

RESPONSES IN BASEFLOW NITROGEN CONCENTRATIONS TO WASTEWATER
MANAGEMENT APPROACHES IN THE NORTH CAROLINA PIEDMONT

By

Jennifer Richardson

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Directors of Thesis: Dr. Michael O'Driscoll and Dr. Natasha Bell

Major Department: Geological Sciences

Abstract

Falls Lake is currently classified as impaired because of elevated chlorophyll-a and turbidity levels associated with excess nutrient inputs. Anthropogenic and natural inputs of nutrients, especially nitrogen (N), into surface water bodies have increased over the last century, degrading water quality, and endangering environmental and public health. This research investigated the relationship between baseflow N concentrations and point and non-point sources of wastewater discharges within selected sub-watersheds of Falls Lake. Twenty-eight streams within sub-watersheds of Falls Lake, consisting of 20 septic and 6 sewer dominated wastewater management approaches (WMAs), were sampled monthly over a 1-year period. The Kruskal-Wallis statistical test and pairwise Wilcoxon rank-sum test were used to determine if there were statistically significant relationships between N concentrations and controlling factors. The results of this research show that median nitrate-nitrogen ($\text{NO}_3\text{-N}$) and total dissolved nitrogen (TDN) concentrations were elevated in septic dominated sub-watersheds, increasing in $\text{NO}_3\text{-N}$ and TDN concentration with increased onsite wastewater treatment systems (OWTS) density. $\delta^{15}\text{N}$ in NO_3^- isotopic ratios indicated that manure and septic/sewer effluent sources of NO_3^- were

present in surface water in all sub-watersheds regardless of WMA. The baseflow stream nitrate and $\delta^{15}\text{N}$ data in the Falls Lake sub-watersheds suggests that OWTS contribute NO_3^- to streams in high-density (HD) septic sub-watersheds. Elevated nitrogen concentrations in two sewer sub-watersheds suggest the possibility of legacy nutrients or leaking sewer lines contributing to in-stream $\text{NO}_3\text{-N}$ concentrations. The comparison between geologic setting and in-stream N concentrations showed significant associations between TDN and Triassic Basin septic sub-watersheds. Overall, the results of this study show that OWTS can contribute N to streams and there appears to be an influence based on geologic setting. The information provided by this study will be useful in further research to determine stream remediation approaches and to identify sensitive areas within this watershed and other urban watersheds, based on the methodology and interpreted relationships.

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Master of Science in Geology

By
Jennifer Richardson
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Director of Thesis: Michael O'Driscoll, PhD and Natasha Bell, PhD
Thesis Committee Members:
Alex Manda, PhD
Guy Iverson, PhD

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LIST OF ABBREVIATIONS

DON	Dissolved Organic Nitrogen	3
HD	High density (≥ 1 system/ha)	9
LD	Low density (<1 system/ha)	9
N	Nitrogen	1
NO ₃ ⁻	Nitrate	1
NO ₃ -N	Nitrate-nitrogen	1
NH ₄ ⁺	Ammonium	5
NH ₄ -N	Ammonium-nitrogen	3
OWTS	Onsite Wastewater Treatment Systems	2
PM	Passmore (site)	9
PR	Park Ridge (site)	9
TN	Total Nitrogen	2
WMA	Wastewater Management Approach	7

1.0 INTRODUCTION

Natural and anthropogenic inputs of nutrients into surface water bodies can lead to eutrophic and anoxic water conditions, causing water quality degradation (Smith et al., 1999). Eutrophication can occur when excess nutrient inputs are loaded to a water body (Gilbert et al., 2005), which may lead to the excess production of harmful algae and oxygen depletion (Carpenter, 2005). Since metropolitan regions and cities often rely on these water bodies as a source of drinking water (Voli, 2012), the effects of eutrophication may lead to changes in taste, odor, and clarity, as well as potentially harmful algal blooms and fish kills, which lead to health concerns for the surrounding population (Delkash et al., 2018). A prominent health concern, mostly affecting infants, that results from excess nitrate (NO_3^-) in potable water is methemoglobinemia or ‘blue baby syndrome,’ which is a result of insufficient amounts of oxygen in the bloodstream that can lead to brain damage or death (Self & Waskom, 1992). Examples of these metropolitan regions and cities in the southern Piedmont that rely on reservoirs for water supply are Raleigh, NC relying on the Falls Lake reservoir (Voli, 2012), Atlanta, GA relying on Lake Lanier and Allatoona Lake (MNGWPD, 2020), and Charlotte, NC relying on Lake Norman and Mountain Island Lake (CCG, 2022).

Falls Lake is currently classified as impaired due to elevated nutrient concentrations (NC DEQ, 2022a), which can facilitate eutrophic conditions and possibly endanger environmental and public health. The United States Environmental Protection Agency (US EPA) has set limits of 10 mg/L on nitrate-nitrogen ($\text{NO}_3\text{-N}$) in drinking water sources (US EPA, 2022a) per the Safe Drinking Water Act to help prevent health issues associated with excess nitrogen (N); however, algal blooms can still occur when N concentrations are much lower (0.5 $\text{NO}_3\text{-N}$ mg/L; Zeng et al., 2016). Because of this low threshold, North Carolina is in the process of developing numeric

nutrient water quality criteria to prevent surface water nutrient pollution (US EPA, 2022b; NC DEQ, 2022b). Based on previous work using loading averages from six sub-watersheds surrounding Falls Lake, average total nitrogen (TN) loads to the lake were estimated to be 825 kg/day with TN predominantly contributed by agricultural land cover (27%), point sources (24%), forested land cover (16%), septic systems (14%), and developed land (13%) sources (NCDENR, 2009). However, the onsite wastewater treatment system (OWTS) nutrient inputs to the lake were estimated using a watershed model and earlier estimates based on the US Census in 1990, introducing potentially high uncertainties in the estimates of nutrient inputs. More research is needed to improve the understanding of nutrient inputs from OWTS and non-point nutrient sources to Falls Lake.

1.1 Nitrogen Inputs

Understanding the association between land cover and nutrient concentration is required for understanding the source of impairment to water bodies and to be able to better determine areas of possible impairment (Tasdighi et al., 2017). Working in the Piedmont region of Maryland, Groffman and others (2004) documented that impervious surfaces significantly increased runoff of nutrients into surface water bodies during storm events but have little influence on nutrient loading during baseflow conditions. Another study completed in the Piedmont region of Maryland determined that $\text{NO}_3\text{-N}$ concentrations in ground water, contributing to baseflow in nearby streams, were higher in agricultural areas than forested and urban areas (McFarland, 1995). Due to increased crop and livestock production in the Piedmont in recent decades (~1960s-recent), the application of fertilizers and manure has also increased, making agricultural activities an increasing non-point source of nitrogen (McFarland, 1995).

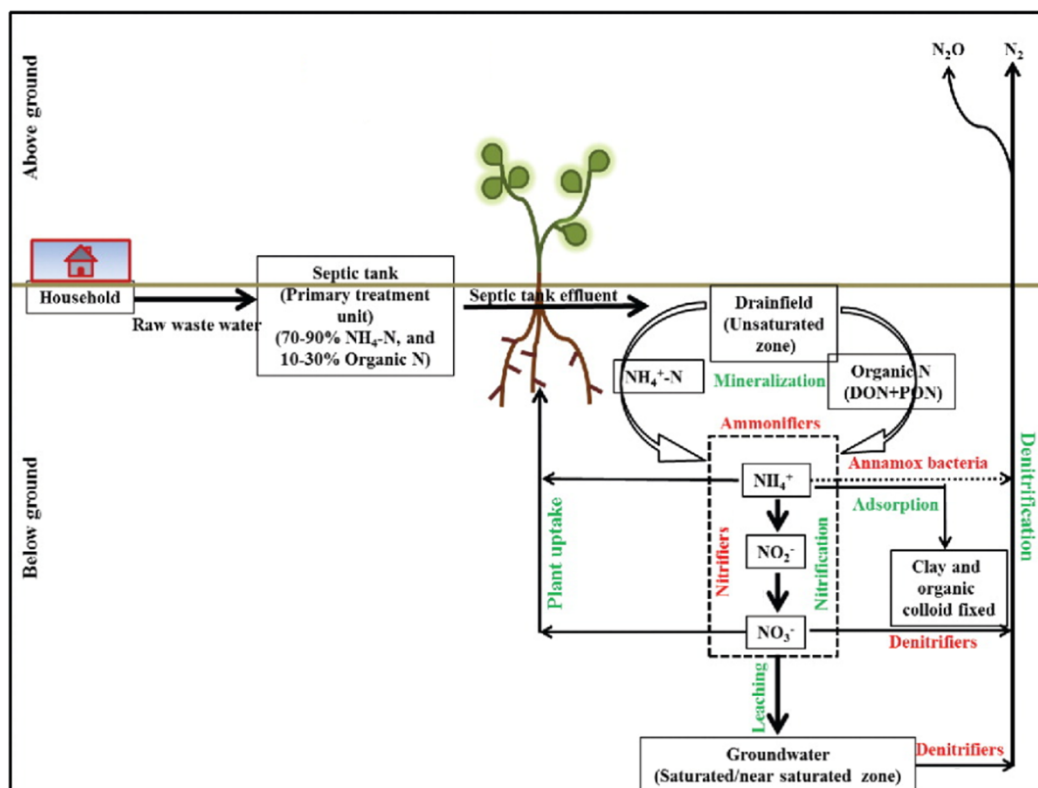
Spatial variations of soil types throughout the sub-watersheds observed in the research conducted in this report also have different effects on the treatment of nutrient rich waters or wastewater. D'Amato and others (2016) completed a study in the Chesapeake Bay, focusing on nitrogen inputs from OWTS, showing a direct association between grain size and TN loading. Sandy soils, with larger grain sizes, allowed around twice the amount of TN (4.2 kg/cap/yr) to load into the estuary than clayey soils (2.3 kg/cap/yr) (D'Amato et al., 2016). TN reduction has been found to be around 76% for clayey soil (Humphrey et al., 2019) and around 35% for sandy soil (D'Amato et al., 2016). However, other work has also shown that fine-grained poorly drained soils do not allow for adequate infiltration, thus contributing excess N to baseflow (USDA, 2021). This decrease in drainage within the soils increases the likelihood of lateral flow on layers of very low permeability, decreasing the functionality of the OWTS. The decrease in residence time due to lateral flow also decreases the amount of ammonium-nitrogen ($\text{NH}_4\text{-N}$) and dissolved organic nitrogen (DON) attenuated in the vadose zone (NC DEQ, 2021; NC DEQ, 2022c).

The North Carolina Division of Water Quality has determined that atmospheric N deposition increases with increasing urbanization and is estimated to supply around 10-40% of new N to N-sensitive waters (NC DEQ, 2008). As a result of this, atmospheric N deposition has been determined to be a significant source of N to the Falls Lake watershed, along with many others throughout North Carolina (NC DEQ, 2008).

Compared to these various non-point sources of nitrogen inputs (e.g., runoff and atmospheric deposition), there is less information on the contribution of nitrogen to ground- and surface water from OWTS due to variability from site to site (NC Policy Collaboratory, 2021).

1.2 Onsite Wastewater Treatment Systems

OWTS treat raw wastewater and release effluent into the subsurface for soil treatment. Eventually, effluent infiltrates into underlying groundwater, which can result in elevated N in downgradient ground- and surface water (Hoghooghi et al., 2017). Within the septic tank, waste separates out into liquid and solid waste, primarily through density stratification of heavier solids and lighter fats, oils, and greases. The tank then acts as an anaerobic bioreactor, where bacteria break down some of the organic matter before moving on to the second step of treatment (US EPA, 2002). The wastewater effluent moves from the septic tank, through the drain-field, where it infiltrates the surrounding soil (Figure 1) and additional treatment can occur through biological processes, oxidation, adsorption to surrounding particles, and filtration before reaching the nearby groundwater (US EPA, 2002).



processes through which nitrogen is transformed and removed are shown in green and the microorganisms which transform or remove the nitrogen are shown in red (modified from Lusk et al., 2017).

1.2.1 Nitrogen Cycling of Wastewater in OWTS

The wastewater within septic tanks contains predominantly ammonium (NH_4^+) and organic nitrogen forms of nitrogen (Wilhelm et al., 1994). Within the septic tank, anoxic conditions allow anaerobic bacteria to break down the organic N converting it to NH_4^+ (Xuan et al., 2012). Once this waste travels through the drain-field to the subsurface, aerobic oxidation occurs, converting NH_4^+ to NO_3^- through nitrifying bacteria. NH_4^+ can also be attenuated in unsaturated soils from cation-exchange processes, which occurs from attraction of the positive charge associated with ammonium to the negative charges on the soils (Buss et al., 2004). After nitrification (NH_4^+ to NO_3^-), the resultant NO_3^- may be removed and converted to N_2 through the process of denitrification (Wilhelm et al., 1994). However, NO_3^- often leaches in the unsaturated zone since the negative charge of the NO_3^- repels the negative charges on the soils (Buss et al., 2004). The denitrification process occurs in anaerobic conditions with the presence of denitrifying bacteria, oxidized forms of N, and highly soluble carbon (C) source (Martens, 2005). However, C is often limited in groundwater, resulting in inadequate nitrogen treatment during the denitrification process, unless the N-rich water is processed by natural or engineered features that contain abundant C (e.g., riparian buffers and denitrifying bioreactors). Since NO_3^- is mobile in groundwater, N transport to local surface water may be increased in subsurface environments where nitrification and denitrification are inadequate or where there are higher hydraulic conductivities, resulting in concentrated plumes of nutrients from contaminant sources (O'Driscoll et al., 2021; Wilhelm et al., 1996).

A previous study within this same study area determined that the mean attenuation of TDN from OWTS to streams was 84.7% and the median attenuation was 97%, suggesting that only 3-15% of OWTS N may be transmitted from these systems to streams during baseflow

conditions (O'Driscoll et al., 2021). If these OWTS do not work properly, they will not remove the adequate amount of nutrients, increasing concentrations in the surrounding ground- and surface waters. However, factors such as water table depth, water use, soil characteristics, forested riparian buffers, and other site conditions can also affect the attenuation. For example, a vertical separation between the water table and the OWTS drain-field is needed for aerobic wastewater treatment (Humphrey et al., 2021). When there is little to no separation, the septic effluent has less interaction with the unsaturated soil and microorganisms within the soil, impairing the nitrification-denitrification process and N treatment (DOH, 1990). This lack of nitrification-denitrification allows for greater amounts of NO_3^- and nitrite (NO_2^-) to accumulate within the subsurface and groundwater, impairing both ground- and surface water.

1.2.2 Wastewater Management Approach

Previous work suggests that general sources of excess nutrient loading within urban watersheds are often wastewater and fertilizers (Delesantro et al., 2022; He & Lu, 2016; Hoghooghi et al., 2016). Iverson et al. (2018) also determined that sub-watersheds with higher OWTS densities (≥ 1 system/ha) reported elevated nutrient exports (4.9 kg-N/year/ha) relative to sub-watersheds with lower (<1 system/ha) OWTS densities (1.0 kg-N/year/ha) and sewer dominated sub-watersheds (1.4 kg-N/year/ha) during baseflow conditions. Delesantro and others (2022) working in the Jordan Lake watershed supported these findings by determining that the primary source of baseflow N in developed catchments was wastewater, contributing around 61% of NO_3^- loading in septic served sub-watersheds and around 49% from sewer drained sub-watersheds.

Other work within a similar setting, an urban watershed in the Atlanta, GA Piedmont region, also showed that septic system density influences baseflow stream nutrient

concentrations, with TN and NO₃-N concentrations linearly increasing with increases in septic system density above a threshold of 75 OWTS per square km (0.75 systems/ha) (Hoghooghi et al., 2016). This same research also determined that the main form of N found in-stream was NO₃-N and was likely a result of the input of septic systems effluent during baseflow conditions, rather than runoff events (Hoghooghi et al., 2016).

Although there are an increasing number of studies conducted in the Piedmont, most of them have occurred at the site scale or within smaller watersheds and include less variables (~4 sample locations) used to understand nitrogen treatment from OWTS (Humphrey et al., 2019), slightly generalizing the watershed. Based on this lack of information on nutrient inputs into this Piedmont water supply at a watershed scale, further work is needed to fill the gap in understanding the main contributors of point and non-point sources of nutrient inputs throughout the watershed.

1.3 Objectives

The primary objective of this research was to evaluate how wastewater management approaches (WMAs) and OWTS density affect in-stream N concentrations during baseflow conditions in sub-watersheds that drain into Falls Lake. Geologic/soil settings and land cover within these sub-watersheds were also analyzed to evaluate their influence on stream N concentrations. The main hypothesis tested during this research was that N concentrations at watershed outlets were positively associated with OWTS density, suggesting that sub-watersheds with elevated OWTS densities contribute more N to Falls Lake. It was also hypothesized that sub-watersheds within the Triassic Basin that contained poorly drained soils would be associated with higher in-stream N concentrations and loading to the lake.

This research aims to provide a better understanding of wastewater-derived point and non-point sources of N inputs to Piedmont water supply reservoirs and identifies areas in which best management practices (BMPs) could potentially reduce N loading. These results can also broadly increase the understanding of the influence of WMAs on N loading in nutrient-sensitive watersheds.

2.0 METHODOLOGY & STUDY AREA

2.1 Site Selection and Sample Collection

2.1.1 Study Area

The Falls Lake watershed, which drains an area of ~200,000 hectares (ha) in the Piedmont region of central North Carolina and covers portions of Durham, Wake, Person, Granville, Franklin, and Orange counties, is underlain by multiple distinct geologic units (Figure 2). Falls Lake begins at the confluence of the Eno, Flat and Little rivers, and is approximately 28 miles in length. It discharges into the Neuse River at the Falls Lake Dam (NC DEQ, 2022c; O'Driscoll et al., 2021). Based on monthly averages from 1981-2010, approximately 47 inches of rainfall per year are deposited within the Falls Lake watershed (US Climate Data, 2021), with median streamflow from the watershed to the lake ranging from around 100 cfs to 1400 cfs depending on the time of year (Devineni et al., 2008; O'Driscoll et al., 2021).

The sites for this study were selected to represent a range of OWTS densities and geologic conditions to evaluate the influence of these factors on stream N concentrations and loading (O'Driscoll et al., 2021). Based on this preliminary research, 28 sites were selected with a range of OWTS density of ~0-2.3 systems/ha and a range sub-watersheds area of ~9-458 ha (Table D2).

Eight of the 28 headwater streams were located within sewer dominated sub-watersheds, acting as the control sites, whereas the remaining 20 streams were located within sub-watersheds that predominantly utilized septic systems. Two of these sites, Park Ridge (PR) and Passmore (PM), were selected to evaluate how nutrient concentrations change downstream as one is downstream of the other in the same sub-watershed. The septic sub-watersheds in this study can be further categorized into WMA groups consisting of high-density (HD) and low-density (LD)

septic by determining the number of OWTS per hectare of land. A sub-watershed scale density threshold of ≥ 1 OWTS per hectare was used to subdivide sub-watersheds into HD and LD. Similar studies conducted in urban, Piedmont regions used thresholds of 0.75 and 1 system per hectare when separating HD and LD sub-watersheds (Hoghooghi et al., 2016; Iverson et al., 2018; O'Driscoll et al., 2021).

