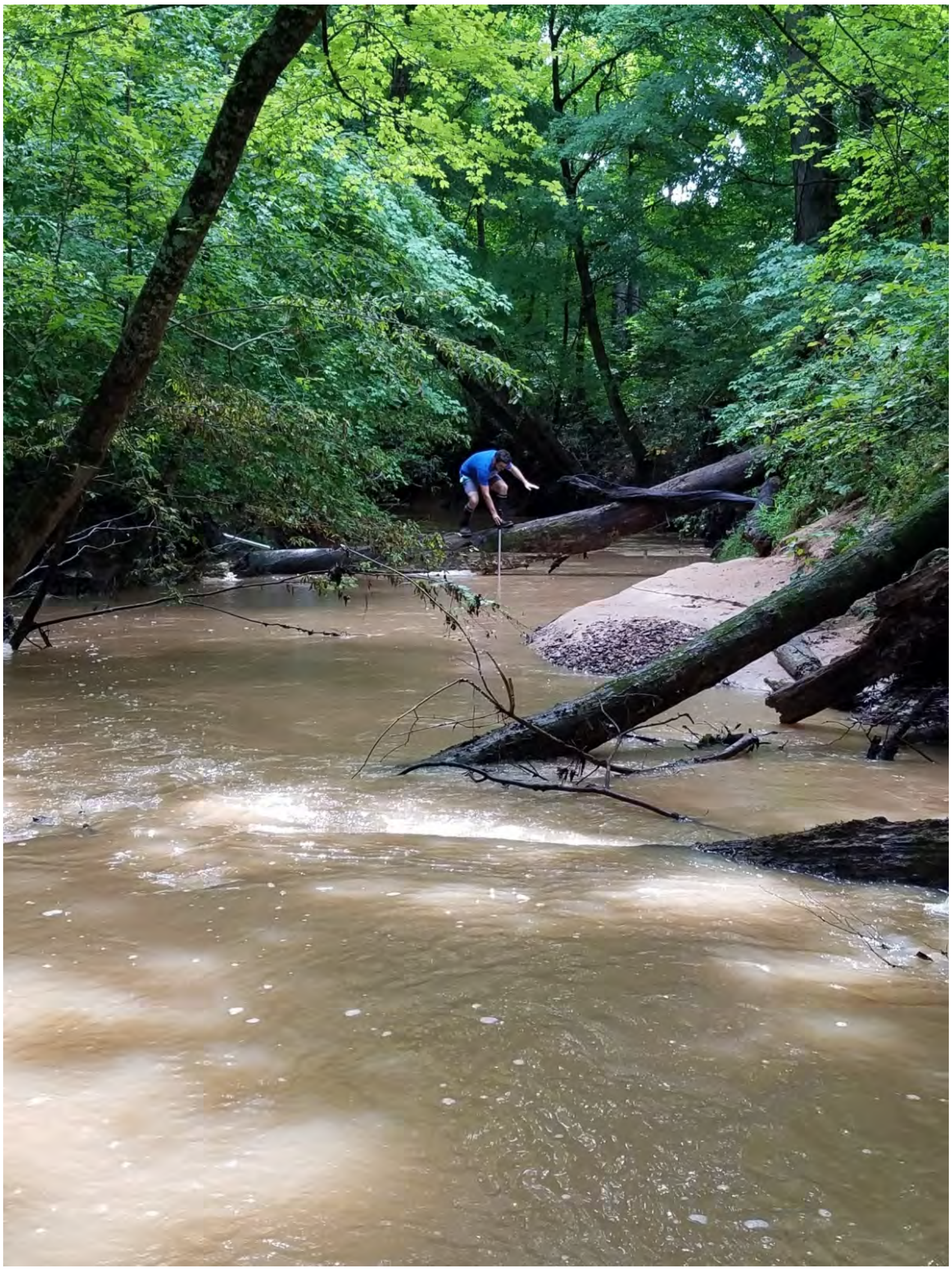
Nutrient Loading from Onsite Wastewater Systems in the Falls Lake Watershed: Evaluating the Potential for Nutrient Load Reductions via Bioreactors

