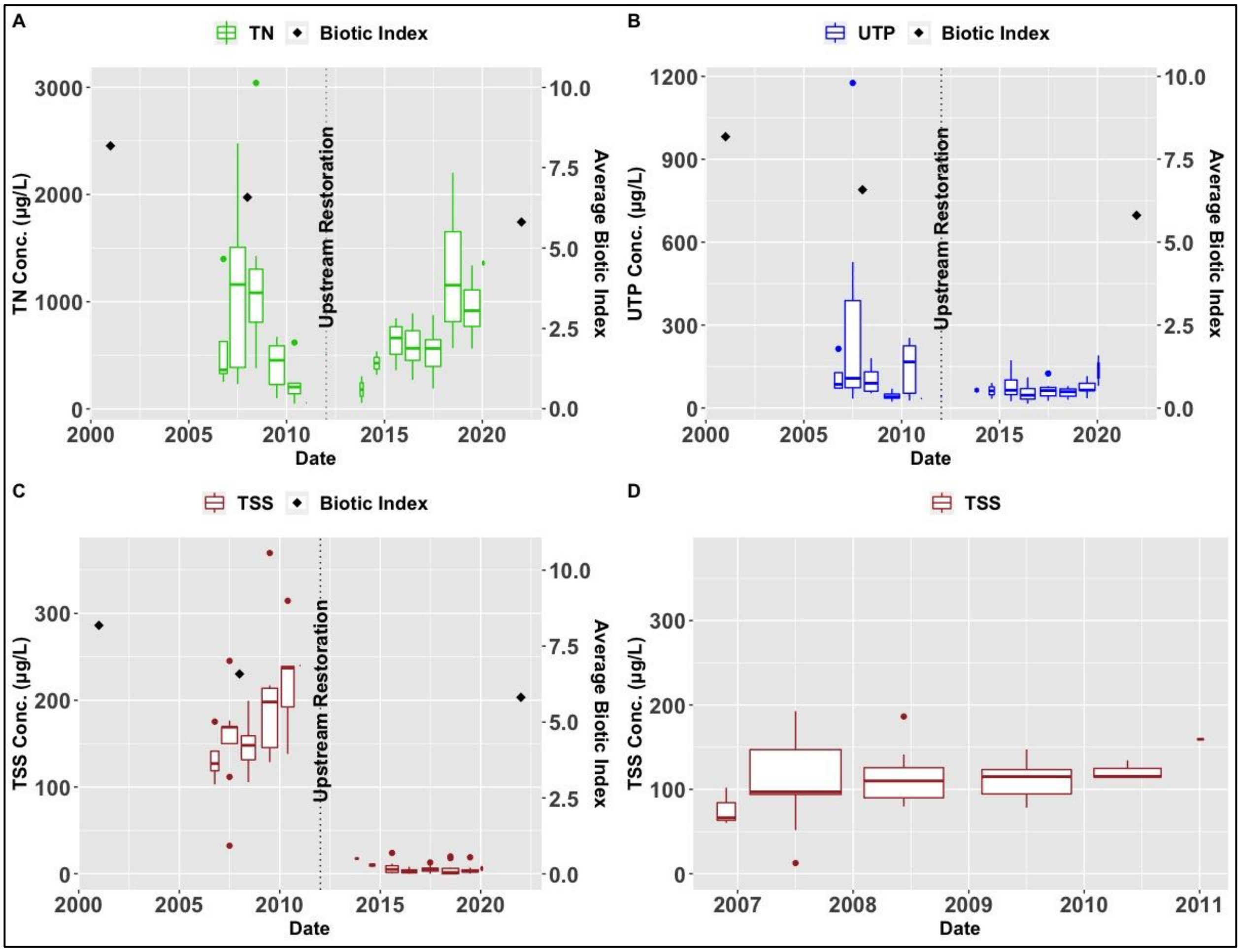

Figure 10: WT1 monthly water quality time series (A: TN, B: UTP, & C: TSS) with WT1 NCBI values. The biotic index is shown as diamonds in black. MC monthly water quality time series during pre-restoration of WT1 time period (D; note different x-axis time period).

all parameters but TN variability has decreased over time, especially for UTP and TSS. The strongest overall decreasing trend is in TSS with averages over time dropping from around 150 mg/L to 10 mg/L. While TN decreased from 2007-11 it showed a large increase in 2018-19, which could have affected macroinvertebrate responses. The peak of TN was around 3000 μg/L in 2008 with many years spent closer to roughly 500 μg/L, but more recently rising to > 1000 μg/L. Besides the one outlier in 2007, UTP has been stable over the period though there were larger peaks prior to the restoration upstream of WT1 in 2012 and 2013. The average biotic index response in relationship to these water quality changes shows a decrease in value over time which follows the decrease in UTP and TSS both in terms of magnitude and variability. MC TSS data is shown to compare differences in variability/magnitude and have average values that are much greater than that of WT1 after restoration (Fig. 10). Because of the short period of data available for MC, drawing long-term trend conclusions is difficult. There is not a guarantee that this trend has continued over time but there has been no restoration upstream of this site, making it unlikely that changes would occur in the water quality over time.