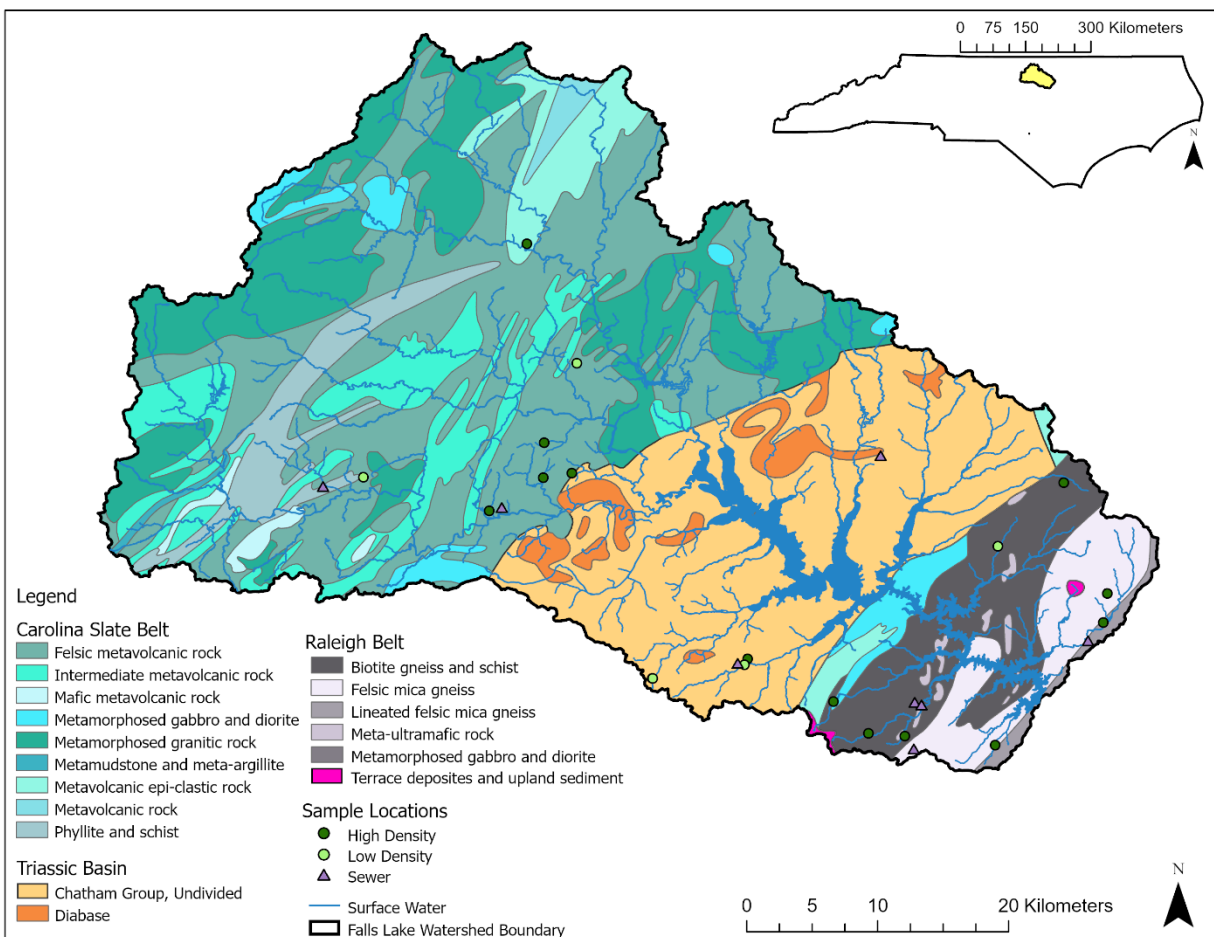


Figure 2. Falls Lake watershed geology and site map with an inset map of the state of North Carolina showing the study area at the state scale. Further site information is provided in Tables B1 and B2 in the Appendix.

2.1.2 Geologic and Soil Setting

The differences in rock and soil types among the three distinct geological regions in the Falls Lake watershed (Figure 2) may influence drainage to streams across the watershed (Boggs et al. 2013). The Carolina Slate Belt, present in the westernmost part of the study area, consists of fine-grained, platy, and moderately to highly fractured igneous rocks with some low-grade metamorphism (Rogers, 2006). Soils in the Slate Belt are prominently Georgeville and Herndon silty loams, hydrologic group B (moderately draining soils with moderately low runoff potential) (Figure A2), characterized by moderate infiltration rates (USDA, 2021), allowing for N to be attenuated or taken up through denitrification (Buss et al., 2005). The Triassic Basin, in the central part of the study area, consists of sedimentary rocks, predominately interbedded conglomerate, sandstone, siltstone, and clay stones, intruded by igneous rocks (Bain & Brown, 1981). Soils in the Triassic Basin are predominantly the Creedmoor, White Store, and Mayodan, which consist of sandy clay loam, hydrologic group C/D (slowly draining soils with high runoff potential) (Figure A2), with a higher possibility of runoff due to the low permeability (USDA, 2021). The easternmost terrane in the watershed is the Raleigh Belt. Rocks in this belt consists of meta-igneous and meta-volcanic rocks, including fractured gneiss, gabbro, granodiorites, and diorites; soils are predominately the Palocet and Cecil sandy loams. These soils consist of hydrologic group A (fast draining soils with low runoff potential) (Figure A2), characterized by highly permeable soils (USDA, 2006), allowing for groundwater flow but may increase N leaching rates into surface water (Buss et al., 2005).

2.1.3 Sample Collection

Sample collection sites were selected based on WMAs, land cover, and their location within the watershed (Figure 2). The land cover data were compiled from *StreamStats 4* using

the 2011 national land cover database (NLCD) (USGS, 2022), which estimates land cover percentages from each sub-watershed (Figure A3; Table D2). Septic system data were obtained from the health departments in Durham, Franklin, Granville, Orange, Person, and Wake Counties where some data were already geospatially referenced in 2020 and additional data were collected through the collaboration with Brown & Caldwell and county health departments. For this research, septic system age and maintenance records were not considered in analysis. However, septic system density was estimated by dividing the number of septic systems per sub-watershed by the area of the sub-watershed. Although the NLCD land cover percentages have not changed much since 2011, one limitation of this work is that new data has been published from data collected in 2019 that may affect regression results comparing land covers and N concentration.

Monthly sample collection of the 28 sites began September 2020 and continued until August 2021. At each site, 500 mL bottles were used to collect water samples during baseflow conditions. Baseflow conditions are characterized by the portion of streamflow that is fed by delayed subsurface pathways which is sustained between precipitation events (Price, 2011) and were selected based on the criteria that there was no precipitation at least one day before the sampling event. Water samples were then stored on ice and transported to the Environmental Research Laboratory at East Carolina University (ECU) where they were analyzed for nutrients, which will be discussed in further detail in the next subsection. In addition to nutrient monitoring, 50 mL samples were collected a total of four times, in February, June, and November 2020 and March 2021, for ^{15}N in NO_3^- analysis. This subset of samples was shipped to the UC Davis Isotope Facility for analysis, which will be discussed in further detail in the next subsection. In total, 331 samples were collected over 12 months.

2.2 Laboratory Analysis

Samples were frozen after collection for preservation (Avanzino & Kennedy, 1993) before being taken to the Environmental Research Lab at East Carolina University, where they were thawed and filtered to remove particulate matter before analysis. These samples went through two rounds of filtration, first through a 1.5 μm ashed glass filter and then through a 0.7 μm ashed glass filter. Filters were ‘ashed’ by thermal treatment via a furnace at high temperature to remove any organic material present on the filter. Filtrate was collected and analyzed for nutrient concentrations (ECU’s Environmental Research Lab) and stable nitrogen (^{15}N) and oxygen (^{18}O) isotopes of $\text{NO}_3\text{-N}$ (UC-Davis’s Stable Isotope Facility).

2.2.1 Nutrient Analysis

The samples were filtered and analyzed for ammonium (NH_4^+), nitrate plus nitrite (NO_3+NO_2), and dissolved Kjeldahl nitrogen (DKN) concentrations on the *Smartchem 170 and 200* Discrete Analyzer (SmartChem 200 Method 375-100E-1; Standard Methods 4500-NH3-H; AMS, 2014), at the Environmental Research Lab. DON was determined by subtracting $\text{NH}_4\text{-N}$ from DKN. Total dissolved nitrogen (TDN) was analyzed using a *Shimadzu* TOC-TN Analyzer (Total Organic Carbon Analyzer User’s Manual, 2010; Shimadzu, 2010). In the case that measured concentrations were below the method detection limit for any of these analytes, the value was replaced with half of the method detection limit (Julian, 2020).

2.2.2 Isotopic Data Analysis: Nitrate Source Identification

Isotopic analysis can be used as an identifier of the source of NO_3^- within water samples (Ding et al., 2014; Korth et al., 2014). In this study, the proportional contributions of NO_3^- from ammonia fertilizer, septic and manure effluent, and soil organic matter (Silva et al., 2002) were estimated. Since each source has a general range of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ratios that represent that

source, plotting the ratio of each sample displays which source may account for most of the NO_3^- in that sample (Silva et al., 2002).

A subset of samples, collected in February, June, and November 2020 and March 2021, were sent to the University of California at Davis Stable Isotope Facility where they were analyzed for the isotopic fractionation of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- -N. This lab compares ^{15}N values vs the average abundance of ^{15}N in air (Air (‰)) and ^{18}O vs the Vienna Standard Mean Ocean Water (VSMOW (‰)), which are international isotopic reference standards (USGS, 2020). The values produced by this process ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^-) signify the composition (reported in per mil; molecules per thousand) of the stable isotopes found in the sample, in comparison to their standards. At the UC Davis Stable Isotope Facility, the ThermoFinnigan GasBench + PreCon trace gas concentration system is interfaced to a ThermoScientific Delta V Plus isotopic-ratio mass spectrometer that was used to measure the isotopic ratios. A bacterial denitrification assay was used to convert NO_3^- to nitrous oxide (N_2O) gas, which was then carried to the Isotope Ratio Mass Spectroscopy system. Isotope ratios were produced and adjusted for the international reference standards (Lennon, 2014).

Because stable nitrogen (^{15}N) and oxygen (^{18}O) isotopes of NO_3^- can be used to determine the source of the NO_3^- (Xue et al., 2009), these data were evaluated following the methods of Silva et al. (2002) to determine if there is a relationship between isotope values and potential sources, which may be influenced by wastewater management systems and/or land cover. Because the range of common $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values vary between fertilizers, soil organic matter, and wastewater, the combined use of both nitrogen and oxygen isotopes of NO_3^- is more reliable when determining source separation (Figure 9). Denitrification along a stream or groundwater flow path can also be evaluated using this method because it leads to enrichment in the $\delta^{15}\text{N}$ and

$\delta^{18}\text{O}$ values of the remaining NO_3^- (Silva et al., 2002). Because common ranges of the stable isotopes have already been determined for each source being observed, plotting the ratio between the isotopes can indicate the source of the NO_3^- .

2.3 Statistical Methodology

Statistical analysis of the collected data allows for comparison of the differences in nutrient concentrations among the study area's various land covers, geologic belts, and WMAs. Since the data didn't exhibit normality based on preliminary statistical analysis (Ghasemi & Zahediasl, 2012; Hoghooghi et al., 2016), the primary statistical methods used were the Kruskal-Wallis statistical test, the non-parametric form of the one-way ANOVA (Hoghooghi et al., 2016), and the post-hoc pairwise Wilcoxon rank-sum test. Rank tests are useful for data which are nonnormal and contain large outliers, as they decrease the probability of type 1 (false positive) or type 2 (false negative) errors (Garland-Campbell, 2018). These tests were used to determine if there are statistical differences when comparing variables in the dataset (Daxue Consulting, 2022). The p-values that result from these tests were used to indicate whether the medians of the different variables were significantly different ($p\text{-value} < 0.05$) or not ($p\text{-value} > 0.05$). When the Kruskal-Wallis test determined there was a significant difference between variables within a dataset, the pairwise Wilcoxon test was run (Daxue Consulting, 2022) to determine which WMAs and geologic belts accounted for variations in nitrogen concentrations.

To further determine which variables impacted the different forms of N, a best subsets regression test was run (Kassambara, 2018), which included the WMA, septic system density, geologic belts, and land cover variables. This test was used to determine which subset of predictors were best at accounting for variations in N concentrations. The variable(s) with the

largest R^2 and the smallest mean standard error values were subsequently the best predictor variables to account for variations in this dataset (Lambert et al., 2018).

3.0 RESULTS AND DISCUSSION

3.1 Nitrogen Compounds

3.1.1 Nitrate-Nitrogen

Statistical tests showed a significant association between median NO₃-N concentrations (Table 1) and OWTS density ($p < 0.001$; Table E2), showing an increase in concentration with increases in OWTS density (Figure 3). However, PR and PM, two sites along the same creek within sewer dominated sub-watersheds in the Raleigh Belt, displayed extremely elevated N concentrations in comparison to the other 6 sewer sites observed, impacting the interquartile range (IQR) values for sewer dominated sub-watersheds which will be discussed in detail in a subsequent section (3.3). There was also one HD septic dominated sub-watershed, Jones Ditch, with NO₃-N concentrations recorded up to 22.17 mg/L, most likely a result of inadequate treatment of septic effluent (Figure 3).

Table 1. Median nitrate-nitrogen (NO₃-N) concentrations during baseflow conditions grouped by wastewater management approach (WMA) (LD = Low density and HD = High density) with letters A and B used to show statistically significant differences between variables. Variables with different letters show statistical difference while variables with the same letter show no statistical difference. Interquartile range (IQR) and standard error (SE) are reported to show variability within the dataset. The number of samples (n) within each WMA is also reported.

WMA	NO ₃ -N Median Concentration (mg/L)	IQR (mg/L)	SE (mg/L)	<i>n</i>
Septic HD	0.78 _A	0.74	0.21	178
Septic LD	0.51 _B	0.45	0.04	59
Sewer	0.52 _B	7.79	0.57	96

The Kruskal-Wallis statistical test showed that NO₃-N concentrations statistically differed by WMA ($p = 0.001$; Table E1). The pairwise Wilcoxon test then showed that median NO₃-N concentrations in HD septic sub-watersheds were significantly ($p < 0.001$; Table E1) higher than in sewer sub-watersheds and LD septic sub-watersheds. A plot comparing median NO₃-N concentrations to WMA within HD, LD, and sewer dominated sub-watersheds (Figure 4)

supports these statistical results, showing that HD sub-watersheds were characterized by elevated median $\text{NO}_3\text{-N}$ concentrations which decrease with decreases in OWTS density.

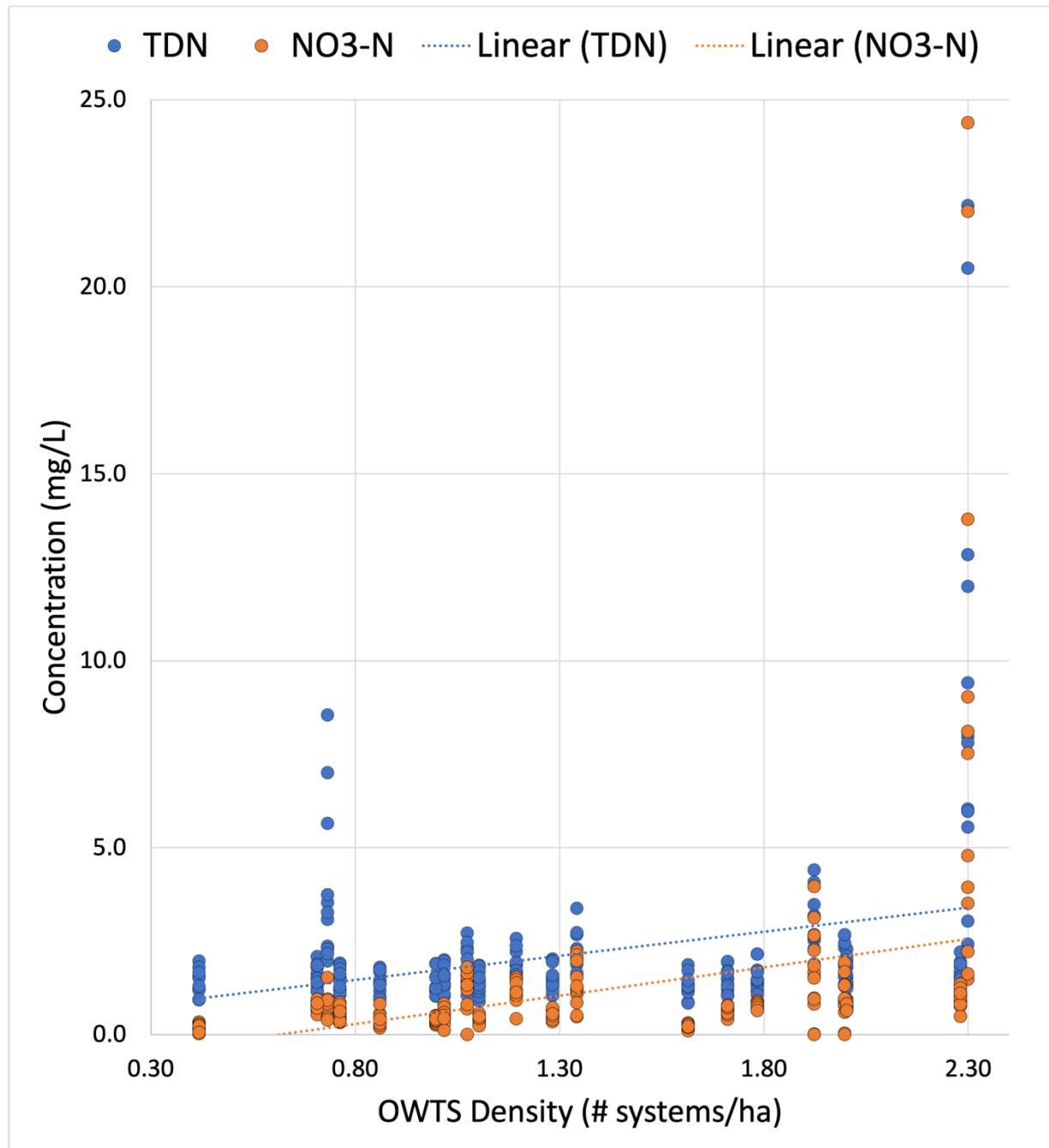


Figure 3. Scatterplot of all septic total dissolved nitrogen (TDN) and nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations compared to the onsite wastewater treatment system (OWTS) density, showing a positive association between the two variables and OWTS density.