Submitted to the NC Policy Collaboratory (30 September 2021)

By: Guy Iverson, Michael O'Driscoll, Charles Humphrey, Natasha Bell, Ann Marie, Lindley, John Hoben, and Jennifer Richardson

East Carolina University



Executive Summary

This study utilized an integrated approach (geographic information system [GIS], literature review, and synoptic sampling) to identify stream reaches in the Falls Lake Watershed that may have elevated nutrient concentrations and to evaluate the potential of bioreactors to reduce nutrient loading to Falls Lake. Septic system locations were analyzed in GIS to identify 30 sub-watersheds that contained a range of high septic system densities (at least 1 systems ha^{-1}). Stream water quality was assessed in December 2020 and February 2021 to quantify nutrient concentrations and if streams contained elevated septic-derived nutrients. A pilot-scale project was launched to evaluate the nutrient treatment efficacy of various media. Denitrification rates were studied between peanut shells, pine bark, and mixed species woodchips under varying hydraulic retention times. Furthermore, each bioreactor cell included a section of expanded slate aggregate to assess phosphate desorption behaviors after reactive sites have reached capacity. A literature review was conducted to evaluate the potential efficacy of utilizing in-stream bioreactors in the Falls Lake Watershed. Data collected from the field study, pilot study, and literature review were coupled to identify optimal locations in the Falls Lake Watershed that may benefit from use of in-stream bioreactors. We have included brief research highlights that address each of the research questions this project addressed.

- Density analysis indicated that the highest septic system densities are located in the southeastern (Wake County) and central (central Durham County) portions of the Falls Lake Watershed.
- All the sub-watersheds contained elevated TN concentrations and 8 of the 30 sub-watersheds contained TN concentrations > 2 mg L^{-1}. TP values were elevated in 23 of the sub-watersheds and 6 of these contained TP concentrations > 0.08 mg L^{-1}.
- Past studies have shown that denitrifying bioreactors can reduce nitrate-nitrogen concentrations by 14 – 98% and coupling bioreactors with phosphate-sorbing media can reduce phosphate-phosphorus concentrations by 10 – 96%. Few studies have quantified in-stream bioreactor treatment efficiencies, but early research has indicated that nitrate and phosphate reductions were 78% and 74%, respectively.
- Results from the pilot study bioreactor found that pine bark at a 2-hour hydraulic retention time was most effective at reducing nitrate, while mitigating releasing other nutrient species. Nitrate reduction in this media was 72% in early trials and estimated annual mass removal was up 15.6 pounds per cubic feet of bioreactor.
- Coupling results from watershed sampling, bioreactor pilot study, and literature review suggested that there are multiple candidates that would be excellent for in-stream bioreactors in the Falls Lake Watershed. Candidate sub-watersheds were all low-order tributaries to Falls Lake and contained elevated nitrate and phosphate concentrations. Furthermore, land cover data suggested that these sub-watersheds tended to have higher developed lands and low percentages of forested lands within 50 ft of the stream reach.

Additional research is suggested to explicate pollutant reduction and removal efficiencies of in-stream bioreactors to manage nutrient-sensitive waters in the North Carolina Piedmont.

Introduction

Onsite wastewater treatment systems (henceforth referred to as septic systems) are commonly used wastewater technologies for individuals that live in suburban or rural areas of North Carolina. In 2010, approximately 4.87 million North Carolinians (approximately 50% of the total population) used septic systems. The last time that the US Census Bureau included questions in their survey regarding septic systems was 1990 and the statewide percent of the population using septic was 49%. These data suggest that the percentage of the population using septic has not greatly shifted over 20 years [1], despite increasing populations and urbanization in North Carolina (NC). In July 2019, the NC population was estimated to be approximately 10.5 million people [2]. It is estimated that 5.24 million North Carolinians were served by septic systems in 2019, assuming the percent using septic systems have not substantially changed from 2010 to 2019. The average people per household in NC was 2.52 in 2019 [2], thus there are approximately 2.1 million septic systems serving residences in NC. In the Falls Lake Watershed, there are an estimated 50,000 septic systems based on estimates from Brown and Caldwell who partnered with Durham, Franklin, Granville, Orange, Person, and Wake Counties and the State of North Carolina to identify septic system locations in the Falls Lake Watershed. These septic systems have the potential to discharge elevated pollutants to groundwater and/or surface waters downgradient from disposal fields, which could ultimately reach Falls Lake and contribute to eutrophication in the Lake.