In addition to the averages of the key water quality parameters, the variability of records for each parameter within each year were also investigated. Both the average levels and the fluctuations of these concentrations are necessary to consider water quality as a driver of the change in NCBI over time. In both cases where the range converges to zero, 2012-2014 for WT1 and in 2011 for MC, there was only one month of reported data for that specific year. There is a consistent trend toward less variability within year variation at the WT1 site following the period of restoration though it is difficult to discern any definite changes in the pattern from year to year (Fig. 11). However, the MC site seems to show no sign of less variation within each year with

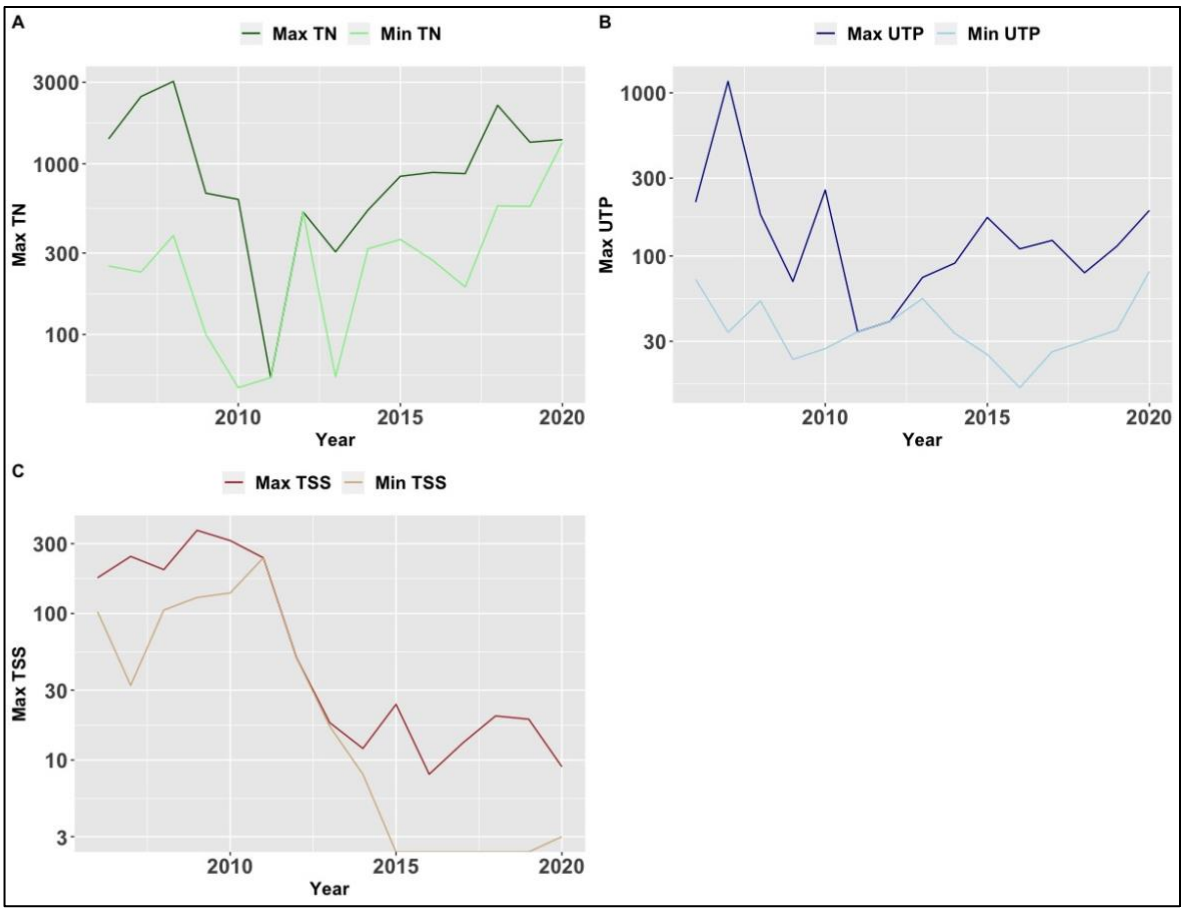

Figure 11: WT1 water quality yearly maximum and minimum time series (A: TN, B: UTP, & C: TSS)

some years having levels varying by as much as a factor of ten (most years for TN; TSS in 2007;