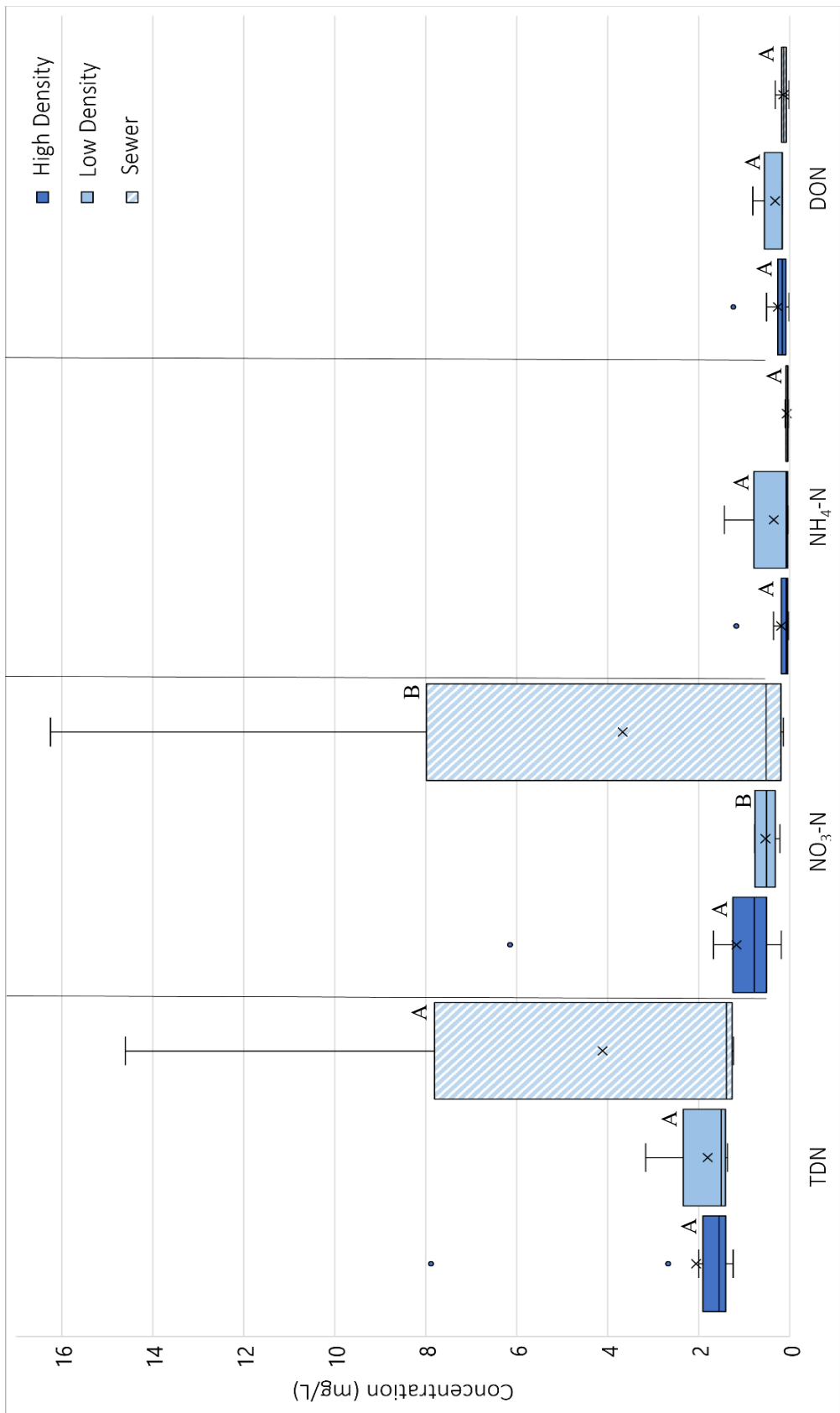


Figure 4. Boxplots of median nitrogen (N) concentrations categorized by wastewater management approach (WMA) (high density (HD), low density (LD) and sewer). The middle line represents the median of the dataset, the box covers the 25th to 75th percentile, the x represents the mean, the tails represent the minimum and maximum observations and filled circles plotted outside of these tails represent outliers. Letters A and B are used to show statistically significant differences between median N concentration of each form of N separately, with HD median nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations shown to be significantly different than both LD and sewer median $\text{NO}_3\text{-N}$ concentrations. All other median concentrations between WMA are not statistically different within the form of N in which concentration is being compared.

Other work within a similar setting in the Piedmont of Georgia (Hoghooghi et al., 2016) showed that septic system density influenced nutrient loading by determining that $\text{NO}_3\text{-N}$ concentrations increased with increases in OWTS density. This research also determined that in sub-watersheds served by both OWTS and sewer systems, leaking sewer lines did not seem to be a primary source of excess $\text{NO}_3\text{-N}$. This supports the evidence that under baseflow conditions in the HD septic sub-watersheds, the elevated $\text{NO}_3\text{-N}$ concentrations were associated with increasing OWTS (Hoghooghi et al., 2016). Another study completed within the coastal plain of NC determined that N concentrations in surface water during baseflow conditions were approximately two times greater in OWTS watersheds ($1.27 \pm 0.46 \text{ mg/L}$) compared to centralized sewer system watersheds, supporting the results of this study (Iverson, 2013). The results of the current study along with those of previous work suggest that the presence of OWTS and increased OWTS density can significantly increase in-stream $\text{NO}_3\text{-N}$ concentrations during baseflow conditions by up to 33% (Table 1).

The Kruskal-Wallis statistical test shows insignificant differences when comparing all $\text{NO}_3\text{-N}$ concentrations of the geologic belts ($p = 0.15$; Table E1). A plot comparing median $\text{NO}_3\text{-N}$ concentrations for sewer and septic dominated sub-watersheds in the three main geologic belts representing this study area displayed that sewer dominated sub-watersheds in the Raleigh Belt had the highest median in-stream N concentrations than all other geologic belts during baseflow conditions (Figure 5), followed by the septic dominated sub-watersheds in the Triassic Basin. The elevated N concentrations in the Raleigh Belt are due to the presence of the two outlier sites Park Ridge and Passmore.

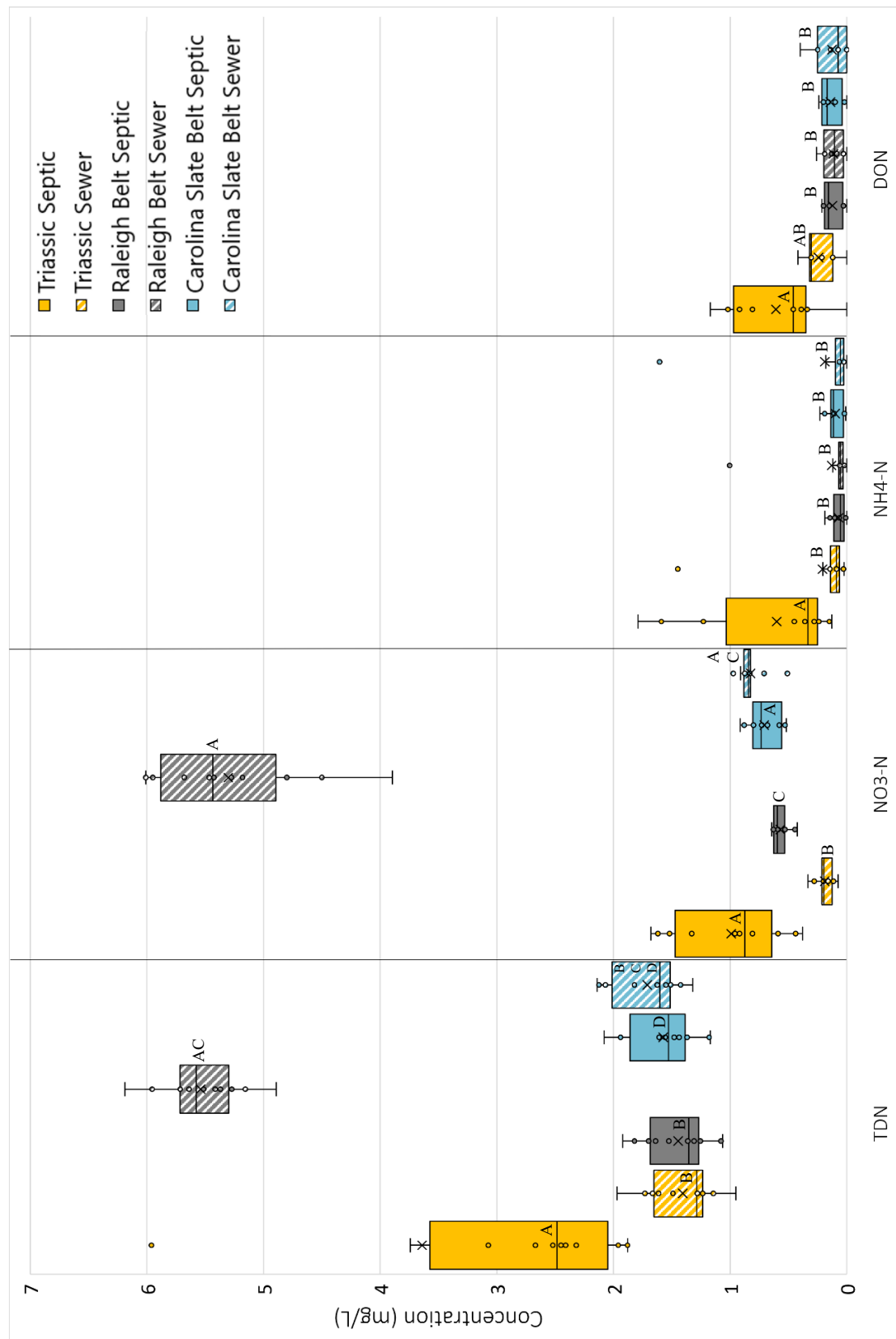


Figure 5. Boxplots of median nitrogen (N) concentrations categorized by geologic belts (Triassic Basin, Raleigh Belt, Carolina Slate Belt). Letters A-D are used to show statistically significant differences between median N concentration of each form of N compared separately. Boxplots labeled with the same letter represent insignificant differences (similar) concentrations between those two variables, while boxplots labeled with different letters represents statistically significant differences between the concentrations of those variables. This figure excludes one data point for the Triassic Basin Septic sub-watersheds with a total dissolved nitrogen (TDN) concentration of 12.83 mg/L.

Although the hypothesis that the highest concentrations of $\text{NO}_3\text{-N}$ present in this study were within the Triassic Basin was not completely supported by the results, $\text{NO}_3\text{-N}$ concentrations in the Triassic Basin were slightly elevated compared to most other geological settings. Previous work supports the hypothesis, showing that the low permeability within the soils of the Triassic Basin could lead to increases in $\text{NO}_3\text{-N}$ concentration as it is more difficult for these soils to transmit water and can decrease the functionality of OWTS (NC DEQ, 2021; NC DEQ, 2022c). Another study determined that mean $\text{NO}_3\text{-N}$ concentrations were higher (0.12 mg/L) in the Triassic Basin compared to the other geologic belts (0.02 mg/L) (Boggs et al., 2013). Since most previous work supports the idea that higher $\text{NO}_3\text{-N}$ concentration would be present within the Triassic Basin instead of the Raleigh Belt, it may be useful to determine site specific variations (riparian vegetation, water table depth, and geochemical conditions) that may be accounting for variability in stream N concentrations (Harden & Spruill, 2008).

The best subsets regression statistical test showed that there is a statistically significant, but weak, positive association between cropland land cover percentage and $\text{NO}_3\text{-N}$ concentration ($p = 0.007$; Table E2, R^2 of 0.22). These results indicate that ~22% of variations in $\text{NO}_3\text{-N}$ concentration can be accounted for by the percent of cropland land cover in that sub-watershed. This statistical test compared all other land cover groups (urban, forested, and other) to $\text{NO}_3\text{-N}$ concentration and no other significant relationships were found. Linear regression plots (Figure 6) comparing monthly median $\text{NO}_3\text{-N}$ concentrations to the cropland land cover percentage in each sub-watershed support these statistical results. Some limitations of these results are that the R^2 is weak, and the regression is violating some assumptions since the presence of the two outliers are what makes this relationship significant.

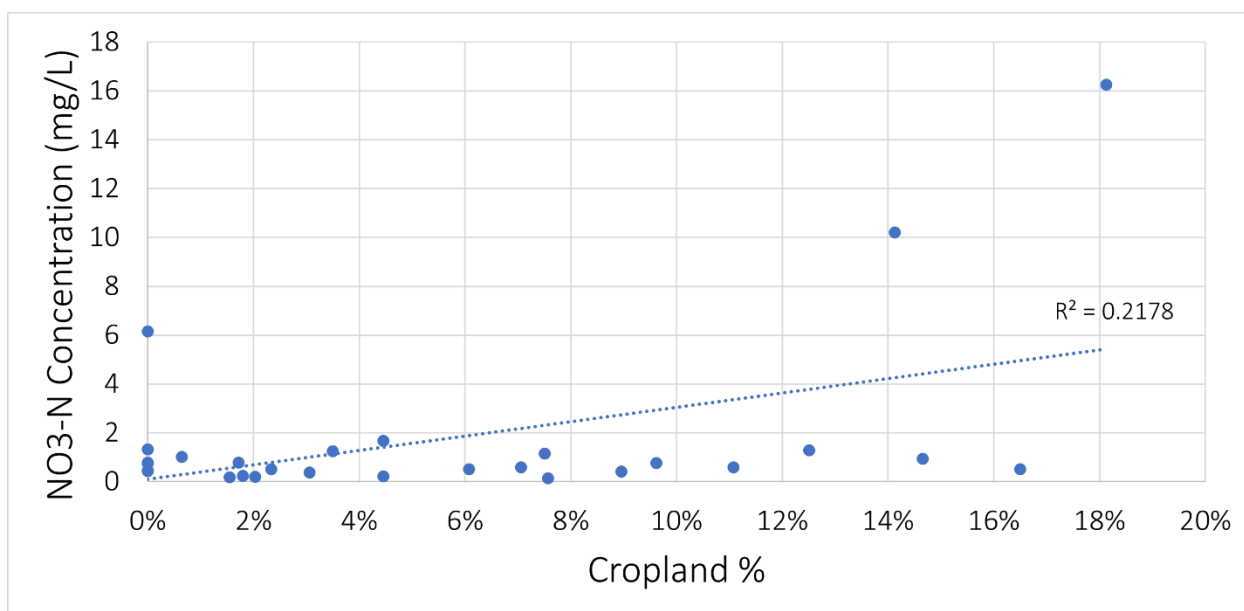


Figure 6. Scatterplot of median nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations compared to the percent of the cropland land cover present within each sub-watershed.

Previous work in an urban watershed in Georgia reported a positive and significant correlation with the percent of range land cover (a component of agricultural land cover) and $\text{NO}_3\text{-N}$ concentration ($r = 0.59$, $p = 0.04$) in LD septic sub-watersheds (Hoghooghi et al., 2016), comparable to the results of this study (Figure 6). Another study in the Maryland Piedmont determined that $\text{NO}_3\text{-N}$ concentration increased linearly with cropland land cover percentage above 20%, with no association before that threshold (Jordan et al., 1997). Limitations across the range of land cover percentages contributed to issues with upholding some linear regression assumptions.

3.1.2 Ammonium-Nitrogen and Dissolved Organic Nitrogen

The Kruskal-Wallis statistical test reported statistically insignificant differences ($p = 0.14$; Table E1) when comparing DON to WMA and significant differences in $\text{NH}_4\text{-N}$ concentrations between WMAs (Table 2). A plot comparing $\text{NH}_4\text{-N}$ and DON concentrations to WMA (Figure 4) shows that the highest concentrations in both species are within the LD

threshold (<1 system/ha), supporting the best subsets regression statistical results and indicating that there is no strong association between DON and NH₄-N concentrations and WMA ($p = 0.071$ and $p = 0.098$, respectively).

Table 2. P-values indicating significant ($p < 0.05$, shown in bold) associations between wastewater management approaches (WMA) and median ammonium-nitrogen (NH₄-N) concentrations and insignificant ($p > 0.05$) associations between WMA and median dissolved organic nitrogen (DON) concentrations during baseflow conditions.

N Concentration vs WMA	p value
DON	0.14
NH₄-N	0.04

A similar study within the Piedmont region of Georgia determined that there was no clear graphical trend when comparing organic N or NH₄-N concentrations with OWTS density in the LD and HD watersheds (Hoghooghi et al., 2016). A two-way ANOVA also determined insignificant associations between organic N and NH₄-N concentrations and OWTS density (Hoghooghi et al., 2016), supporting the results of the current study. Hoghooghi and others (2016) determined that these NH₄-N and DON concentrations are consistent with groundwater sources of N. The lower surface water N concentrations for these species relative to NO₃-N, suggest that NH₄-N and DON have likely sorbed to the soil or nitrified into NO₃-N in the subsurface.

Statistical tests from the current study reported statistically significant differences when comparing NH₄-N and DON concentrations of the geologic belts ($p < 0.001$; Table E1). NH₄-N and DON concentrations were significantly higher within the Triassic Basin septic sites as compared to sub-watershed sites within the Raleigh Belt and Carolina Slate Belt but are not significantly different when comparing the Raleigh Belt to the Carolina Slate Belt (Table E1). A plot comparing median NH₄-N and DON concentrations for sewer and septic dominated sub-watersheds in the three main geologic belts representing this study area displayed that septic

dominated sub-watersheds in the Triassic Basin had higher in-stream N concentrations than all other geologic belts (Figure 5).

Prior work conducted in this same watershed determined that elevated median $\text{NH}_4\text{-N}$ and DON concentrations were predominately present within the Triassic Basin compared to all other geologic belts and were restricted mainly to sites within the septic dominated sub-watersheds (O'Driscoll et al., 2021). Another study in the Carolina Slate Belt and Triassic Basin determined that $\text{NH}_4\text{-N}$ annual mean concentrations were four times higher in the Triassic Basin (0.004 mg/L) than the Carolina Slate Belt (0.001 mg/L) during their 2009 sample period (Boggs et al., 2013). The results of these studies support the hypothesis that N concentrations will be higher in sub-watersheds in the Triassic Basin, which could be a result of poorly drained soils increasing the likelihood of lateral flow across layers of low permeability, contributing N-rich water to baseflow. This lateral flow would decrease the residence time, also decreasing the amount of $\text{NH}_4\text{-N}$ and DON attenuated in the vadose zone.

The best subsets regression statistical test reported no statistically significant associations between land cover percentage and $\text{NH}_4\text{-N}$ and DON concentrations. Since there was a significant association between the cropland land cover and $\text{NO}_3\text{-N}$ concentrations, the same test was run comparing $\text{NH}_4\text{-N}$ and DON concentrations and cropland land cover percentage. The results of this test are shown in table 3 and indicate that only ~8% and ~3% of variations in $\text{NH}_4\text{-N}$ and DON concentrations, respectively, can be accounted for by the percent of cropland land cover in that sub-watershed.

Table 3. P-values indicating insignificant ($p > 0.05$) associations between the cropland land cover percentage and median ammonium-nitrogen ($\text{NH}_4\text{-N}$) and dissolved organic nitrogen (DON) concentrations during baseflow conditions. R^2 values are used to determine which predictor variables best account for variations in this dataset.

N Concentration vs Cropland %	p	R^2
DON	0.45	0.03
$\text{NH}_4\text{-N}$	0.25	0.08

Previous work determining patterns in water quality parameters in fresh waters compared to land use and management (Yates et al., 2022) discovered that both $\text{NH}_4\text{-N}$ and DON concentrations are slightly correlated with agricultural land cover, with DON having a much more significant correlation ($r = \sim 0.4$; $p = 0.001$) than $\text{NH}_4\text{-N}$ ($r = \sim 0.2$; $p = 0.05$). Although this previous research covered a much larger area, increasing the probability of variations in other controlling factors, their results still support the results of this study, indicating very little to no association between land use and $\text{NH}_4\text{-N}$ and DON concentrations. The EPA (2022a) has also reported that fertilizer type may play an important role in $\text{NH}_4\text{-N}$ dynamics, leading to increased in-stream concentrations. Some research also shows that associations exist between forested land cover and median DON concentrations (Willett et al., 2004); however, linear regressions created to compare the data within this report resulted in a p value of 0.42. This value indicates an insignificant association between median concentrations and forested land use, meaning little to no changes in concentration can be accounted for by changes in this land use.