Septic systems have potential to be substantial contributors of nitrogen [3-9] and phosphorus [6, 7, 10-12] to downgradient groundwaters and surface waters. Over the past several years, there have been several studies conducted to quantify nutrient transport from septic systems at the site [13, 14] and watershed [15] scales within the Falls Lake Watershed. Furthermore, several more studies have been published that characterized septic system transport of nutrients in the Georgia Piedmont [5, 16]. Previous research determined that the mass reduction of nitrogen and phosphorus from the drainfield to the stream was 77% and 94%, respectively. The annual mass of nitrogen and phosphorus loaded to the stream was estimated to be 1.0 and 0.04 kg person^{-1} yr^{-1}, respectively [17]. The number of people served by septic systems in the Falls Lake Watershed was estimated to be >100,000 [17]. If estimates from prior research are representative of all septic systems in the watershed, there is potentially at least 100,000 kg of nitrogen and 4,000 kg of phosphorus loaded to the Falls Lake Watershed each year. These data suggest that stream reaches draining watersheds served predominantly by septic systems may contain elevated nutrient concentrations. Thus, more work is needed to evaluate the nutrient transport of septic systems at the watershed scale and to consider potential management strategies for nutrient-sensitive watersheds.

The goal of this study was to identify tributaries of the Falls Lake Watershed with elevated septic system nutrient loads using an integrated approach (geographic information system (GIS), literature review, and synoptic sampling) and evaluate the potential for in-stream bioreactors for reducing nutrient loads to Falls Lake. Based on GIS data on septic system densities, this study focused on Durham and Wake Counties since those areas of the Falls Lake Watershed contain the most elevated septic system densities. More specifically, this study aimed to address the following questions:

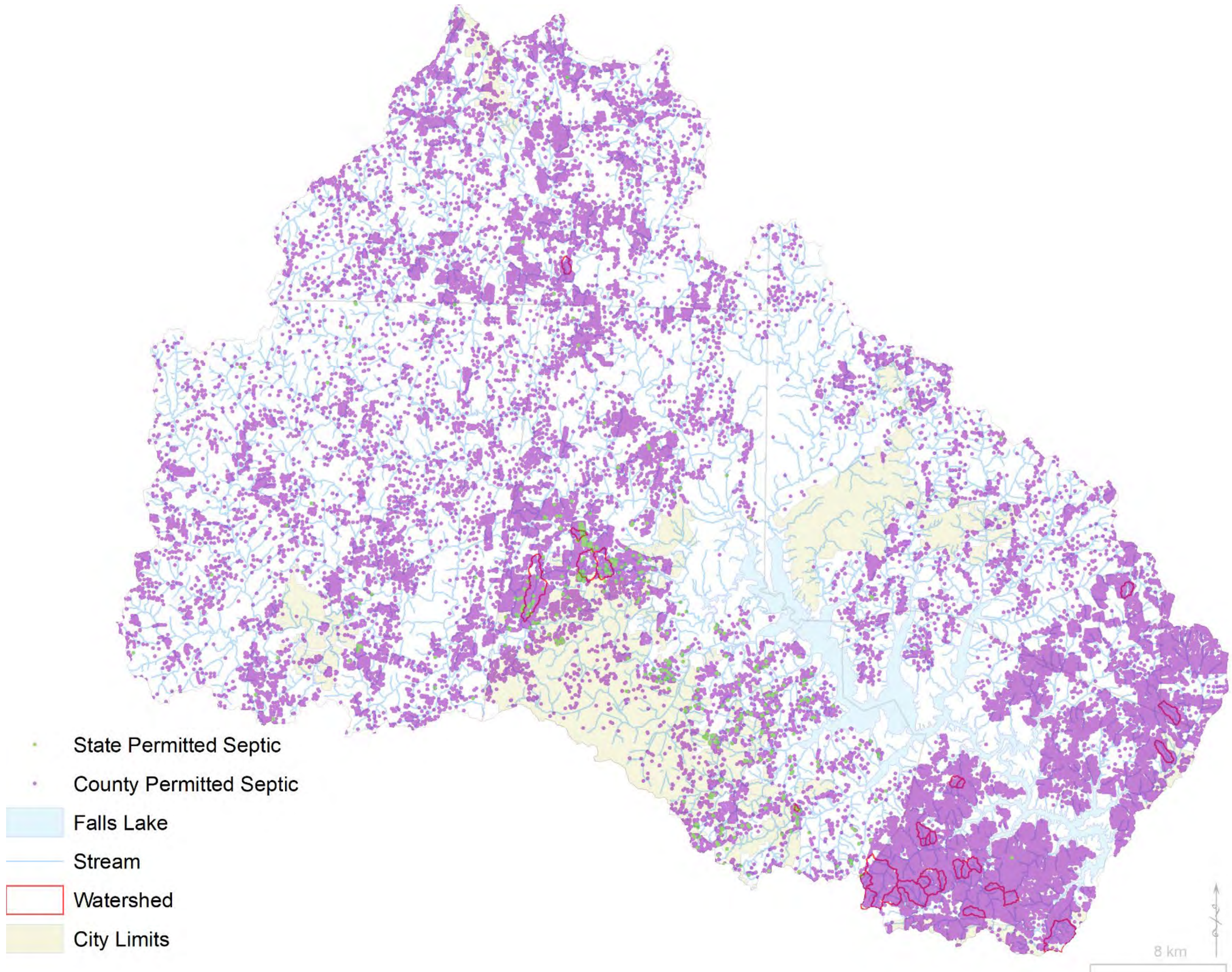
1. Which stream reaches in the Falls Lake watershed are most vulnerable to excess nutrient loading from onsite wastewater treatment system inputs?
2. Which sub-watersheds in the Falls Lake watershed that have elevated septic system densities (> 1.5 systems/ha) have elevated baseflow nutrient concentrations?
3. Does the published literature suggest that in-stream bioreactors can reduce nutrient inputs to Falls Lake, and if so what types of systems are most likely to be effective and what are the potential reductions?
4. What bioreactor porous media are most effective at reducing onsite nutrient transport?
5. What are the optimal locations for bioreactors along low-order streams to reduce nutrient inputs to Falls Lake?