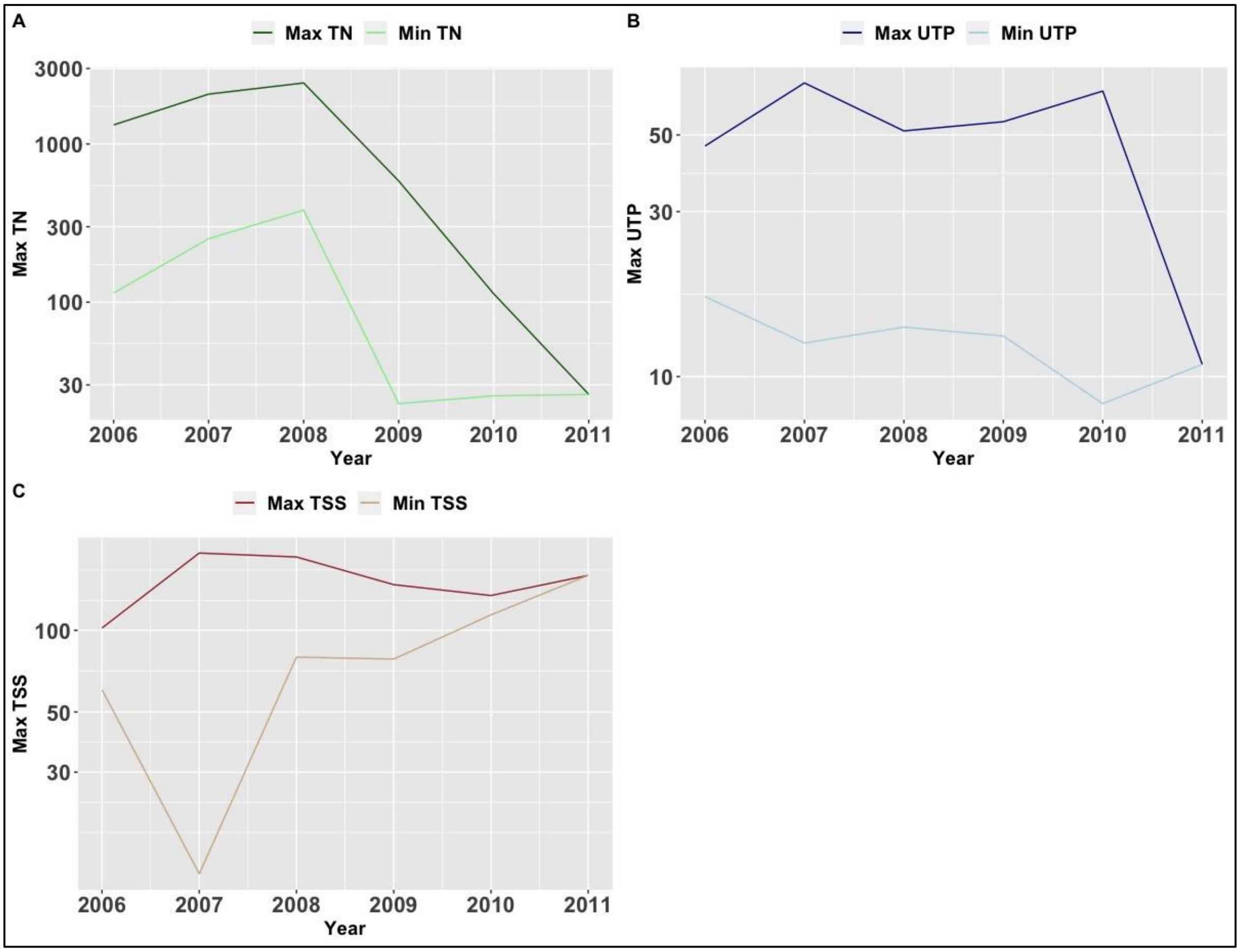

Figure 12: MC water quality yearly maximum and minimum time series (A: TN, B: UTP, & C: TSS)

Fig. 12). Though the monitoring period is shorter for MC, the magnitude of difference between maximum and minimum parameter levels seems greater than those in comparable WT1 monitoring periods.

Discussion:

Restored sites showed much higher abundances overall than that of the reference site (Fig. 4), though the reference site had higher diversity at the family level (Fig. 6). In comparison to previous studies conducted on SWAMP, diversity seems to have trended down while the number of orders present has increased (DUWC data). In 2004 and 2005 (Roberts 2005) there were only 4 and 5 insect orders, respectively, compared to there being 7 different orders