3.1.3 Total Dissolved Nitrogen

The Kruskal-Wallis and best subsets regression statistical tests show significant associations between OWTS density and median TDN concentrations ($p < 0.001$; Table E2) and insignificant associations between WMA and TDN concentrations. A plot (Figure 4; Table E1) and Table (4) comparing median TDN concentrations to WMA support these statistical results, showing that median concentrations increase with increasing OWTS density (Figure 3) but show

no statistical difference across WMA. Table 4 also shows that sewer dominated sub-watersheds have a significantly larger variability within the dataset than the other WMA due to the presence of outlier sites PR and PM.

Table 4. Median total dissolved nitrogen (TDN) concentrations during baseflow conditions grouped by wastewater management approach (WMA) (LD = Low density and HD = High density). Interquartile range (IQR) and standard error (SE) are reported to show variability within the dataset.

WMA	Median TDN Concentration (mg/L)	IQR (mg/L)	SE (mg/L)
Septic HD	1.56	0.50	0.20
Septic LD	1.51	0.92	0.74
Sewer	1.39	6.54	0.48

A similar study supports these results by determining TN concentrations compared to OWTS density follows the same trend as NO₃-N concentrations, increasing with OWTS density in HD watersheds (Hoghooghi et al., 2016). A two-way ANOVA test also determined significant associations between TN concentrations and OWTS density (Hoghooghi et al., 2016). Another study conducted in the Coastal Plain of North Carolina measured around double the amount of TN in streams draining OWTS sub-watersheds compared to those draining sewer sub-watersheds (Iverson, 2013). These studies support the results provided by this study, also supporting the hypothesis that increasing the density of OWTS in a sub-watershed increases the concentrations of TDN in-stream, likely as a result of undertreated septic effluent reaching surface water.

The Kruskal-Wallis statistical test reported statistically significant differences when comparing TDN concentrations of the geologic belts for all sub-watersheds ($p = 0.002$; Table E1). The pairwise Wilcoxon test then reported no statistical difference when comparing the Triassic Basin septic sub-watersheds to the Raleigh Belt sewer dominated sub-watersheds (Table E1). A plot comparing median TDN concentrations for sewer and septic dominated sub-watersheds and the three main geologic belts representing this study area shows elevated in-

stream N concentrations in both the Triassic Basin septic and Raleigh Belt sewer dominated sub-watersheds (Figure 5).

As hypothesized, the highest concentrations of TDN were present within the Triassic Basin due to the low permeability within the soils of that region (NC DEQ, 2022c). However, the elevated concentrations in the Raleigh Belt sewer sites are a result of the outlier sites Park Ridge and Passmore and will be discussed further in a subsequent section (3.3). Previous work within the same study area shows that TDN concentrations were elevated in the Triassic Basin compared to all other geologic belts and 97% greater in OWTS sub-watersheds compared to sewer sub-watersheds (O'Driscoll et al., 2021), supporting the results of this study. The decreased permeability within the Triassic Basin soils is thought to reduce the amount of water that can infiltrate and be transmitted through the soil (NC DEQ, 2022c; O'Driscoll et al., 2021), potentially decreasing nitrification and denitrification and increasing in-stream N concentrations.

The best subsets regression statistical test reported that there are no statistically significant associations between land cover percentage and TDN concentration. When comparing TDN concentration and the cropland land cover using a linear regression (Figure 7), the resultant R^2 value was 0.17, mostly related to the concentrations recorded at the two outlier sites PR and PM. The resultant R^2 value indicates that ~17% of variations in TDN concentration can be accounted for by the percent of cropland land cover in the sub-watersheds, indicating that other variables than land cover account for most of the variations in TDN concentrations.

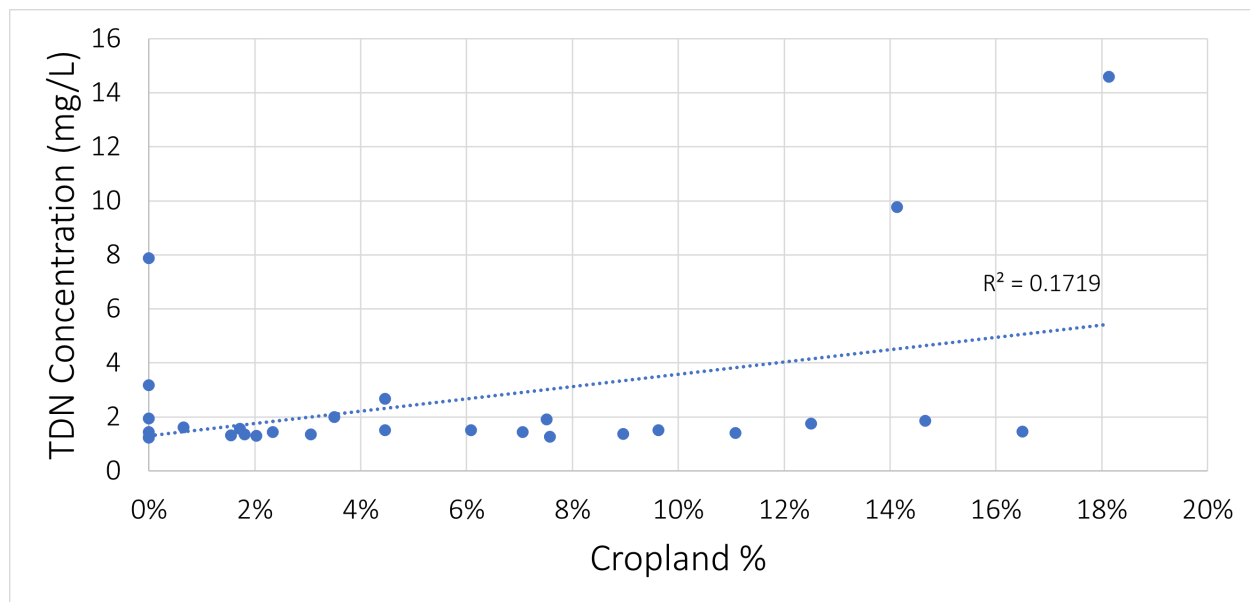


Figure 7. Scatterplot of median total dissolved nitrogen (TDN) concentrations compared to the percent of the cropland land cover present within each sub-watershed.

Previous work researched the association between TN concentrations and land cover in urban streams, determining that there was a positive relationship between agricultural land cover and TN concentrations but a slightly stronger association between urban land cover and TN concentrations (Tasdighi et al., 2017; Halstead et al., 2014). Another study conducted in the Jordan Lake watershed, neighboring the Falls Lake watershed, reported that this trend is explained by much lower percentages of agricultural land cover (up to ~30%) compared to urban land cover (up to ~75%) and that studies where cropland percentages were higher than urban land cover percentages had stronger and more significant associations with TN concentration (Tasdighi et al., 2017). Although it was hypothesized that sub-watersheds with higher percentages of urban land cover would be associated with higher in-stream TDN concentrations, it was determined that the cropland land cover had a much stronger relationship, although not considered significant. However, there are limitations in the comparison between the results of

previous work and of this study since TN also includes particulate species of N, although it is unlikely that PN will contribute to substantial variations in concentrations during baseflow. To understand the association more completely between the cropland land cover and TDN concentration, headwater streams in sub-watersheds with higher percentages of cropland percentage should be studied.

Seasonal variations, especially in temperature, can also have a major impact on in-stream N concentrations. A line graph showing median TDN concentrations over time, separated by WMA, shows seasonal variations (Figure 8) with the highest concentrations recorded in the colder winter months (Dec.-Feb.) and the lowest in the warmer spring months (March-May). One way that these changes in concentration can be accounted for is by changes in nutrient uptake from plants throughout the year (VA DEQ, 1997). During the fall and early winter (Sept.-Dec.), the dormancy period begins where vegetative growth slows or stops, decreasing the need for nutrient uptake from plants and increasing the concentrations recorded in-stream (Nilsson, 2022). Towards the end of winter and beginning of spring (February or March), the growing season begins and vegetative growth increases, increasing the need for more nutrients and decreasing the concentrations recorded in-stream (Nilsson, 2022). Median TDN concentrations were also consistently higher in the HD septic dominated sub-watersheds compared to the sewer dominated sub-watersheds, supporting the hypothesis that OWTS density is associated with in-stream N concentrations.

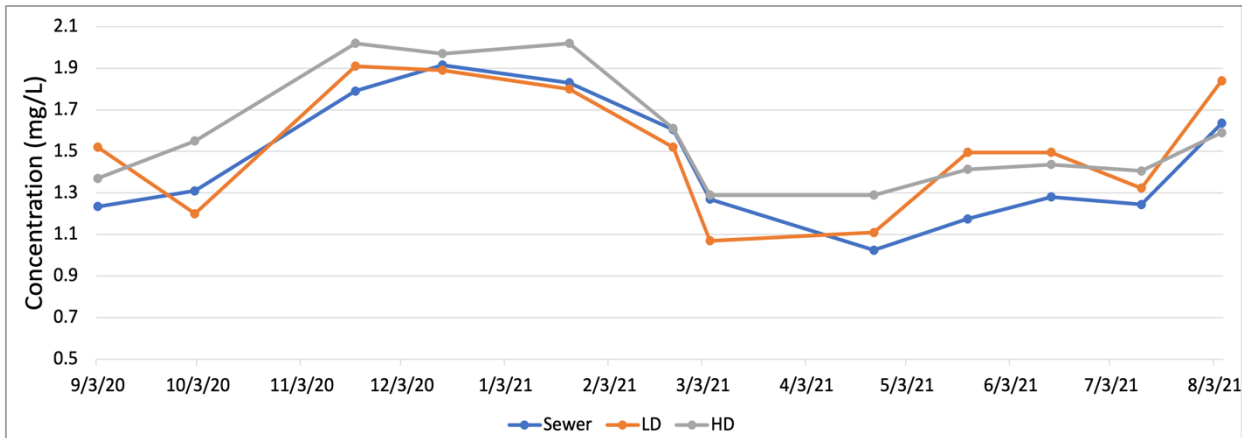


Figure 8. Line graph showing the variations in median total dissolved nitrogen (TDN) concentrations over time, separated by wastewater management approach (WMA) (LD = low density septic and HD = high density septic). The highest concentrations were recorded in the winter months (Dec.-Feb) with the lowest recorded in the spring months (March-May).

3.2 Nitrogen and Oxygen Stable Isotope Indicators of the Sources of Nitrate-Nitrogen

A plot comparing $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ stable isotope indicators showed that $\delta^{15}\text{N}$ in NO_3^- values differed slightly in HD, LD, and sewer dominated sub-watersheds samples from February, June, and November 2020 and March 2021 (Figure 9). Some of the plotted $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ stable isotopes from the HD septic sub-watersheds fall outside of the manure and septic effluent range (Figure 9A), indicating possible variability of OWTS performance for nitrogen processing. For example, OWTS with high treatment efficiencies may be masking the animal/human waste NO_3^- signature, subsequently plotting in the soil organic matter or ammonia fertilizer range. On the other hand, some of the plotted $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ stable isotopes from the sewer sub-watersheds fall inside of the manure and septic/sewer effluent range, indicating possible sewer leakage or increased animal waste. Based on the $\delta^{15}\text{N}$ signature in Figure 9A, sewer sites PR, PM, and Cloverhill indicate possible leakage.

Median values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ in NO_3^- (Table 5) in LD sub-watersheds indicate a predominately soil and organic matter source of NO_3^- in the surface water samples while HD

sub-watersheds indicate predominately manure and septic effluent sources of NO_3^- (Figure 9B).

The median values for sewer sub-watersheds are plotted within the overlap of the soil organic matter and manure and sewer effluent sources of NO_3^- (Figure 9B), likely a result of the enriched $\delta^{15}\text{N}$ found at outlier sites PR and PM (Figure 9A).

Table 5. Median values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for septic high density (HD), septic low density (LD), and sewer sub-watersheds.

WMA	$\delta^{15}\text{N}$ vs Air	$\delta^{18}\text{O}$ vs V-SMOW
Septic HD	8.81	5.54
Septic LD	7.42	6.09
Sewer	8.27	6.72

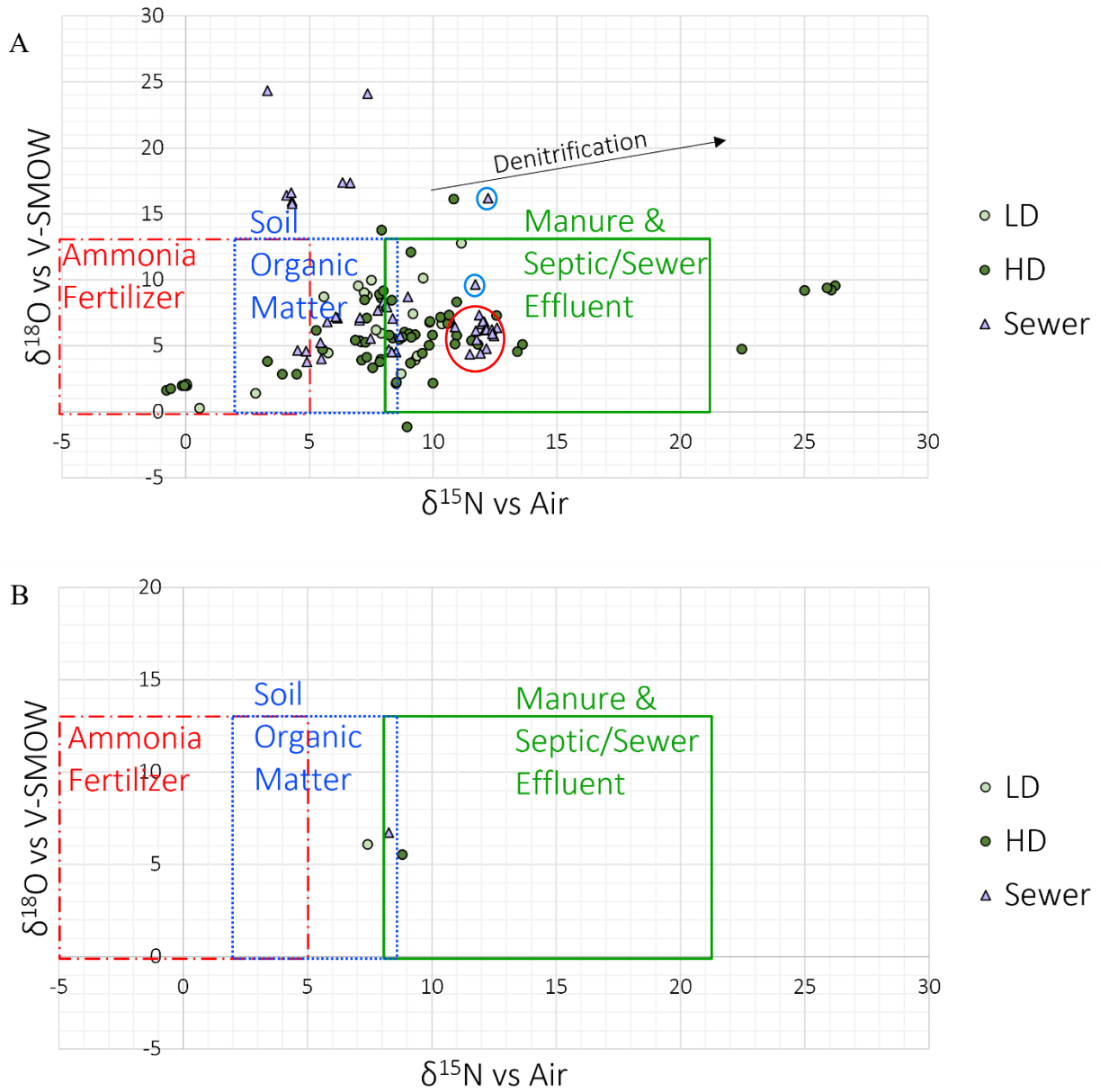


Figure 9. Isotopic fractionation of $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate. A) Graph showing $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values for each sample analyzed from February, June, and November 2020 and March 2021. Outlier sites Park Ridge (PR) and Passmore (PM) are represented by the purple triangles within the red circle and site Cloverhill is represented by purple triangles within the blue circles. B) Median values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for high density (HD), low density (LD), and sewer dominated sub-watersheds. Boundaries for these nitrate contributors were selected based on Silva et al. (2002).

Due to the range of factors contributing to in-stream N concentrations throughout the sites in this study, stable isotope indicators were used to further characterize the source of NO_3^- -N dominant at each site. Previous work in urban watersheds determined that isotopic analysis is a reliable method of determining the source of NO_3^- , particularly manure and septic effluent and fertilizers, in urban watersheds due to the distinct signatures of the isotopes (Silva et al., 2002). Silva and others (2002) also determined that NO_3^- -N concentrations were variable but the identified source of NO_3^- -N during baseflow conditions was dominated by manure and septic effluent and during stormflow conditions was dominated by synthetic NO_3^- fertilizer.

Other work during baseflow conditions in the North Carolina Coastal Plain showed that $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ signatures varied with WMA (Iverson, 2013). This previous work determined $\delta^{15}\text{N}$ - NO_3^- values in septic sub-watersheds indicated a manure and septic effluent source of NO_3^- in surface water, whereas $\delta^{15}\text{N}$ - NO_3^- values in sewerage sub-watersheds indicated ammonia fertilizer and soil organic matter sources of NO_3^- in surface water (Iverson, 2013). Since there were little to no cropland or livestock land covers, the prominent source of this NO_3^- -N is expected to be septic effluent, also supported by the results of Iverson (2013).

3.3 Possible Sources of Excess N in Sewer Dominated Sub-watersheds

Sites PR and PM are located within Wake County and are in sub-watersheds that are dominated by the sewer WMA (Figure 10). These sites are also within the Raleigh Belt geologic belts, consisting of soils predominantly in hydrologic group A (fast draining soils with low runoff potential), and are located within sub-watersheds that contain 14.13% and 18.13%

cropland land cover for PR and PM, respectively. As mentioned previously, these sites had the highest N concentrations compared to all other sites within this research.

The results of this research generally show the trend that OWTS density can influence in-stream nitrogen concentration. Since sites PR and PM are located within sub-watersheds dominated by the sewer WMA, which were intended to act as controls, it was expected that the in-stream median TDN concentrations would range from 0.91 mg/L to 2.39 mg/L (Hoghooghi et al., 2016; Iverson, 2013). However, median TDN concentrations for PR and PM were 9.77 mg/L and 14.60 mg/L, respectively. Since these sites also contain some of the largest percentages of cropland, it would be expected from previous work that TDN concentrations would be elevated compared to sites with smaller percentages of cropland land cover (Tasdighi et al., 2017).