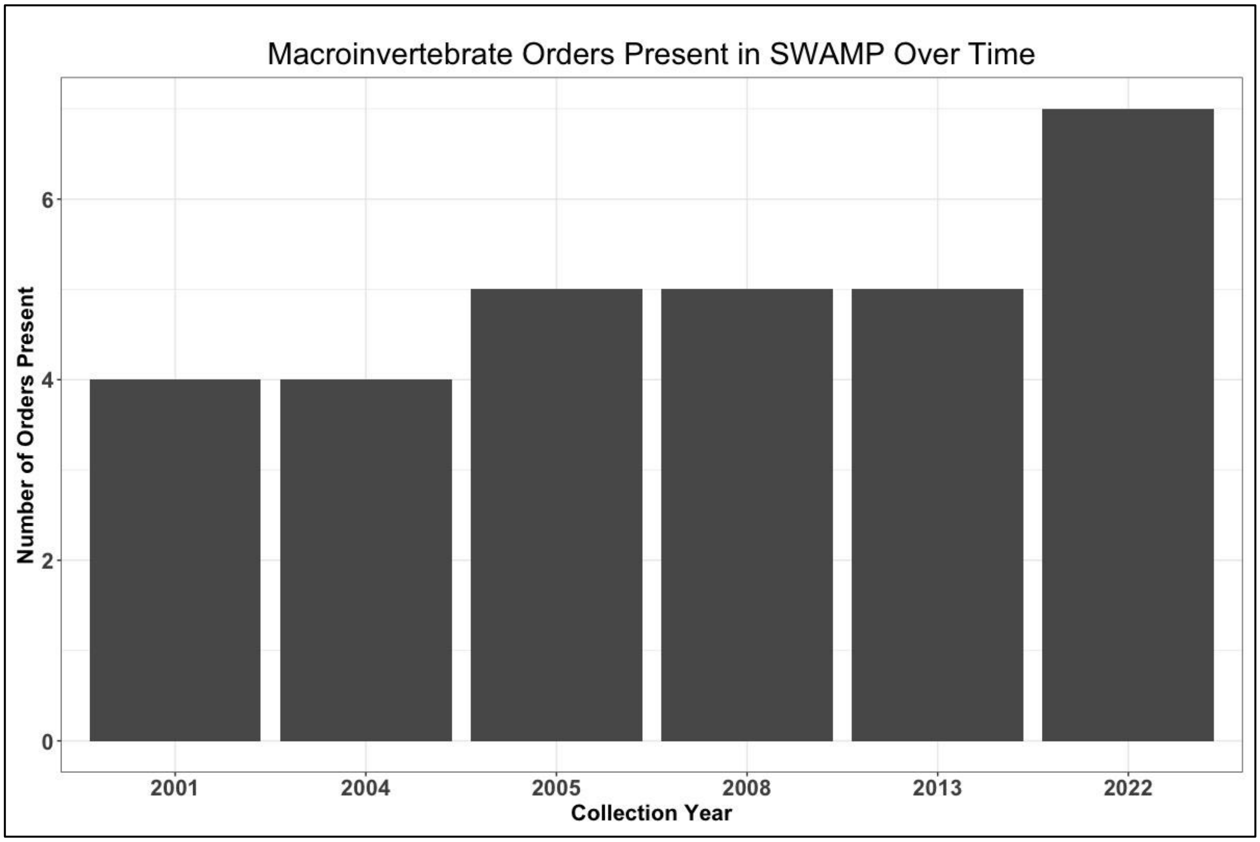

Figure 13: Number of macroinvertebrate orders collection in SWAMP in various years since prior to restoration (2001-2004), compared to post restoration years (2005-22).

collected at SWAMP sites in 2022 (Fig. 13). An increase in the number of orders could reflect improvement in habitat, though this would be more likely for orders in the EPT categories, commonly known as indicators of better water quality.

Percent EPT was quite variable among all sites included in this study but showed trends of being higher in the restored sites, specifically WT1, compared to that of the reference site (Fig. 5). In more instances there were EPT orders, especially Ephemeroptera and Trichoptera, present in the WT1 site than the MC site, though this relationship is likely not significant. Signs of improvement in EPT order populations are encouraging for the success of the restoration project and it is important to understand the timescales that it may take for restored streams to repopulate sensitive taxa in landscapes that are largely surrounded by other impaired waterways. Larger areas of impaired streams would likely delay recolonization by these species. Both order presence/abundance and percent EPT are commonly used as indicators of the quality of water and stream habitat. These parameters seem to show trends of increased order abundance and EPT percentage in the restored sites compared to the reference site, but these trends are not significant enough to draw conclusions on.

Unlike the trends previously outlined, the Shannon Diversity Index of WT1 showed slightly lower values than that of the reference site. However, when comparing diversity index values over time there is a trend in both WT1 and MC sites of decreasing diversity. As mentioned previously the decrease in diversity index at the reference site was of greater magnitude than the decrease in diversity at the restored site (60% and 50%, respectively). This could indicate a benefit from the restoration and be a result of the increased number of orders, and in turn families, present as well as improved abundance numbers.

The NCBI, like percent EPT, is commonly used as a proxy for water quality. Here I compare the NCBI of restored and reference sites in the spring and fall sampling periods of 2022. I also compare them temporally, in some cases over the span of the restoration project, to better understand the impacts of the restoration on macroinvertebrate communities and water quality as a potential driver of them. As seen in Figure 7, the NCBI of restored sites are all slightly higher than the reference site in the spring with the opposite being true for the fall 2022 sampling period, though this is not a significant trend. In this case similar results would indicate similar taxa sensitivity for each of the sites which is the goal of habitat restoration and ecological recovery. The NCBI not being significantly different indicates a return to the reference levels of taxa sensitivity, and water quality via proxy, in the restored reaches of Sandy Creek. The key goals of the SWAMP project center around nutrient and turbidity water quality issues that plague urban streams around the world. This decrease in NCBI could be driven by improvement in water quality parameters, addressed below.

NCBI values for both reference and restored sites decreased over time since the restoration and monitoring of macroinvertebrate assemblages began in 2001, with larger decreases at the WT1 site compared to the MC site. The overall decreasing trend among all sites through the years of monitoring is likely a result of increased water quality monitoring and protection in the watershed through programs put in place by the City of Durham. Many of these water quality standards and programs are outlined in Durham's Stormwater Management Plan (2019). It is likely that the management plan and other legislation for environmental protection put in place over the last two decades has contributed to overall improvements in NCBI, but this improvement has not been equal among restored and reference sites, indicating potential benefits resulting from the restoration project's improvements of habitat and water quality, particularly decreasing TSS.

To confirm the pattern of changing NCBI values as a proxy for water quality, water quality data from WT1 and MC was analyzed and compared to the NCBI values over time. Varying trends arose from the water quality data for WT1 including less variation in phosphorus concentrations, a large decrease with a subsequent recent increase in nitrogen concentrations, but the starkest trend was the large decrease in suspended solids (Fig. 10). This curbing of suspended solids is largely due to increased residence times resulting from reconnection of the stream/floodplain, riparian wetlands, constructed wetland cells, and BMP retention ponds upstream in 2012-13 (Fig.10). The substantial decrease in suspended solids is likely to be an important driver of improved NCBI at this site as this is the most dramatic change in water quality parameter trend over all parameters and sites. Increased residence times are likely also responsible for decreases in UTP as it allows more plant uptake and retention by the soils in the floodplain and wetland areas. All these trends are pivoting at the time upstream restoration is performed at WT1 in 2012 and 2013. The shift in pattern is likely a result of better floodplain water treatment occurring upstream, improving the water quality at this sampling site in the years following this construction, especially for suspended solids. Although there is less data for the MC site, the trends in each parameter likely carry to present day without much deviation as there have been no drastic changes that would affect its treatment of stormwater like that occurring in the upstream reaches of WT1. The lack of change in water quality parameters could be the reason behind less improvement in NCBI values over the research period.

The variability of monthly values within each year were compared for each site's sampling period to further understand potential drivers of macroinvertebrate assemblages. The effective ranges of WT1's water quality, specifically phosphorus and suspended solids show both a decrease in magnitude of maximums and minimums but also a shrinking of the range of concentrations following the restoration of areas upstream of this site (Fig. 10). This pattern is not present for nitrogen as the maximums drop slightly while the minimums actually increased slightly. The use of nitrogen fertilizers on urban lawns is a potential explanation for less reduction of nitrogen levels following the restoration. The range/variability of nitrogen decreased following the restoration efforts upstream, until the last few years (Fig. 10). There was some reduction due to the uptake, processing, and sequestration of nitrogen in the reconnected floodplains and wetlands, but additional inputs could be curbing that benefit.

Macroinvertebrate assemblages have long been used as a proxy for both water quality, which directly impacts their survivability and likelihood of colonization, as well as overall ecosystem production as they are a foundational portion of the food chain in these habitats. A variety of parameters can be used to measure this proxy, many of which are included in this study. Though abundances are an important part of this, the indices calculated from them can often be more useful as they consider multiple factors pertaining to these populations. The NCBI utilized here shows large improvements in both restored and reference sites since the SWAMP project began however, the magnitude of the improvements was greater for the restored stream. The restored site larger improvement may be due to the improvements of water quality parameters resulting from the upstream restoration on Sandy Creek. Results of this study seem to indicate a trend toward habitat recovery and repopulation of sensitive taxa in the restored sites, returning them closer to the reference site comparison, though it is difficult to make direct causal claims. The goal of restoration is to improve the ecology of impaired systems, and even though the restored sites in an urban setting may not be an example of a pristine waterway of abundant diversity, they do seem to be approaching or meeting the levels of the reference site included in this study. Incorporation of more water quality data and the investigation of more specific causality between water quality parameters and macroinvertebrate populations could improve the ability for conclusions to be made about impacts that the restoration has had on this urban system. A return to reference site biotic populations in the restored reaches of a stream is an important goal of any restoration project and the new data and long-term comparisons in SWAMP indicate restoration has improved both macroinvertebrate habitat and water quality. Returning streams to a near-natural state in an urban watershed is an ever-moving and nearly impossible target to achieve in an anthropogenically impacted world and this must be taken into consideration when judging the success of this restoration project and others like it.

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Appendix A:

Below are all the data collected among all three collection methods, for each site, in both the spring and fall of 2022. Also included are their relative abundances, collection dates, and family-level tolerance values.