There are many possible sources of this excess N in the streams of sites PR and PM based on land cover and WMA. As indicated by the isotopic fractionation graph (Figure 9A), the primary source of $\text{NO}_3\text{-N}$ at these sites is fecal matter. Based on the increased percentage of the cropland land cover at these sites compared to the other sites within this study, it could be assumed that possible legacy nutrients from agriculture or livestock may be a source of excess $\text{NO}_3\text{-N}$ concentrations in these sub-watersheds (Van Meter and Basu, 2015). Legacy nutrients are excess nutrients from anthropogenic sources, in this case from cropland land cover, which infiltrated the watershed in previous years and are being stored, having the potential to contribute to the surface water many years after the initial input (Chen et al., 2018). Since some cropland land covers do not use waste-derived fertilizers, the most likely agriculture-based sources of this isotopic signature would be livestock rearing or row-crop operations using waste-derived fertilizers (US EPA, 2022c). This hypothesis is also supported by aerial imagery, showing that an upstream farm was in use until around 2004, when residential development began on the prior

agricultural land (Figures G1 and G2). Another possible source could be leaking sewer lines as there is evidence that this may be common in urban watersheds, but there is little information quantifying the amount of N making it into headwater streams (Divers et al., 2013). Since in-stream $\text{NO}_3\text{-N}$ and TDN concentrations were consistently higher at site PM compared to PR (Figure 11), this possible source may likely be found upstream of PM with N attenuation occurring as the water flows downstream. Preliminary data collected on the mainstem of the creek and the tributary that flows into the creek at Passmore Court, indicated that the elevated $\text{NO}_3\text{-N}$ was observed upstream along the mainstem of the creek and not the tributary, suggesting that further work in upstream areas could help to isolate the source of elevated nitrates.

Further work at these sites may be useful in isolating the source of excess N and quantifying the inputs of that source. To conduct this research, samples should be taken upstream of PM to determine where the highest concentrations of N can be found, and to possibly identify the source of this excess N. Earlier work showed that $\text{NO}_3\text{-N}$ was diluted during stormflow, suggesting groundwater transport of $\text{NO}_3\text{-N}$ to the creek (O'Driscoll et al., 2021). Samples should also be taken at multiple points downstream of sites PM and PR, at least to the Lower Barton Creek, which is a higher order stream, to determine how in-stream processes affect N concentrations (Fono et al., 2006; Alexander et al., 2007). Sampling should occur monthly for a year or at a higher frequency for a shorter period of time during baseflow conditions (Clinton & Vose, 2005; Fono et al., 2006). This future research will be helpful in better understanding the source of this N and to aid in the development of possible best management practices.

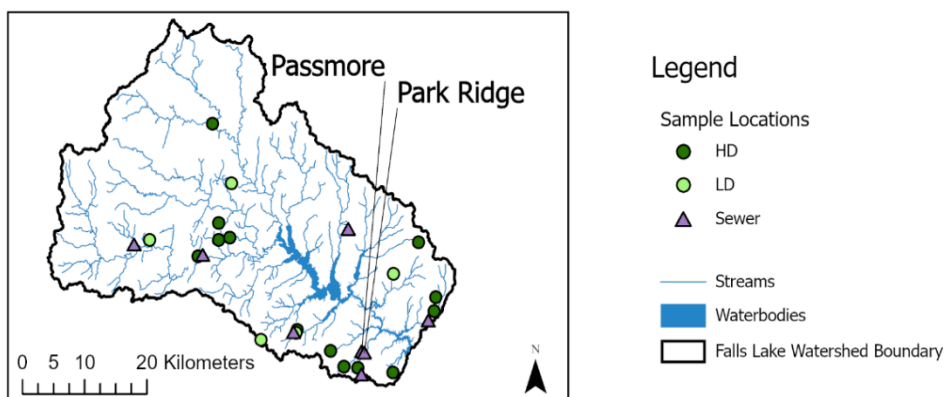


Figure 10. Park Ridge and Passmore site map with an inset map of the Falls Lake watershed.

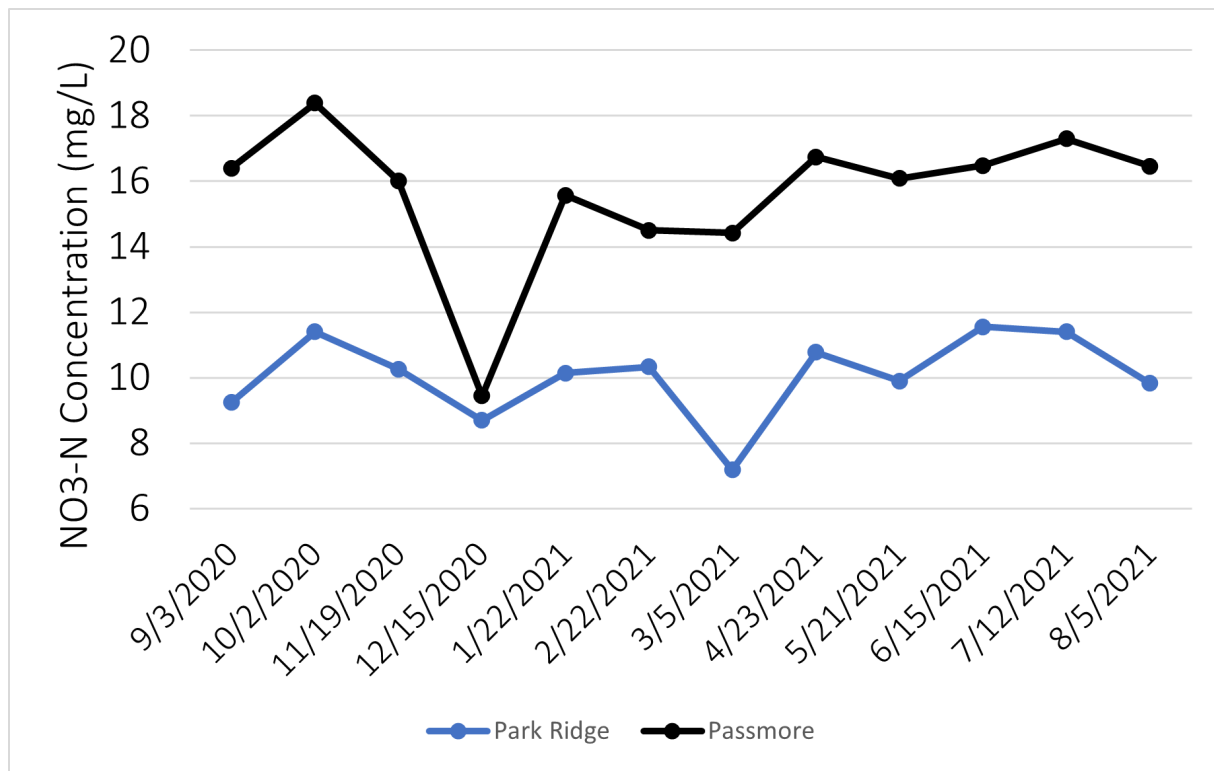


Figure 11. Plot of median nitrate-nitrogen ($\text{NO}_3\text{-N}$) concentrations during baseflow conditions over the 1-year sampling period at extreme outlier sites Park Ridge and Passmore, showing in-stream concentrations were always lower at the downstream site (Park Ridge) indicating possible attenuation or denitrification from Passmore to Park Ridge.

4.0 CONCLUSIONS

The results of this research show that median $\text{NO}_3\text{-N}$ and TDN concentrations were elevated in septic dominated sub-watersheds, with significant increases in $\text{NO}_3\text{-N}$ and TDN concentrations with OWTs density. $\delta^{15}\text{N}$ in NO_3^- isotopic ratios suggested that in-stream concentrations of $\text{NO}_3\text{-N}$ originated from manure and septic/sewer sources within sewer and high-density septic dominated sub-watersheds. The low percentage of cropland land cover percentage supports the idea that OWTs were the major source of in-stream $\text{NO}_3\text{-N}$ concentrations in HD septic sub-watersheds. However, possible legacy nutrients from agricultural or livestock land covers or leaking sewer lines may be a source of in-stream $\text{NO}_3\text{-N}$ in sub-watersheds PR and PM, which have historically been comprised of relatively large percentages of cropland land cover. Soil organic matter was also a dominant source of $\text{NO}_3\text{-N}$ in sewer and LD septic sub-watersheds. When comparing land use to N concentrations, the only statistically significant association that could be made was between $\text{NO}_3\text{-N}$ concentrations and the cropland land use. Although there is a significant association between these variables ($p = 0.007$), previous work (Jordan et al., 1997) shows that further research in sub-watersheds containing higher cropland land cover percentages is needed to better understand this relationship. The comparison between geologic belts and in-stream N concentration showed that significant associations were found when compared to TDN, $\text{NH}_4\text{-N}$, and DON concentrations. TDN concentrations were significantly elevated in the Raleigh Belt sewer sub-watersheds and Triassic Basin septic sub-watersheds and $\text{NH}_4\text{-N}$, and DON concentrations were only significantly elevated in the Triassic Basin septic sub-watersheds. The results of this research also show that seasonal variability can also impact in-stream TDN concentrations throughout the year.

Since the data were collected in headwater streams during baseflow conditions, processes such as nitrogen attenuation may occur moving downstream, and can result in variability of in-stream N concentrations but are not accounted for within this dataset. Future work may be done to include more watersheds with a larger range of cropland land cover percentage, likely improving regression results, and to characterize the amount of nitrogen attenuated between the headwater streams and the lake. Other work is also needed to better understand in-stream nitrogen concentrations during stormflow and changes in attenuation as a result of stormflow conditions.

These findings support the importance of increased in-stream nutrient monitoring and the influence of wastewater management approach and geologic and soil settings on in-stream nitrogen concentrations in suburban watersheds. Based on the results of this research, there are several management implications that would be ideal to implement in this area. To decrease the likelihood of elevated $\text{NO}_3\text{-N}$ concentrations in septic sub-watersheds, land use planning can be implemented to keep the density of OWTS to less than one system per hectare. Since septic systems in the Triassic Basin were more likely to have an influence on stream $\text{NO}_3\text{-N}$ concentrations than the other geologic basins observed, advanced OWTS or in-stream bioreactors could be implemented to reduce the in-stream $\text{NO}_3\text{-N}$ concentrations. Another management implementation could be to install in-stream bioreactors upstream of the PM site to reduce the impacts of the excess N in downstream waters. More research is still needed to better account for the variations within in-stream N concentrations, including the relationship between land cover percentage, water table depth, and how N sinks (e.g., riparian buffers/forest cover and the atmosphere) relate to in-stream nitrogen, to better understand the source of this excess nitrogen.

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5.0 APPENDICES

APPENDIX A: SITE MAPS



Figure A1. Falls Lake watershed site map with an inset map of the state of North Carolina showing the study area at the state scale (HD = High density septic sites and LD = Low density septic sites).

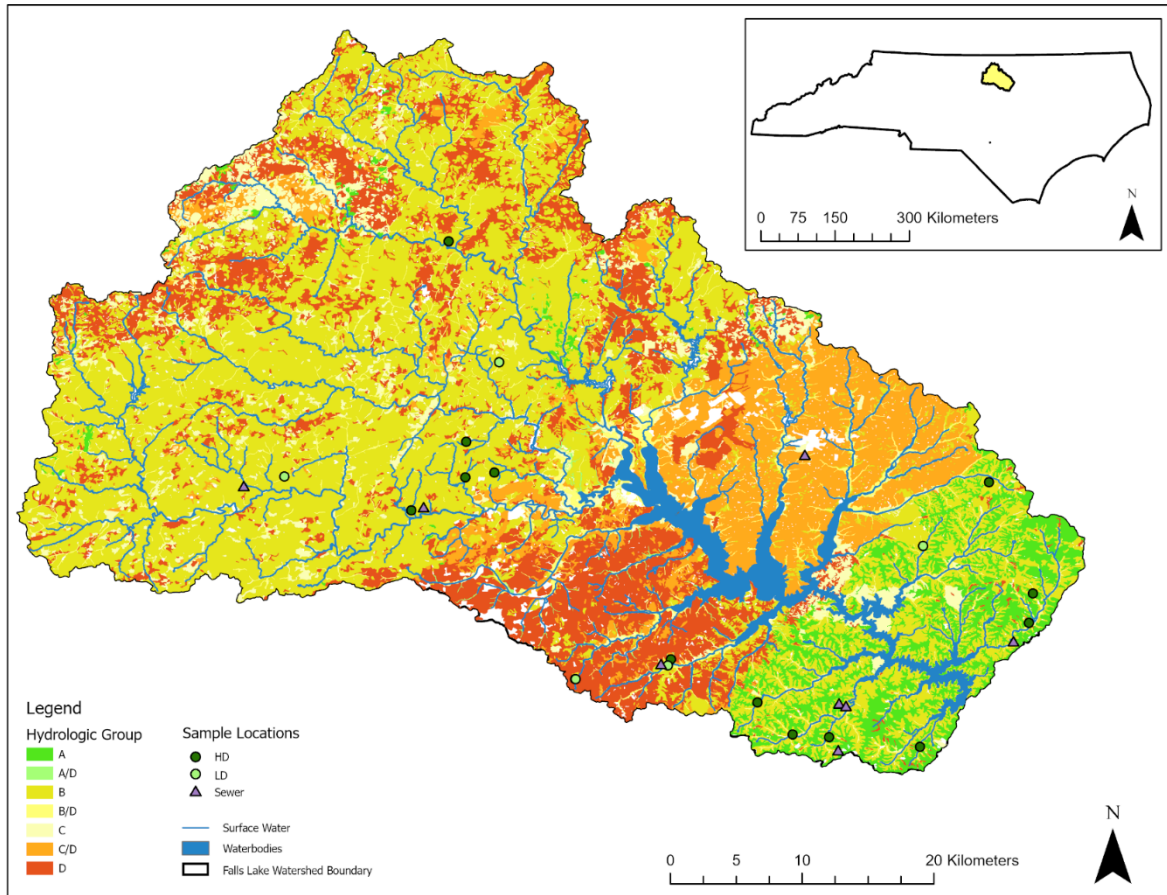


Figure A2. Falls Lake hydrologic group map with an inset map of the state of North Carolina showing the study area at the state scale (HD = High density septic sites and LD = Low density septic sites).

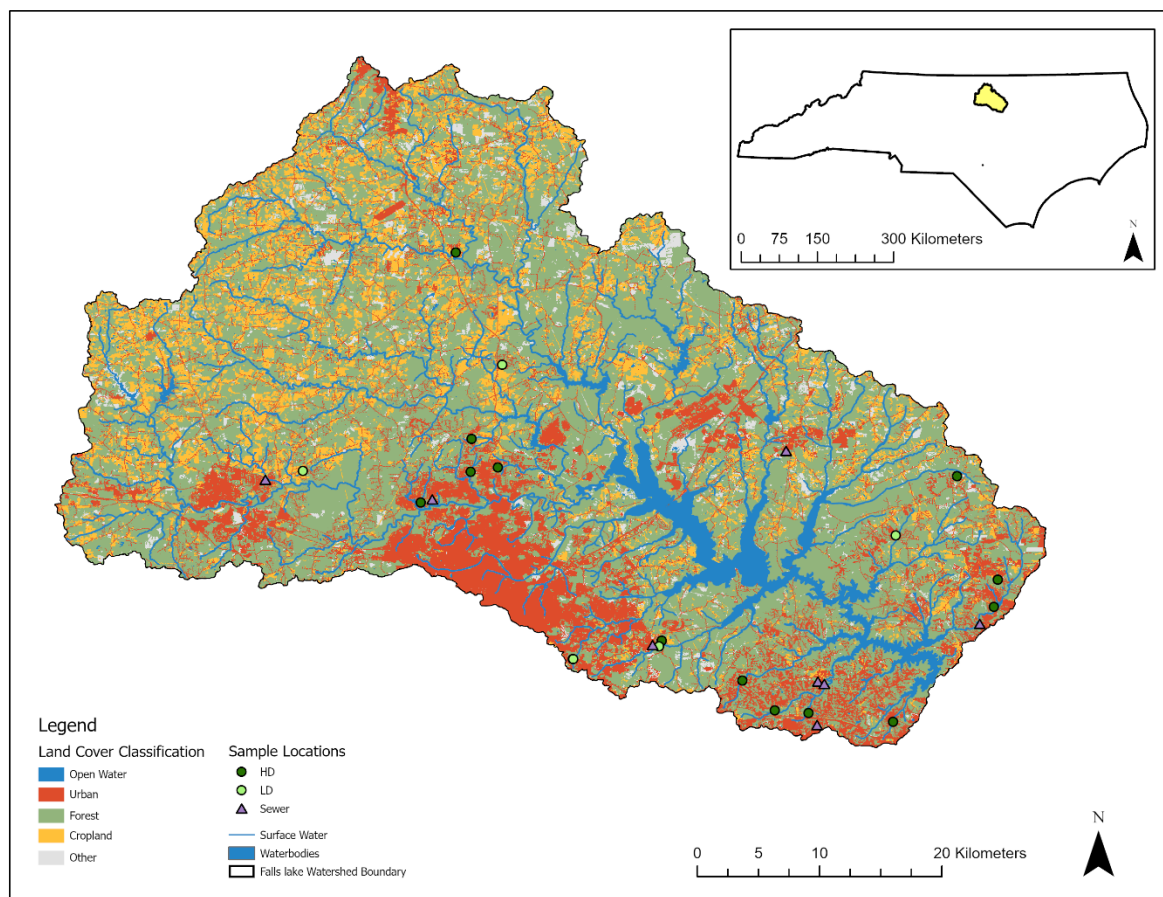


Figure A3. Falls Lake watershed land cover map showing the major land covers throughout the watershed with an inset map of the state of North Carolina showing the study area at the state scale (HD = High density septic sites and LD = Low density septic sites).

APPENDIX B: SITE CHARACTERISTICS

Table B1. Site characteristics including nearest municipality, dominant soil series, geologic unit, and basin slope percentage for each sample location.