Site	Collection Date	Collection Method	Order	Family	Tolerance Value	Abundance
AS	3/16/22	D-Net	Ephemeroptera	Baetidae	4	1
AS	3/16/22	D-Net	Diptera	Chaoboridae	NA	1
AS	3/16/22	D-Net	Diptera	Chironomidae	5.7	20
AS	3/16/22	D-Net	Odonata	Coenagrionidae	8.8	1
AS	3/16/22	D-Net	Collembola	Isotomidae	10	1
AS	3/16/22	D-Net	Oligachaete	Oligachaete	8.4	1
AS	3/16/22	D-Net	Diptera	Trichoceridae	NA	1
DS1	3/16/22	D-Net	Diptera	Chaoboridae	NA	4
DS1	3/16/22	D-Net	Trichoptera	Hydropsychidae	2.9	1
MC	3/16/22	D-Net	Odonata	Calopterygidae	6.2	1
MC	3/16/22	D-Net	Diptera	Chaoboridae	NA	14
MC	3/16/22	D-Net	Diptera	Chironomidae	5.7	6
MC	3/16/22	D-Net	Diptera	Culicidae	8.6	1
MC	3/16/22	D-Net	Hemiptera	Mesoveliidae	NA	1
MC	3/16/22	D-Net	Oligachaete	Oligachaete	8.4	1
WT1	3/16/22	D-Net	Diptera	Chaoboridae	NA	4
WT1	3/16/22	D-Net	Diptera	Chironomidae	5.7	26
WT1	3/16/22	D-Net	Odonata	Coenagrionidae	8.8	4
AS	3/30/22	Kick-Net	Diptera	Chaoboridae	NA	2
AS	3/30/22	Kick-Net	Diptera	Chironomidae	5.7	49
AS	3/30/22	Kick-Net	Oligachaete	Oligachaete	8.4	6
DS1	3/30/22	Kick-Net	Diptera	Chironomidae	5.7	3
MC	3/30/22	Kick-Net	Ephemeroptera	Ameletidae	2.4	5
MC	3/30/22	Kick-Net	Ephemeroptera	Baetidae	4	9
MC	3/30/22	Kick-Net	Diptera	Chaoboridae	NA	9
MC	3/30/22	Kick-Net	Diptera	Chironomidae	5.7	45
MC	3/30/22	Kick-Net	Coleoptera	Hydrochidae	NA	1
MC	3/30/22	Kick-Net	Trichoptera	Hydropsychidae	2.9	1
MC	3/30/22	Kick-Net	Collembola	Isotomidae	10	4

MC	3/30/22	Kick-Net	Trichoptera	Lepidostomatidae	1	2
MC	3/30/22	Kick-Net	Diptera	Simuliidae	5.9	1
MC	3/30/22	Kick-Net	Diptera	Tipulidae	5.3	1
WT1	3/30/22	Kick-Net	Diptera	Chaoboridae	NA	5
WT1	3/30/22	Kick-Net	Diptera	Chironomidae	5.7	57
WT1	3/30/22	Kick-Net	Trichoptera	Hydropsychidae	2.9	18
WT1	3/30/22	Kick-Net	Collembola	Isotomidae	10	1
WT1	3/30/22	Kick-Net	Trichoptera	Lepidostomatidae	1	3
AS	4/12/22	Leaf Pack 1.1	Diptera	Chaoboridae	NA	3
AS	4/12/22	Leaf Pack 1.1	Diptera	Chironomidae	5.7	27
AS	4/12/22	Leaf Pack 1.1	Amphipoda	Gammaridae	4	1
AS	4/12/22	Leaf Pack 1.1	Collembola	Isotomidae	10	1
AS	4/12/22	Leaf Pack 1.1	Oligachaete	Oligachaete	8.4	2
DS1	4/12/22	Leaf Pack 1.1	Diptera	Chironomidae	5.7	11
DS1	4/12/22	Leaf Pack 1.1	Coleoptera	Hydrochidae	NA	1
DS1	4/12/22	Leaf Pack 1.1	Oligachaete	Oligachaete	8.4	11
MC	4/12/22	Leaf Pack 1.1	Diptera	Chironomidae	5.7	17
MC	4/12/22	Leaf Pack 1.1	Coleoptera	Hydrophilidae	NA	1
MC	4/12/22	Leaf Pack 1.1	Trichoptera	Hydropsychidae	2.9	1
MC	4/12/22	Leaf Pack 1.1	Oligachaete	Oligachaete	8.4	2
MC	4/12/22	Leaf Pack 1.1	Diptera	Tipulidae	5.3	1
WT1	4/12/22	Leaf Pack 1.1	Diptera	Chaoboridae	NA	5
WT1	4/12/22	Leaf Pack 1.1	Diptera	Chironomidae	5.7	25
WT1	4/12/22	Leaf Pack 1.1	Diptera	Culicidae	8.6	1
WT1	4/12/22	Leaf Pack 1.1	Trichoptera	Hydropsychidae	2.9	2
WT1	4/12/22	Leaf Pack 1.1	Oligachaete	Oligachaete	8.4	2
AS	4/14/22	Kick-Net	Diptera	Chironomidae	5.7	67
AS	4/14/22	Kick-Net	Amphipoda	Gammaridae	4	2
AS	4/14/22	Kick-Net	Hemiptera	Hebridae	NA	2
AS	4/14/22	Kick-Net	Oligachaete	Oligachaete	8.4	2
AS	4/14/22	Kick-Net	Diptera	Simuliidae	5.9	4
DS1	4/14/22	Kick-Net	Diptera	Chironomidae	5.7	2
DS1	4/14/22	Kick-Net	Odonata	Coenagrionidae	8.8	1
DS1	4/14/22	Kick-Net	Oligachaete	Oligachaete	8.4	2
MC	4/14/22	Kick-Net	Odonata	Calopterygidae	6.2	4
MC	4/14/22	Kick-Net	Diptera	Chironomidae	5.7	46
MC	4/14/22	Kick-Net	Hemiptera	Hebridae	NA	1
MC	4/14/22	Kick-Net	Coleoptera	Hydrochidae	NA	2
MC	4/14/22	Kick-Net	Plecoptera	Perlodidae	2.1	3
MC	4/14/22	Kick-Net	Diptera	Simuliidae	5.9	1

WT1	4/14/22	Kick-Net	Diptera	Chironomidae	5.7	53
WT1	4/14/22	Kick-Net	Ephemeroptera	Heptageniidae	2.4	2
WT1	4/14/22	Kick-Net	Trichoptera	Hydropsychidae	2.9	6
WT1	4/14/22	Kick-Net	Oligachaete	Oligachaete	8.4	1
AS	4/19/22	Leaf Pack 1.2	Diptera	Chironomidae	5.7	55
AS	4/19/22	Leaf Pack 1.2	Odonata	Coenagrionidae	8.8	1
AS	4/19/22	Leaf Pack 1.2	Oligachaete	Oligachaete	8.4	18
DS1	4/19/22	Leaf Pack 1.2	Diptera	Chaoboridae	NA	1
DS1	4/19/22	Leaf Pack 1.2	Diptera	Chironomidae	5.7	40
DS1	4/19/22	Leaf Pack 1.2	Oligachaete	Oligachaete	8.4	11
MC	4/19/22	Leaf Pack 1.2	Diptera	Ceratopogonidae	6.8	4
MC	4/19/22	Leaf Pack 1.2	Diptera	Chironomidae	5.7	12
MC	4/19/22	Leaf Pack 1.2	Oligachaete	Oligachaete	8.4	1
WT1	4/19/22	Leaf Pack 1.2	Diptera	Chironomidae	5.7	9
WT1	4/19/22	Leaf Pack 1.2	Oligachaete	Oligachaete	8.4	2
AS	4/25/22	D-Net	Ephemeroptera	Baetidae	4	1
AS	4/25/22	D-Net	Odonata	Calopterygidae	6.2	2
AS	4/25/22	D-Net	Diptera	Chaoboridae	NA	8
AS	4/25/22	D-Net	Diptera	Chironomidae	5.7	39
AS	4/25/22	D-Net	Odonata	Libellulidae	9.6	1
AS	4/25/22	D-Net	Oligachaete	Oligachaete	8.4	3
DS1	4/25/22	D-Net	Diptera	Chironomidae	5.7	3
DS1	4/25/22	D-Net	Oligachaete	Oligachaete	8.4	3
DS1	4/25/22	D-Net	Amphipoda	Talitridae	5.5	13
MC	4/25/22	D-Net	Odonata	Calopterygidae	6.2	2
MC	4/25/22	D-Net	Diptera	Chaoboridae	NA	2
MC	4/25/22	D-Net	Diptera	Chironomidae	5.7	14
MC	4/25/22	D-Net	Amphipoda	Gammaridae	4	1
MC	4/25/22	D-Net	Ephemeroptera	Heptageniidae	2.4	2
MC	4/25/22	D-Net	Collembola	Isotomidae	10	1
MC	4/25/22	D-Net	Hemiptera	Mesoveliidae	NA	1
WT1	4/25/22	D-Net	Odonata	Calopterygidae	6.2	2
WT1	4/25/22	D-Net	Diptera	Chaoboridae	NA	4
WT1	4/25/22	D-Net	Diptera	Chironomidae	5.7	9
WT1	4/25/22	D-Net	Odonata	Coenagrionidae	8.8	4
WT1	4/25/22	D-Net	Diptera	Culicidae	8.6	1
WT1	4/25/22	D-Net	Odonata	Libellulidae	9.6	1
WT1	4/25/22	D-Net	Oligachaete	Oligachaete	8.4	1
AS	4/26/22	Leaf Pack 1.3	Diptera	Chironomidae	5.7	18
AS	4/26/22	Leaf Pack 1.3	Oligachaete	Oligachaete	8.4	9