			Source: USDA NRCS web soil survey	Source: NCDEQ interactive geologic maps	Source: Stream Stats Watershed Delineation Tool
Site Name	Nearest Municipality	Coordinates	Dominant Soil Series	Geologic Unit	Basin Slope (%)
Mayview	Creedmoor	36.11023, - 78.69185	Creedmoor, coarse sandy loam; Chewacla and Wehadkee soils	Triassic Conglomerate	5.14
Penfold	Wake Forest	35.98323, - 78.54961	Cecil, urban land complex; Chewacla and Wehadkee soils; Palocet sandy loam	Cambrian/Late Proterozoic lineated felsic mica gneiss	7.22
Churton Grove	Hillsbouroug h	36.08906, - 79.07431	Herndon, silt loam; Georgeville, silt loam; Chewacla, loam; Lignum, silt loam	Cambrian/Late Proterozoic phyllitic schist	7.26
Cloverhill	Durham	36.074625, - 78.951728	Chewacla and Wehadkee soils; Palocet, sandy loam	Cambrian/Late Proterozoic biotite gneiss rock	5.81
Ashburn	Durham	35.967738, - 78.7899	Pinkston, fine sandy loam; White Store, sandy loam; Chewacla and Wehadkee soils; Mayodan, sandy loam	Triassic Conglomerate	6.93
Baileywick	Raleigh	35.909053, - 78.669106	Cecil, urban land complex; Palocet, urban land complex	Cambrian/Late Proterozoic felsic gneiss	6.4
Jones	Durham	35.957578, - 78.848201	White Store, sandy loam	Triassic Conglomerate	5.73
Jones Ditch	Durham	35.9574, - 78.8484	White Store, sandy loam	Triassic Conglomerate	5.73
Rondelay	Raleigh	35.9668, - 78.7852	Chewacla and Wehadkee soils; Mayodan, sandy loam; Pinkston, fine sandy loam	Triassic Conglomerate	6.87
Olive Branch	Raleigh	35.9668, - 78.7852	Chewacla and Wehadkee soils; Mayodan, sandy loam; Pinkston, fine sandy loam	Triassic Conglomerate	6.87
Pegasus	Bahama	36.1735, - 78.90027	Georgeville, silt loam; Wehadkee, silt loam; Herndon, silt loam	Cambrian/Late Proterozoic felsic metavolcanic rock	7.89
Buckhorn	Grissom	36.04825, - 78.61138	Chewacla and Wehadkee soils; Palocet, sandy loam	Cambrian/Late Proterozoic biotite gneiss and schist	9.16

Passmore	Raleigh	35.941, - 78.6685	Chewacla and Wehadkee soils; Palocet, sandy loam; Palocet, urban land complex; Cecil, sandy loam	Cambrian/Late Proterozoic biotite gneiss rock	7.42
Prophet	Hillsbourough	36.09556, - 79.04676	Chewacla loam; Georgeville, silt loam; Herndon, silt loam	Cambrian/Late Proterozoic felsic metavolcanic rock	5.08
Durant	Raleigh	35.91151, - 78.61337	Chewacla and Wehadkee soils; Palocet, sandy loam; Cecil, sandy loam	Cambrian/Late Proterozoic felsic gneiss rock	6.33
Victory Church	Raleigh	35.94169, - 78.72411	Chewacla and Wehadkee soils; Palocet, sandy loam; Palocet, urban land complex	Permian metamorphosed gabbro/diorite, Cambrian/Late Proterozoic biotite gneiss and schist	10
Appaloosa	Creedmoor and Youngsville	36.09157, - 78.56619	Palocet, sandy loam; Chewacla and Wehadkee soils; Cecil, clay loam	Cambrian/Late Proterozoic biotite gneiss and schist	8.4
Woody	Timberlake	36.25569, - 78.93454	Georgeville, silt loam; Chewacla and Wehadkee soils	Cambrian/Late Proterozoic metavolcanic epi- clastic rock	4.78
Barclay	Bahama and Hillsborough	36.09822, - 78.90353	Herndon, silt loam; Mayodan, sandy loam; Wehadkee silt loam	Cambrian/Late Proterozoic felsic metavolcanic rock	5.19
Green Bay	Durham	36.095, - 78.9234	Nason, silt loam; Herndon, silt loam	Cambrian/Late Proterozoic biotite gneiss rock	5.48
November	Durham	36.0725, - 78.96024	Nason, silt loam; Herndon, silt loam	Cambrian/Late Proterozoic felsic metavolcanic rock	8.12
Park Ridge	Raleigh	35.9392, - 78.6638	Chewacla and Wehadkee soils; Palocet, urban land complex; Palocet, sandy loam	Cambrian/Late Proterozoic biotite gneiss rock, Paleozoic/Late Proterozoic metamorphic rock	8.67
Brookfield	Raleigh	35.918, - 78.6754	Cecil, urban land complex; Chewacla and Wehadkee soils; Palocet sandy loam	Cambrian/Late Proterozoic felsic gneiss, Cambrian/Late Proterozoic biotite gneiss	8.12
Harold	Durham and Bahama	36.1191, - 78.9228	Georgeville silt loam; Iredell loam; Wehadkee silt loam	Cambrian/Late Proterozoic felsic metavolcanic rock	7.25
Macon	Raleigh	35.9199, - 78.7003	Chewacla and Wehadkee soils; Palocet sandy loam;	Cambrian/Late Proterozoic biotite gneiss rock	8.03

			Palocet urban land complex		
Jenkins	Wake Forest	35.9959, -78.53913	Chewacla and Wehadkee soils; Palocet, sandy loam; Cecil, sandy loam	Cambrian/Late Proterozoic lineated felsic mica gneiss	5.7
Asbury	Durham	35.9709, -78.7832	Chewacla and Wehadkee soils; Mayodan, sandy loam; Pinkston, fine sandy loam; Creedmoor, sandy loam	Triassic Conglomerate	5.09
Donlin	Wake Forest	36.01579, -78.53638	Chewacla and Wehadkee soils; Palocet, sandy loam; Cecil, sandy loam	Cambrian/Late Proterozoic felsic mica gneiss	6.1

Table B2. Site characteristics including watershed area, land cover percentages, and septic system density for each sample location.

		Land Cover (%) Source: 2019 National Land Cover Database				Source: (O'Driscoll et al., 2021)	WMA
Site Name	Watershed Area (ha)	Urban	Forest	Cropland	Other	System Density (# systems/ha)	
Mayview	21.34	82.00%	15.95%	1.80%	0.25%	0.00	Sewer
Penfold	21.91	81.50%	8.97%	7.06%	2.47%	0.37	Sewer
Churton Grove	21.66	63.80%	16.61%	0.00%	19.59%	0.05	Sewer
Cloverhill	21.57	92.90%	5.54%	1.55%	0.01%	0.51	Sewer
Ashburn	65.00	63.80%	18.29%	7.57%	10.34%	0.20	Sewer
Baileywick	38.52	54.30%	33.11%	0.00%	12.59%	0.93	Sewer
Jones	83.30	52.40%	44.85%	0.00%	2.75%	0.73	LD Septic
Jones Ditch	10.00	52.40%	44.85%	0.00%	2.75%	2.30	HD Septic
Rondelay	19.13	63.30%	25.66%	4.46%	6.58%	0.42	LD Septic
Olive Branch	15.08	63.30%	25.66%	4.46%	6.58%	1.92	HD Septic
Pegasus	199.41	13.50%	73.69%	9.62%	3.19%	0.71	LD Septic

Buckhorn	62.95	12.50%	58.56%	16.50%	12.44%	0.76	LD Septic
Passmore	29.80	35.40%	42.87%	18.13%	3.60%	0.30	Sewer
Prophet	39.52	4.81%	82.59%	8.96%	3.64%	0.86	LD Septic
Durant	229.48	48.80%	48.98%	0.00%	2.22%	1.00	HD Septic
Victory Church	458.12	41.20%	54.40%	2.03%	2.37%	1.62	HD Septic
Appaloosa	48.91	16.70%	62.96%	3.06%	17.28%	1.10	HD Septic
Woody	40.04	41.40%	42.20%	12.51%	3.89%	1.07	HD Septic
Barclay	125.55	54.50%	35.69%	7.51%	2.30%	1.19	HD Septic
Green Bay	159.14	34.50%	52.50%	6.08%	6.92%	1.02	HD Septic
November	268.91	43.20%	50.55%	2.34%	3.91%	1.28	HD Septic
Park Ridge	46.21	53.40%	30.08%	14.13%	2.39%	0.93	Sewer
Brookfield	52.60	43.10%	52.70%	0.65%	3.55%	2.28	HD Septic
Harold	32.03	29.10%	61.86%	3.50%	5.54%	1.34	HD Septic
Macon	152.44	50.60%	47.84%	0.00%	1.56%	1.78	HD Septic
Jenkins	64.27	24.50%	54.86%	11.08%	9.56%	1.71	HD Septic
Asbury	8.51	29.60%	50.73%	14.66%	5.01%	2.00	HD Septic
Donlin	79.88	72.70%	25.59%	1.72%	0.00%	2.00	HD Septic

APPENDIX C: ANALYTICAL METHODS AND LABORATORY SURFACE WATER DATA

Streamflow and channel dimensions were estimated using a Global Water flow probe. Field water quality parameters, including temperature, specific conductivity (SPC), dissolved oxygen (DO, percent and concentration), pH, oxidation reduction potential (ORP) and turbidity were measured at each stream sampling site using a YSI ProDSS (digital sampling system) handheld multiparameter meter.

Table C1. Analytical methods used in the Environmental Research Lab at East Carolina University to determine the concentrations of total dissolved nitrogen (TDN), nitrate + nitrite (NO_3+NO_2) and ammonium (NH_4).

Analyte	Analytical Instrument	Analytical Method	Source	Method Detection Limit (MDL)
TDN	Shimadzu TOC-TN Analyzer	Total Organic Carbon Analyzer User's Manual, 2010	Shimadzu Corporation. 2010. Total Organic Carbon Analyzer User's Manual	0.0001 μM
NO_3+NO_2	Smartchem 170 and 200 Discrete Analyzer	SmartChem 200 Method 375-100E-1; Standard Methods 4500-NH3-H	Westco Scientific Instruments, Inc. 2008. SmartChem 200 Method 375-100E-1, revised July 2008.	0.0001 μM
NH_4	Smartchem 170 and 200 Discrete Analyzer	SmartChem 200 Method 375-100E-1; Standard Methods 4500-NH3-H	Westco Scientific Instruments, Inc. 2008. SmartChem 200 Method 375-100E-1, revised July 2008.	0.0001 μM
DKN	Smartchem 170 and 200 Discrete Analyzer	SmartChem 200 Method 375-100E-1; Standard Methods 4500-NH3-H	Westco Scientific Instruments, Inc. 2008. SmartChem 200 Method 375-100E-1, revised July 2008.	4.0698 μM

Table C2. Surface water concentration of nitrate + nitrite (NO_3+NO_2) during baseflow conditions of a 1-year sampling period (9/2020 – 8/2021) analyzed in the Environmental Research Lab at East Carolina University.

	NO3+NO2 (mg/L)											
	9/3	10/2	11/19	12/15	1/22	2/22	3/5	4/23	5/21	6/15	7/12	8/5
Mayview	0.25	0.27	0.18	0.24	0.21	0.12	0.10	0.27	0.26	0.35	0.27	0.20
Penfold	0.36	0.50	0.59	0.29	0.75	0.60	0.61	0.58	0.32	0.46	0.62	0.72
Churton Grove	1.01	1.53	1.36	1.28	1.24	1.24	1.16	1.29	1.49	1.53	1.48	1.63
Cloverhill	0.01	0.13	0.06	0.54	0.53	0.71	0.59	0.43	0.16	0.15	0.19	0.06
Ashburn	0.14	0.09	0.05	0.19	0.11	0.11	0.05	0.15	0.30	0.32	0.14	0.20
Baileywick	0.36	0.50	0.45	0.30	0.46	0.39	0.42	0.50	0.46	0.47	0.43	0.52
Park Ridge	9.24	11.40	10.26	8.70	10.14	10.33	7.18	10.78	9.90	11.55	11.40	9.83
Passmore	16.40	18.39	16.02	9.44	15.57	14.50	14.42	16.74	16.09	16.48	17.30	16.46
Jones	0.50	0.92	0.95	0.41	0.92	0.47	0.71	0.83	0.44	1.52	0.91	0.38
Jones Ditch	1.48	1.62	9.03	4.78	8.10	3.94	3.50	7.52	2.22	24.38	22.01	13.78
Rondelay	0.02	0.07	0.33	0.29	0.22	0.25	0.25	0.07		0.17	0.22	0.05
Olive Branch	3.12	2.65	1.68	2.25	1.51	0.97	0.81	0.95	0.01	3.96	1.84	
Pegasus	0.78	0.64	0.88	0.53	0.74	0.75	0.73	0.76	0.94	0.94	0.71	0.84
Buckhorn	0.36	0.32	0.48	0.34	0.61	0.42	0.54	0.69	0.85	0.79	0.35	0.62
Prophet	0.43	0.23	0.18	0.24	0.41	0.41	0.33	0.30	0.56	0.50	0.41	0.81
Durant	0.25	0.29	0.32	0.36	0.47	0.51	0.45	0.49	0.48	0.44	0.37	0.41
Victory Church	0.19	0.31	0.15	0.23	0.13	0.15	0.12	0.10	0.19	0.28	0.25	0.21
Appaloosa	0.53	0.48	0.38	0.35	0.27	0.29	0.26	0.33	0.23	0.43	0.56	0.47
Woody	0.00	1.24	1.76	1.20	1.77	1.53	1.64	1.32	0.68	0.80	1.80	0.00
Barclay	1.11	1.01	1.51	0.91	1.51	1.05	1.19	1.52	1.47	1.38	1.12	0.42
Green Bay	0.40	0.81	0.63	0.49	0.73	0.58	0.27	0.11	0.51	0.51		0.43
November	0.52	0.34	0.48	0.55	0.50	0.48	0.40	0.54	0.64	0.72	0.50	0.55
Brookfield	0.76	0.88	1.12	0.49	1.11	0.89	1.05	1.34	1.24	0.97	0.80	1.10
Harold	2.21	2.13	1.27	0.47	1.08	0.51	0.85	1.18	1.23	1.53	1.97	1.29
Macon	0.67	0.74	0.70	0.82	0.89	0.86	0.74	0.83	0.66	0.78	0.72	0.64
Jenkins	0.78	0.41	0.58	0.68	0.59	0.58	0.59	0.60	0.53	0.57	0.72	0.76
Asbury	0.59	1.92	1.68	0.92	1.33	0.81	1.31	0.03		0.77	0.96	
Donlin	0.73	0.78	0.94	0.73	0.94	0.84	0.78	0.89	0.65	0.67	0.81	0.64

Table C3. Surface water concentration of dissolved organic nitrogen (DON) during baseflow conditions of a 1-year sampling period (9/2020 – 8/2021) analyzed in the Environmental Research Lab at East Carolina University.

	DON (mg/L)								
	11/19	12/15	2/22	3/5	4/23	5/21	6/15	7/12	8/5
Mayview				0.00	0.18	0.24	0.12	0.31	0.10
Penfold				0.00	0.08	0.14	0.00	0.23	0.22
Churton Grove				0.00	0.02	0.00	0.03	0.34	0.00
Cloverhill				0.00	0.13	0.00	0.02	0.46	0.28
Ashburn				0.00	0.25	0.37	0.12	0.32	0.54
Baileywick				0.00	0.00	0.00	0.03	0.16	0.24
Jones		0.26		0.00	0.09	0.06	0.03	0.22	0.15
Jones Ditch				0.00	0.13	0.00	0.03	0.08	0.18
Rondelay				0.00	0.39	4.04	0.00	1.03	0.81
Olive Branch				0.00	0.04	1.21	3.25	2.21	1.30
Pegasus	0.31			0.00	0.00	0.32		0.86	0.40
Buckhorn	0.51			0.00	0.50	0.64	0.34	0.92	1.42
Passmore				0.00	0.22	0.10	0.01	0.16	0.16
Prophet				0.00	0.18	0.09	0.00	0.20	0.27
Durant				0.00	0.20	0.00	0.00	0.26	0.26
Victory Church		0.25		0.00	0.19	0.08	0.03	0.27	0.14
Appaloosa		0.27		0.00	0.25	0.03	0.03	0.47	0.33
Woody		0.03		0.00	0.11	0.00	0.03	0.32	0.19
Barclay		0.11		0.08	0.13	0.00	0.03	0.01	0.10
Green Bay		0.29		0.21	0.07	0.09	0.03	0.00	0.29
November		0.21		0.00	0.25	0.39	0.02	0.00	
Park Ridge		0.22		0.05	0.15	0.14	0.02	0.26	0.22
Brookfield		0.18		0.00	0.00	0.04	0.03	0.10	0.13
Harold		0.24		0.00	0.33	0.23	0.13	0.19	0.33
Macon		0.17		0.02	0.12	0.13	0.01	0.23	0.11
Jenkins		0.00		0.00	0.09	0.02	0.37	0.02	0.00
Asbury	0.36	0.46		0.00	0.42	1.02		0.82	0.53
Donlin		0.17		0.02	0.23	0.18	0.45	0.15	0.43

Table C4. Surface water concentration of ammonium-nitrogen ($\text{NH}_4\text{-N}$) during baseflow conditions of a 1-year sampling period (9/2020 – 8/2021) analyzed in the Environmental Research Lab at East Carolina University.

	$\text{NH}_4\text{-N}$ (mg/L)											
	9/3	10/2	11/19	12/15	1/22	2/22	3/5	4/23	5/21	6/15	7/12	8/5
Mayview	0.03	0.09	0.11	0.28	0.09	1.14	0.08	0.06	0.02	0.13	0.03	0.03
Penfold	0.03	0.06	0.04	0.15	0.33	1.15	0.03	0.10	0.04	0.04	0.03	0.04
Churton Grove	0.00	0.07	0.04	0.10	0.06	1.55	0.02	0.17	0.00	0.01	0.03	0.01
Cloverhill	0.08	0.11	0.09	0.10	0.05	1.66	0.03	0.19	0.00	0.10	0.02	0.05
Ashburn	0.02	0.09	0.14	0.13	0.09	1.76	0.03	0.12	0.19	0.05	0.03	0.26
Baileywick	0.00	0.05	0.05	0.06	0.04	1.29	0.14	0.19	0.00	0.02	0.04	0.34
Park Ridge	0.01	0.11	0.07	0.10	0.07	0.36	0.10	0.04	0.00	0.04	0.03	0.05
Passmore	1.60	0.07	0.05	0.14	0.05	0.86	0.03	0.04	0.00	0.08	0.02	0.03
Jones	1.23	0.88	3.00	0.40	1.23	1.23	1.28	3.28	7.35	1.89	1.87	1.59
Jones Ditch	0.12	0.07	1.58	2.97	0.20	2.41	0.78	0.53	1.70	1.57	2.91	0.14
Rondelay	0.18	0.08	0.03	0.14	0.07	1.57	0.31	0.04		0.37	0.03	6.03
Olive Branch		0.13	0.28	0.36	0.35	1.16	0.18	0.30	1.79	0.45	0.38	
Pegasus	0.02	0.11	0.32	0.12	0.13	1.02	0.09	0.04	0.02	0.03	0.02	0.07
Buckhorn	0.00	0.09	0.11	0.12	0.30	1.05	0.08	0.04	0.01	0.03	0.02	0.02
Prophet	0.03	0.09	0.05	0.13	0.06	0.74	0.02	0.05	0.03	0.03	0.02	0.03
Durant	0.00	0.04	0.06	0.07	0.04	0.65	0.01	0.10	0.00	0.03	0.02	0.01
Victory Church	0.02	0.14	0.82	0.09	0.12	0.45	0.03	0.18	0.00	0.03	0.02	0.00
Appaloosa	0.00	0.09	0.12	0.17	0.17	0.22	0.13	0.27	0.00	0.06	0.04	0.03
Woody	0.03	0.10	0.30	0.11	0.13	0.09	0.19	0.16	0.00	0.02	0.04	0.11
Barclay	0.03	0.11	0.13	0.16	0.12	0.14	0.36	0.12	0.00	0.03	0.02	0.09
Green Bay	0.03	0.11	0.07	0.15	0.08	0.23	0.21	0.13	0.01	0.05		0.05
November	0.00	0.06	0.06	0.14	0.07	0.20	0.16	0.17	0.01	0.01	0.01	0.04
Brookfield	0.01	0.05	0.05	0.08	0.05	0.14	0.10	0.04	0.00	0.02	0.04	0.03
Harold	0.78	0.32	0.44	0.25	0.16	0.31	0.66	0.16	0.26	0.12	0.29	0.05
Macon	0.01	0.13	0.39	0.09	0.11	0.14	0.06	0.03	0.04	0.03	0.02	0.03
Jenkins	0.07	0.08	0.06	0.15	0.28	0.16	0.02	0.04	0.00	0.03	0.03	0.02
Asbury	0.11	0.13	0.17	0.34	0.24	0.42	0.20	0.00		0.04	0.25	
Donlin	0.02	0.13	0.13	0.16	0.39	0.11	0.02	0.09	0.01	0.03	0.05	0.06

Table C5. Surface water concentration of total dissolved nitrogen (TDN) during baseflow conditions of a 1-year sampling period (9/2020 – 8/2021) analyzed in the Environmental Research Lab at East Carolina University.

	TDN (mg/L)											
	9/3	10/2	11/19	12/15	1/22	2/22	3/5	4/23	5/21	6/15	7/12	8/5
Mayview	1.28	1.31	1.83	2.08	1.59	1.40	1.38	0.92	1.17	1.28	1.25	1.47
Penfold	1.23	1.29	1.93	1.78	1.80	1.53	1.57	1.05	1.16	1.28	1.35	1.71
Churton Grove	1.61	1.82	2.39	2.31	2.30	1.96	1.89	1.60	1.95	1.92	1.90	2.31
Cloverhill	1.24	1.43	1.75	1.97	1.95	1.68	1.16	1.04	1.07	1.24	1.21	1.38
Ashburn	1.19	1.21	1.15	1.86	1.74	2.06	0.91	0.98	1.42	1.28	1.23	1.76
Baileywick	1.14	1.31	1.21	1.84	1.86	1.42	1.00	1.01	1.18	1.23	1.24	1.56
Jones	2.32	2.36	5.64	2.28	3.53	1.96	2.16	7.00	8.54	3.74	3.07	3.26
Jones Ditch	3.03	2.41	11.98	7.97	9.40	6.03	5.54	7.80	5.96	22.17	20.49	12.83
Rondelay	1.54	1.20	1.19	1.96	1.80	1.51	0.93	0.94		1.66	1.28	44.86
Olive Branch	4.40	3.17	2.52	3.47	2.61	2.29	1.58	1.88	2.67	4.07	3.18	
Pegasus	1.52	1.32	2.08	1.89	1.80	1.63	1.18	1.15	1.50	1.50	1.40	1.84
Buckhorn	1.19	1.20	1.91	1.85	1.75	1.52	1.07	1.11	1.49	1.43	1.27	1.62
Passmore	12.86	15.70	14.60	9.41	15.17	13.76	14.60	15.04	15.41	13.67	13.76	14.72
Prophet	1.34	1.17	1.76	1.80	1.67	1.45	1.01	0.95	1.33	1.39	1.32	1.73
Durant	1.15	1.20	1.22	1.90	1.89	1.53	1.03	1.04	1.26	1.25	1.24	1.52
Victory Church	1.31	1.31	1.16	1.86	1.71	1.36	0.86	0.84	1.14	1.26	1.28	1.45
Appaloosa	1.36	1.31	1.85	1.82	1.69	1.43	0.88	1.01	1.16	1.24	1.33	1.52
Woody	1.05	1.69	2.71	2.30	2.46	2.20	1.81	1.61	1.38	1.44	2.01	1.33
Barclay	1.90	1.63	2.57	2.21	2.37	1.92	1.65	1.85	1.91	1.92	1.71	1.59
Green Bay	1.27	1.50	2.00	1.95	1.85	1.61	1.02	1.08	1.36	1.34		1.58
November	1.37	1.28	2.02	1.97	1.94	1.54	1.04	1.17	1.48	1.41	1.33	1.59
Park Ridge	9.60	9.97	9.98	7.94	10.52	9.90	9.86	9.69	9.85	9.03	9.19	9.60
Brookfield	1.37	1.55	1.55	1.94	2.20	1.82	1.49	1.66	1.71	1.55	1.48	1.89
Harold	3.37	2.67	2.72	1.97	2.02	1.58	1.87	1.64	2.00	2.00	2.30	1.99
Macon	1.33	1.46	1.40	2.15	2.15	1.72	1.17	1.28	1.43	1.47	1.07	1.67
Jenkins	1.49	1.20	1.94	1.95	1.70	1.50	1.06	1.05	1.33	1.28	1.03	1.66
Asbury	1.57	2.66	2.33	2.45	2.67	1.34	1.88	1.29		1.82	1.55	
Donlin	1.52	1.59	2.28	2.09	1.98	1.80	1.29	1.32	1.40	1.39	1.24	1.89

APPENDIX D: STABLE ISOTOPE DATA

Table D1. Isotopic fractionation of $^{15}\text{N}/^{14}\text{N}$ and $^{18}\text{O}/^{16}\text{O}$ of nitrate analyzed at UC Davis's Stable Isotope Facility for the Feb., June, Nov. 2020 and March 2021 sampling periods.

Date	Sample ID	$\delta^{15}\text{N}_{\text{Air}}$ (‰)	$\delta^{18}\text{O}_{\text{VSMOW}}$ (‰)
Feb 2020-1	Baileywick	7.98	8.30
Feb 2020-1 rep	Baileywick	7.76	7.69
Feb 2020-1	Baileywick	8.10	8.09
Feb 2020-1 rep	Baileywick	8.14	7.94
Feb 2020-2	Brookfield	8.23	5.82
Feb 2020-2	Brookfield	8.85	6.06
Feb 2020-2 rep	Brookfield	9.04	5.93
Feb 2020-3	Passmore	10.88	6.42
Feb 2020-3	Passmore	12.58	6.37
Feb 2020-3	Passmore	12.15	6.22
Feb 2020-3 rep	Passmore	12.15	6.23
Feb 2020-4	Park Ridge	11.87	7.33
Feb 2020-4 rep	Park Ridge	11.73	6.13
Feb 2020-4	Park Ridge	12.07	6.83
Feb 2020-4	Park Ridge	11.95	6.64
Feb 2020-4 rep	Park Ridge	12.01	6.84
Feb 2020-5	Macon Rd	9.98	5.82
Feb 2020-5	Macon Rd	9.86	6.77
Feb 2020-5 rep	Macon Rd	9.87	6.85
Feb 2020-6	Asbury	7.56	3.34
Feb 2020-6	Asbury	7.89	3.98
Feb 2020-6 rep	Asbury	7.85	3.78
Feb 2020-7	Rondelay	7.34	8.82
Feb 2020-7	Rondelay	7.22	9.02
Feb 2020-7	Rondelay	6.98	9.56
Feb 2020-7 rep	Rondelay	7.50	9.98
Feb 2020-8	Ashburn	4.31	15.97
Feb 2020-8	Ashburn	4.30	15.76
Feb 2020-8	Ashburn	4.07	16.41
Feb 2020-8 rep	Ashburn	4.27	16.61
Feb 2020-9	Jones	7.10	5.30
Feb 2020-9	Jones	7.03	5.39
Feb 2020-9 rep	Jones	7.06	5.35

Feb 2020-10	Ditch at Jones	25.02	9.21
Feb 2020-10	Ditch at Jones	26.25	9.56
Feb 2020-10	Ditch at Jones	26.09	9.23
Feb 2020-10 rep	Ditch at Jones	25.93	9.39
Feb 2020-11	Harold	-0.77	1.64
Feb 2020-11 rep	Harold	-0.60	1.76
Feb 2020-11	Harold	-0.14	1.99
Feb 2020-11 rep	Harold	0.05	1.98
Feb 2020-11	Harold	0.03	2.12
Feb 2020-11 rep	Harold	-0.05	1.98
Feb 2020-12	Green Bay	7.23	8.49
Feb 2020-12	Green Bay	7.90	8.62
Feb 2020-12	Green Bay	7.84	8.92
Feb 2020-12 rep	Green Bay	7.99	9.17
Feb 2020-13	Cloverhill	5.72	6.81
Feb 2020-13	Cloverhill	6.14	7.10
Feb 2020-13 rep	Cloverhill	6.06	7.19
June 2020-1	Baileywick	8.21	4.72
June 2020-1 rep	Baileywick	8.34	4.54
June 2020-2	Brookfield	9.56	4.44
June 2020-3	Passmore	12.38	6.25
June 2020-4	Park Ridge	12.09	6.22
June 2020-5	Macon Rd	11.55	5.41
June 2020-6	Asbury	10.00	2.18
June 2020-7	Rondelay	8.71	2.88
June 2020-8	Ashburn	4.52	4.66
June 2020-8 rep	Ashburn	4.90	3.79
June 2020-8 rep	Ashburn	4.86	4.58
June 2020-9	Jones	0.56	0.26
June 2020-10	Ditch at Jones	8.95	-1.15
June 2020-11	Harold	8.50	2.15
June 2020-11 rep	Harold	8.51	2.25
June 2020-12	Green Bay	12.57	7.30
June 2020-13	Cloverhill	8.98	8.72
Nov 2020-1	Mayview	7.03	6.93
Nov 2020-1 rep	Mayview	7.04	7.15
Nov 2020-2	Penfold	5.49	4.01
Nov 2020-3	Churton Grove	8.51	4.53
Nov 2020-4	Cloverhill	12.23	16.18

Nov 2020-5	Ashburn	3.30	24.33
Nov 2020-6	Baileywick	8.67	5.73
Nov 2020-7	Jones	2.82	1.41
Nov 2020-8	Jones Ditch	13.62	5.12
Nov 2020-9	Pegasus	7.90	5.96
Nov 2020-10	Buckhorn	10.36	6.66
Nov 2020-11	Passmore	12.47	5.75
Nov 2020-11 rep	Passmore	12.40	5.91
Nov 2020-12	Prophet	11.15	12.78
Nov 2020-13	Durant	10.31	7.18
Nov 2020-14	Victory Church	5.53	4.72
Nov 2020-15	Appaloosa	3.30	3.81
Nov 2020-16	Woody	8.36	5.60
Nov 2020-17	Barclay	10.67	6.88
Nov 2020-18	Green Bay	10.95	8.33
Nov 2020-19	November	9.84	5.03
Nov 2020-20	Park Ridge	11.76	5.47
Nov 2020-21	Brookfield	9.27	5.86
Nov 2020-21 rep	Brookfield	9.12	5.66
Nov 2020-22	Harold	4.49	2.86
Nov 2020-23	Macon	10.96	5.79
Nov 2020-24	Jenkins	7.27	5.28
Nov 2020-25	Donlin	7.12	3.93
March 2021-1	Mayview	6.65	17.35
March 2021-1 rep	Mayview	6.34	17.39
March 2021-2	Penfold	5.45	5.25
March 2021-3	Churton Grove	7.48	5.55
March 2021-4	Cloverhill	11.70	9.64
March 2021-5	Ashburn	7.35	24.13
March 2021-6	Baileywick	8.36	7.07
March 2021-7	Jones	5.77	4.48
March 2021-8	Jones Ditch	22.48	4.76
March 2021-9	Pegasus	7.70	6.21
March 2021-10	Buckhorn	9.18	7.44
March 2021-11	Passmore	12.16	4.79
March 2021-11 rep	Passmore	11.91	4.42
March 2021-12	Prophet	9.60	10.12
March 2021-13	Durant	8.32	8.45
March 2021-14	Victory Church	7.92	13.79

March 2021-15	Appaloosa	5.27	6.18
March 2021-16	Woody	7.32	4.15
March 2021-17	Barclay	10.59	6.69
March 2021-18	Green Bay	10.83	16.11
March 2021-19	November	9.11	12.09
March 2021-20	Park Ridge	11.49	4.35
March 2021-21	Brookfield	8.64	5.48
March 2021-21 rep	Brookfield	8.77	5.64
March 2021-22	Harold	3.90	2.86
March 2021-23	Macon	10.65	7.32
March 2021-24	Jenkins	6.85	5.43
March 2021-25	Donlin	7.31	7.12
March 2021-26	Olive Branch	11.82	5.10
March 2021-27	Asbury	9.09	3.68
March 2021-28	Rondelay	5.58	8.73
March 2021-29	Olive Branch- Nov	13.41	4.57
March 2021-30	Asbury- Nov	10.88	5.15
March 2021-31	Rondelay-Nov	9.27	3.92
March 2021-31 rep	Rondelay-Nov	9.35	4.23

APPENDIX E: STATISTICAL RESULTS

Table E1. Statistical results (p values) from the Kruskal-Wallis (KW) and pairwise Wilcoxon (PW) tests comparing N concentrations to wastewater management approach (WMA) and geologic belts (GEOL) showing statistically significant differences when $p < 0.05$ and insignificant differences when $p > 0.05$. (HD = high density, LD = low density, C = Carolina Slate Belt, R = Raleigh Belt, T = Triassic Basin, n = number of samples)

	WMA vs NO ₃ - N	WM A vs TDN	WMA vs NH ₄ -N	WMA vs DON	GEOL vs NO ₃ -N	GEOL vs TDN	GEOL vs NH ₄ - N	GEOL vs DON
KW Chi ²	13.49	1.30	6.82	4.78	3.86	12.54	52.82	24.83
KW df	2	2	2	2	2	2	2	2
KW p-value	1.18E- 03	0.52	0.04	0.14	0.15	1.90E- 03	3.71E- 12	1.18E- 06
PW p-value HD vs LD or C vs R	1.20E- 04		0.21			8.75E- 02	0.05	0.55
PW p-value HD vs Sewer or C vs T	0.38		0.09			8.70E- 03	6.90E- 08	2.00E- 05
PW p-value LD vs Sewer or R vs T	0.38		0.09			8.70E- 03	1.10E- 11	3.40E- 06
n	331	331	330	208	331	331	330	208

Table E2. Statistical results (p values) from the best subsets regression test comparing N concentrations to septic system density (SSDEN) and land use percentages, showing statistically significant differences when $p < 0.05$ and insignificant differences when $p > 0.05$.

	SSDEN	Cropland %	Urban %	Forested %	Other %
NO ₃ -N	2.00e-16	0.01	0.32	0.31	0.60
TDN	4.08e-08	0.08	0.49	0.48	0.86
NH ₄ -N	0.10	0.25	0.80	0.92	0.39
DON	0.07	0.45	0.40	0.42	0.42

APPENDIX F: SITE PICTURES

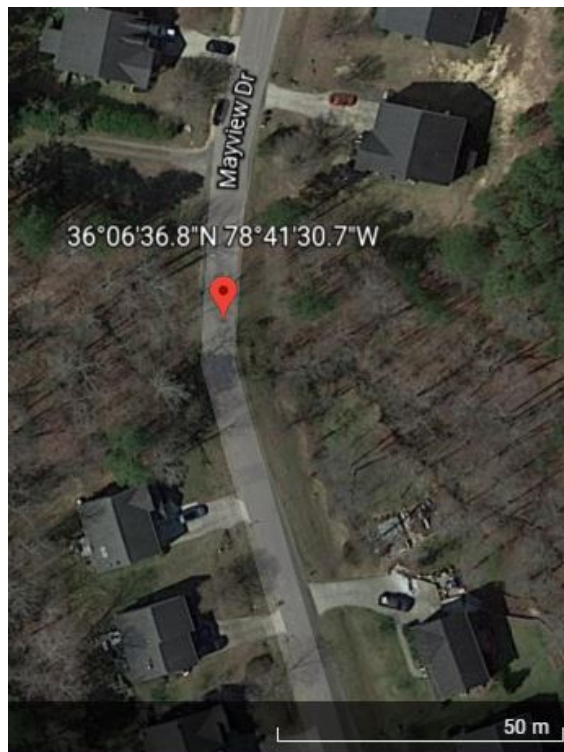


Figure F1. Site Mayview in Granville County

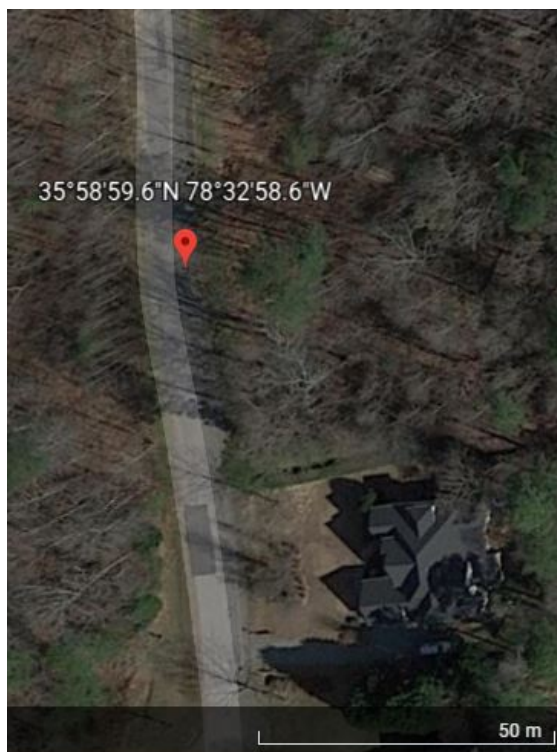


Figure F2. Site Penfold in Wake County

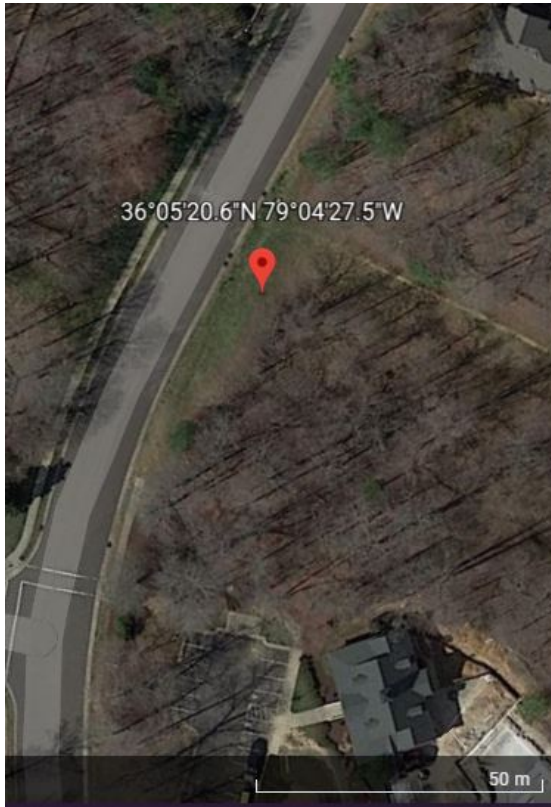


Figure F3. Site Churton Grove in Orange County.



Figure F4. Site Cloverhill in Durham County.



Figure F5. Site Ashburn in Durham County.

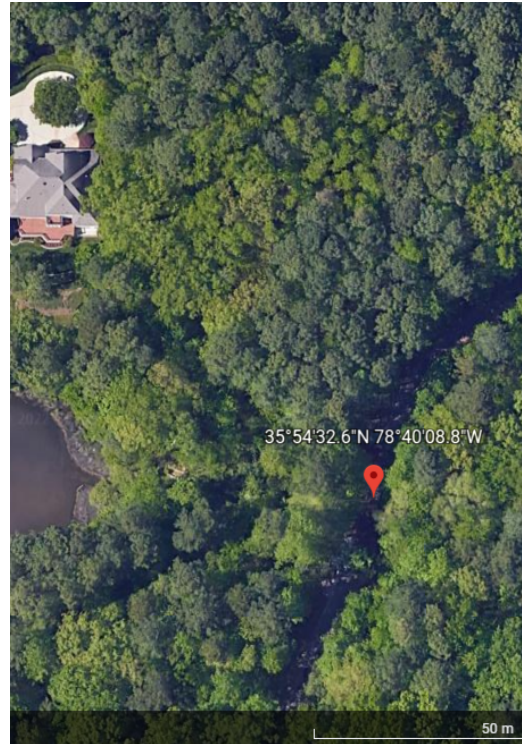


Figure F6. Site Baileywick in Wake County.