WT1	4/26/22	Leaf Pack 1.3	Diptera	Chironomidae	5.7	14
WT1	4/26/22	Leaf Pack 1.3	Oligachaete	Oligachaete	8.4	1
AS	9/16/22	D-Net	Odonata	Calopterygidae	6.2	4
AS	9/16/22	D-Net	Diptera	Chironomidae	5.7	4
AS	9/16/22	D-Net	Odonata	Coenagrionidae	8.8	1
AS	9/16/22	D-Net	Hemiptera	Corixidae	8.7	1
AS	9/16/22	D-Net	Hemiptera	Gerridae	8	1
AS	9/16/22	D-Net	Odonata	Gomphidae	4.4	1
AS	9/16/22	D-Net	Diptera	Sciomyzidae	10	3
DS1	9/16/22	D-Net	Diptera	Chironomidae	5.7	2
DS1	9/16/22	D-Net	Hemiptera	Corixidae	8.7	1
DS1	9/16/22	D-Net	Odonata	Gomphidae	4.4	1
MC	9/16/22	D-Net	Diptera	Chironomidae	5.7	1
MC	9/16/22	D-Net	Odonata	Coenagrionidae	8.8	1
MC	9/16/22	D-Net	Hemiptera	Corixidae	8.7	1
MC	9/16/22	D-Net	Hemiptera	Gerridae	8	2
MC	9/16/22	D-Net	Hemiptera	Veliidae	9	9
WT1	9/16/22	D-Net	Ephemeroptera	Baetidae	4	1
WT1	9/16/22	D-Net	Odonata	Calopterygidae	6.2	1
WT1	9/16/22	D-Net	Diptera	Chironomdae	5.7	1
WT1	9/16/22	D-Net	Odonata	Coenagrionidae	8.8	9
WT1	9/16/22	D-Net	Diptera	Culicidae	8.6	1
WT1	9/16/22	D-Net	Odonata	Gomphidae	4.4	1
WT1	9/16/22	D-Net	Trichoptera	Hydropsychidae	2.9	1
WT1	9/16/22	D-Net	Hemiptera	Veliidae	9	29
AS	9/30/22	Leaf Pack 2.1	Diptera	Chironomidae	5.7	1
DS1	9/30/22	Leaf Pack 2.1	Diptera	Chironomidae	5.7	9
DS1	9/30/22	Leaf Pack 2.1	Trichoptera	Hydropsychidae	2.9	2
WT1	9/30/22	Leaf Pack 2.1	Diptera	Chironomidae	5.7	10
WT1	9/30/22	Leaf Pack 2.1	Trichoptera	Hydropsychidae	2.9	4
MC	10/1/22	Leaf Pack 2.1	Oligachaete	Oligachaete	8.4	2
AS	10/7/22	Leaf Pack 2.2	Diptera	Chironomidae	5.7	1
AS	10/7/22	Leaf Pack 2.2	Odonata	Coenagrionidae	8.8	13
AS	10/7/22	Leaf Pack 2.2	Trichoptera	Hydropsychidae	2.9	1
AS	10/7/22	Leaf Pack 2.2	Oligachaete	Oligachaete	8.4	1
DS1	10/7/22	Leaf Pack 2.2	Diptera	Chironomidae	5.7	1
MC	10/7/22	Leaf Pack 2.2	Oligachaete	Oligachaete	8.4	10
WT1	10/7/22	Leaf Pack 2.2	Trichoptera	Hydropsychidae	2.9	1
WT1	10/7/22	Leaf Pack 2.2	Diptera	Tipulidae	5.3	5
AS	10/14/22	Kick-Net	Odonata	Calopterygidae	6.2	1

AS	10/14/22	Kick-Net	Diptera	Chironomidae	5.7	13
AS	10/14/22	Kick-Net	Odonata	Gomphidae	4.4	1
AS	10/14/22	Kick-Net	Trichoptera	Hydropsychidae	2.9	1
DS1	10/14/22	Kick-Net	Diptera	Chironomidae	5.7	4
DS1	10/14/22	Kick-Net	Diptera	Tipulidae	5.3	2
MC	10/14/22	Kick-Net	Diptera	Chironomidae	5.7	1
MC	10/14/22	Kick-Net	Hemiptera	Gerridae	8	1
MC	10/14/22	Kick-Net	Oligachaete	Oligachaete	8.4	1
MC	10/14/22	Kick-Net	Diptera	Tipulidae	5.3	2
WT1	10/14/22	Kick-Net	Ephemeroptera	Baetidae	4	53
WT1	10/14/22	Kick-Net	Diptera	Chironomidae	5.7	1
WT1	10/14/22	Kick-Net	Trichoptera	Hydropsychidae	2.9	1
WT1	10/14/22	Kick-Net	Collembola	Isotomidae	10	3
WT1	10/14/22	Kick-Net	Diptera	Simulidae	5.9	3
WT1	10/14/22	Kick-Net	Diptera	Tipulidae	5.3	1
WT1	10/14/22	Kick-Net	Hemiptera	Veliidae	9	44
AS	10/14/22	Leaf Pack 2.3	Diptera	Chironomidae	5.7	1
AS	10/14/22	Leaf Pack 2.3	Oligachaete	Oligachaete	8.4	1
DS1	10/14/22	Leaf Pack 2.3	Diptera	Chironomidae	5.7	1
DS1	10/14/22	Leaf Pack 2.3	Hemiptera	Gerridae	8	1
MC	10/14/22	Leaf Pack 2.3	Diptera	Chironomidae	5.7	3
MC	10/14/22	Leaf Pack 2.3	Collembola	Isotomidae	10	1
WT1	10/14/22	Leaf Pack 2.3	Diptera	Chironomidae	5.7	1
WT1	10/14/22	Leaf Pack 2.3	Trichoptera	Hydropsychidae	2.9	2