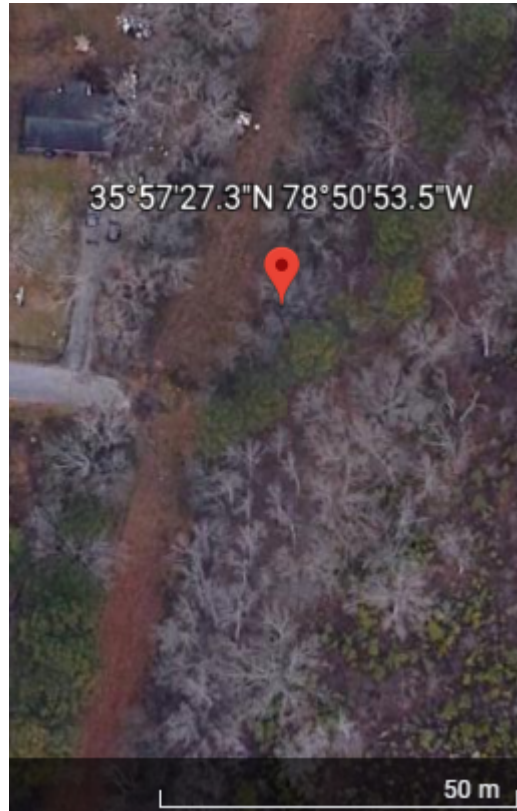


Figure F7. Site Jones in Durham County.

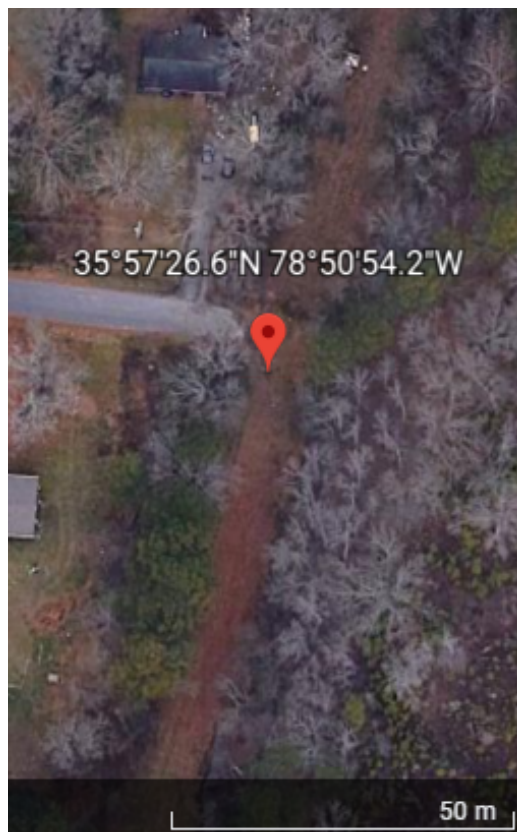


Figure F8. Site Jones Ditch in Durham County.



Figure F9. Site Rondelay in Durham County.

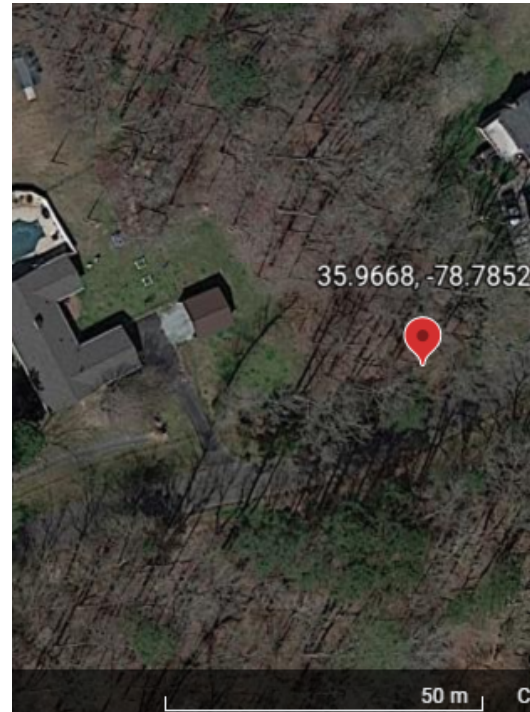


Figure F10. Site Olive Branch in Durham County.

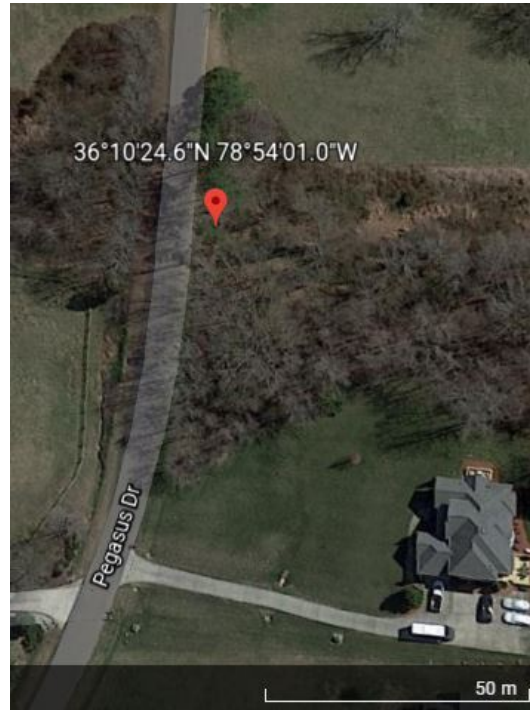


Figure F11. Site Pegasus in Durham County.



Figure F12. Site Buckhorn in Granville County.

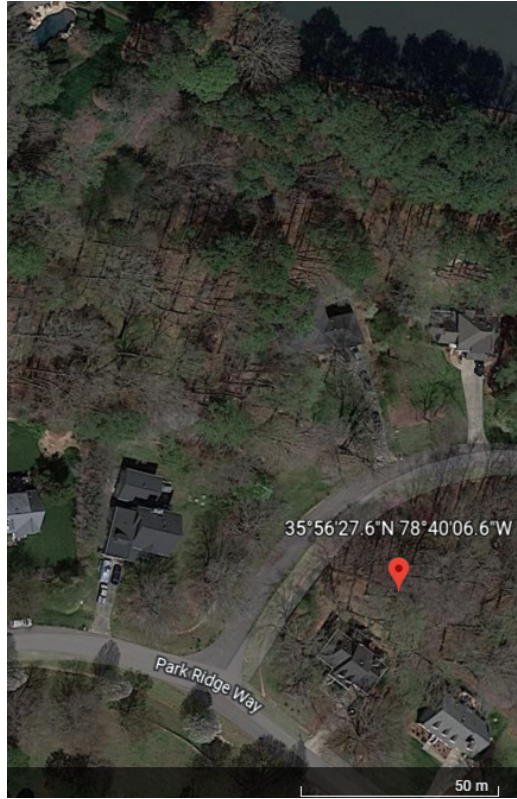


Figure F13. Site Passmore in Wake County.

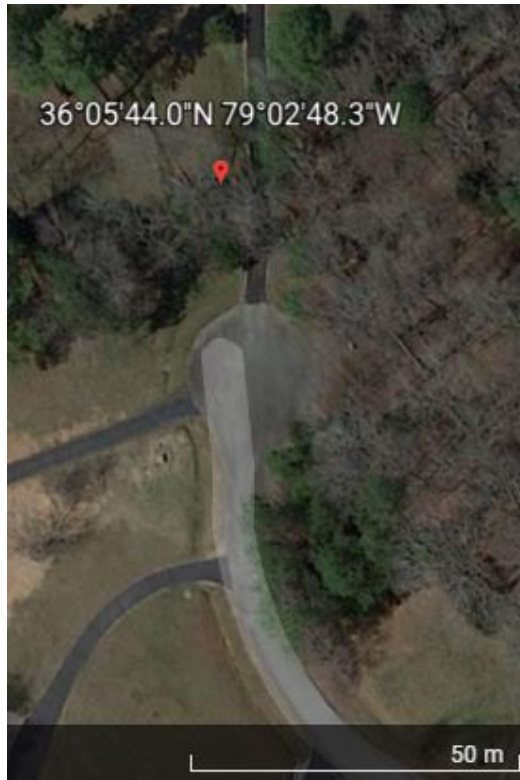


Figure F14. Site Prophet in Orange County.

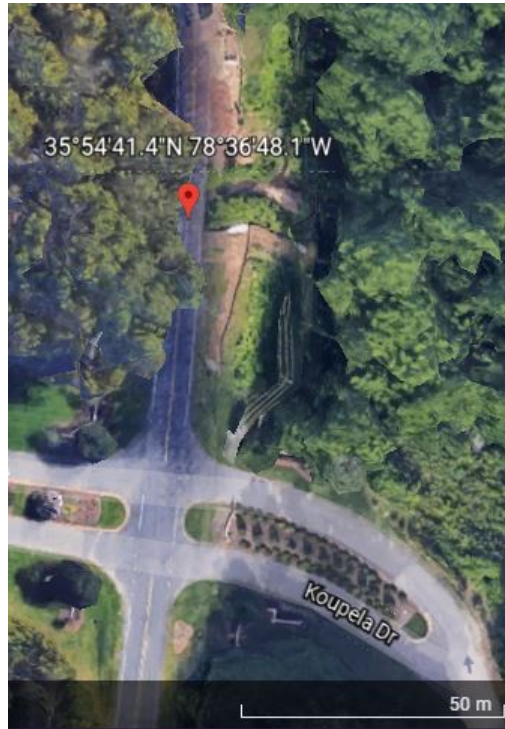


Figure F15. Site Durant in Wake County.



Figure F16. Site Victory Church in Wake County.



Figure F17. Site Appaloosa in Granville County.



Figure F18. Site Woody in Person County.



Figure F19. Site Barclay in Durham County.

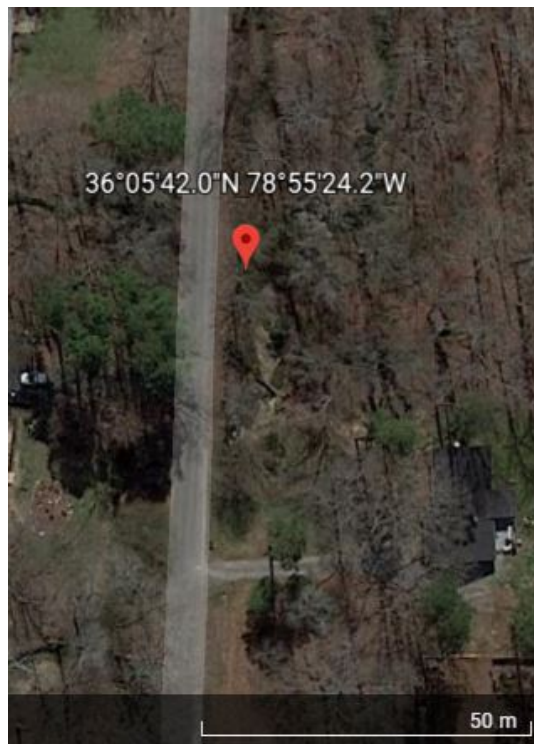


Figure F20. Site Green Bay in Durham County.

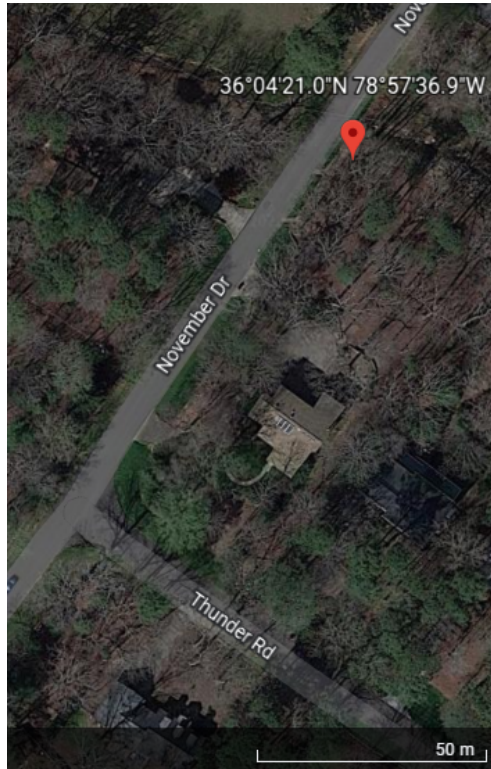


Figure F21. Site November in Durham County.

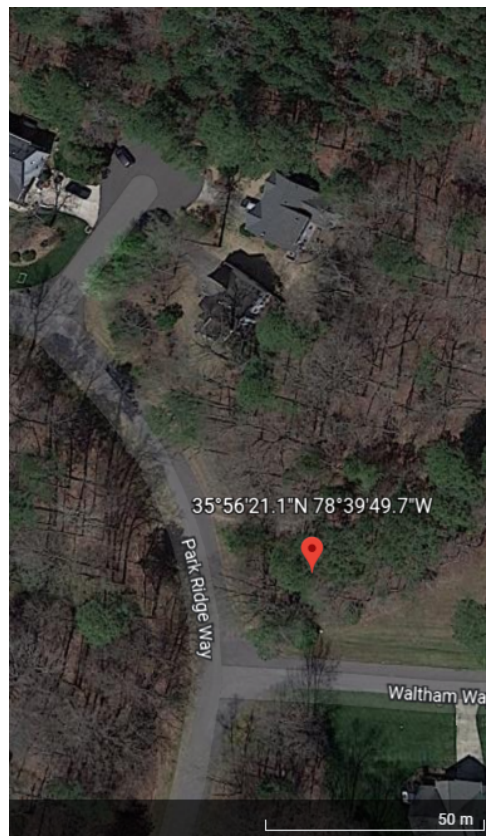


Figure F22. Site Park Ridge in Wake County.

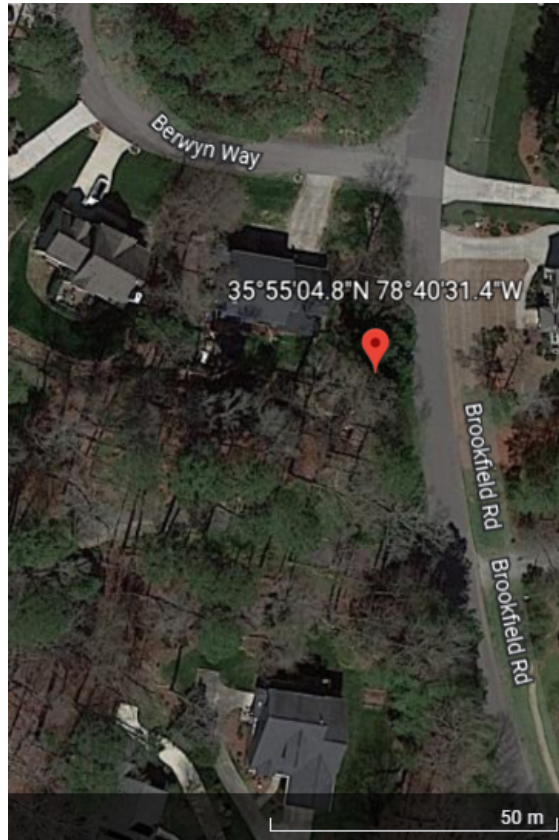


Figure F23. Site Brookfield in Wake County.



Figure F24. Site Harold in Durham County.



Figure F25. Site Macon in Wake County.

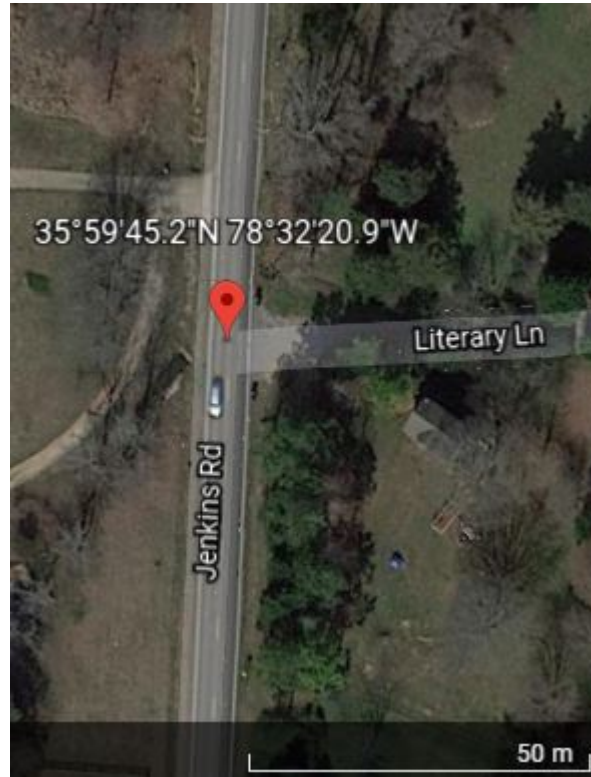


Figure F26. Site Jenkins in Wake County.



Figure F27. Site Asbury in Durham County.

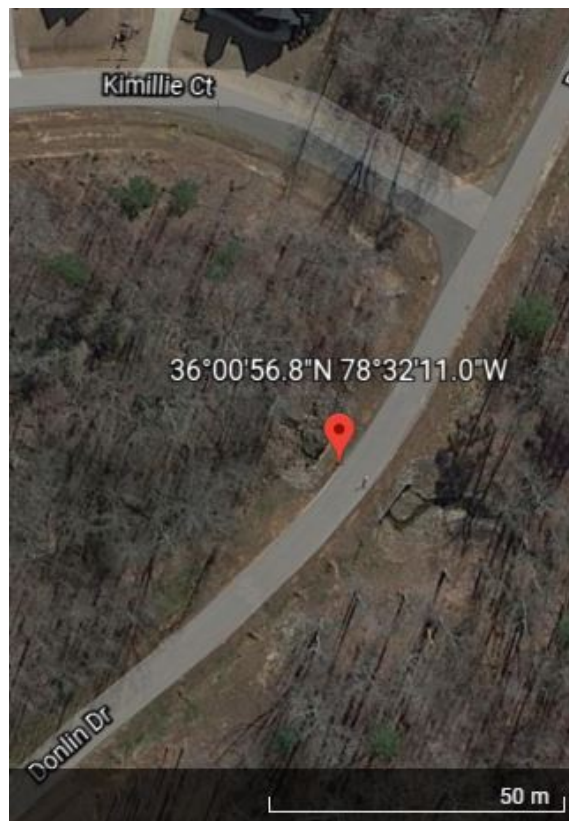


Figure F28. Site Donlin in Wake County.

APPENDIX G: PASSMORE AND PARK RIDGE SITES OVER TIME



Figure G1. Aerial imagery of the agricultural/cropland land cover upstream of outlier sites Park Ridge and Passmore before residential development. The top image was taken in 1993 and the bottom image was taken in 2002 right before development.



Figure G2. Aerial imagery of the agricultural/cropland land cover upstream of outlier sites Park Ridge and Passmore after residential development. The top image was taken in 2004 at the beginning of development and the bottom image is the most recent aerial image taken in 2022.