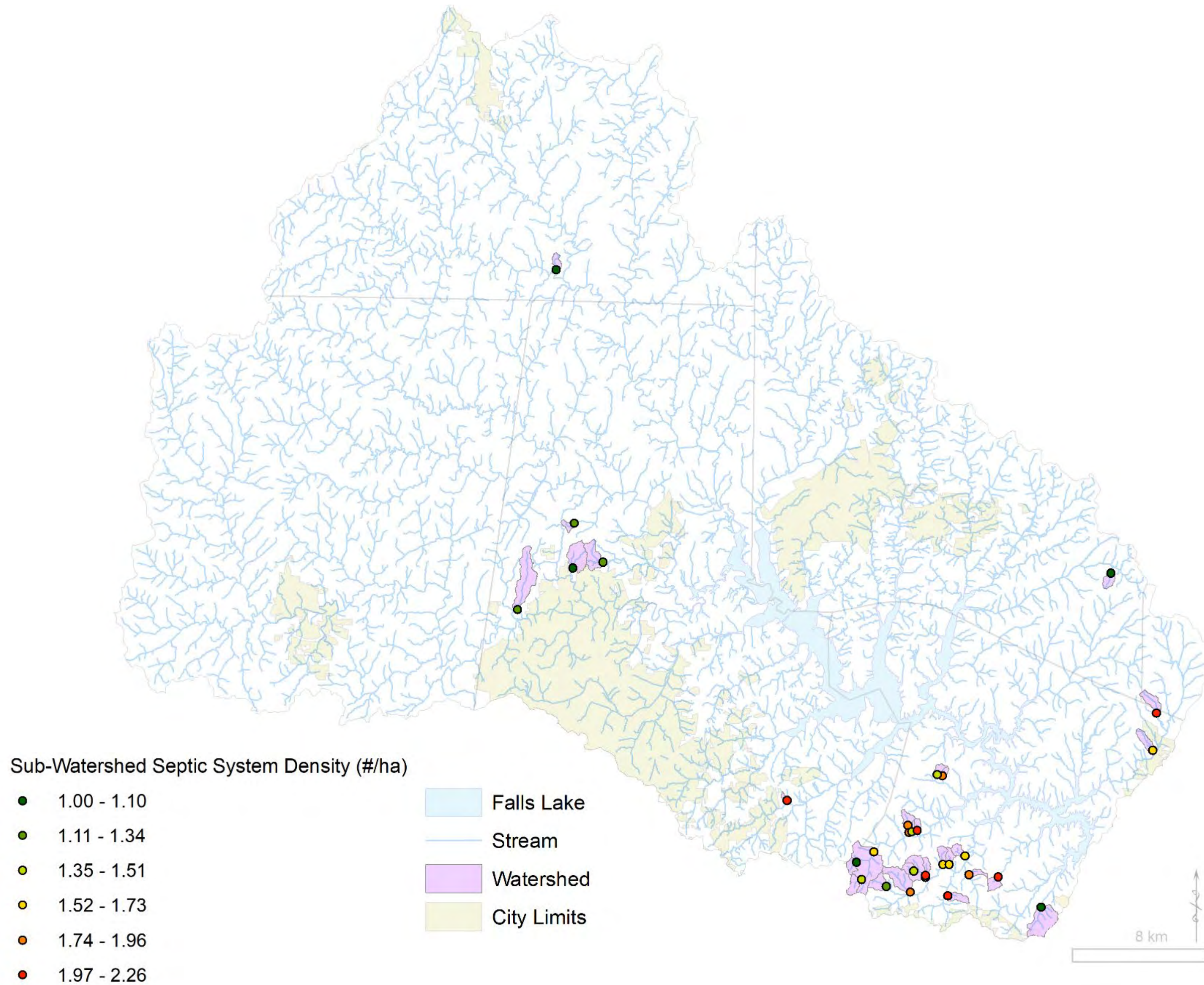
Research Methods

GIS Analysis: Septic system locations were provided by Brown & Caldwell who worked with county health departments and the State of North Carolina to estimate locations of permitted septic systems in the Falls Lake Watershed. These data were integrated into ArcMap 10.8.1 and potential sub-watersheds were identified based on where these data clustered (Figs. 1 and 2). *StreamStats 4.0* (https://streamstats.usgs.gov/ss/) was used to delineate low-order sub-watersheds with high densities of septic systems. A total of 30 sub-watersheds that contained a septic system density > 1 system ha^{-1} or greater were identified and these data were overlain with fine-resolution hydrogeological and road networks. Sites were selected based on septic system density, presence of measurable stream feature, and access. Sub-watershed characteristics of the final sites are summarized in Table 1 and mapped in Figure 2. Soil data were downloaded from the USDA Web Soil Survey (https://websoilsurvey.sc.egov.usda.gov/App/WebSoilSurvey.aspx) and the dominant hydrologic soil group was mapped across the watershed to guide site evaluation. The 2019 National Land Cover Database (https://www.mrlc.gov/data) was used to evaluate dominant land cover in the Falls Lake Watershed. Furthermore, the presence of riparian buffers and wetlands adjacent to streams were assessed. These land cover data were used to estimate potential locations for in-stream bioreactors, riparian buffer restoration, or other best management practices (BMPs) designed to enhance nutrient reduction. Most streams in the selected sub-watersheds were approximately 0.5 – 2.5 m (1.5 – 8 ft) wide and between 3 and 22 cm deep on average. These channel dimensions are favorable for BMPs to facilitate denitrification and phosphate adsorption.

Table 1. Watershed characteristics for the 30 studied sub-watersheds.

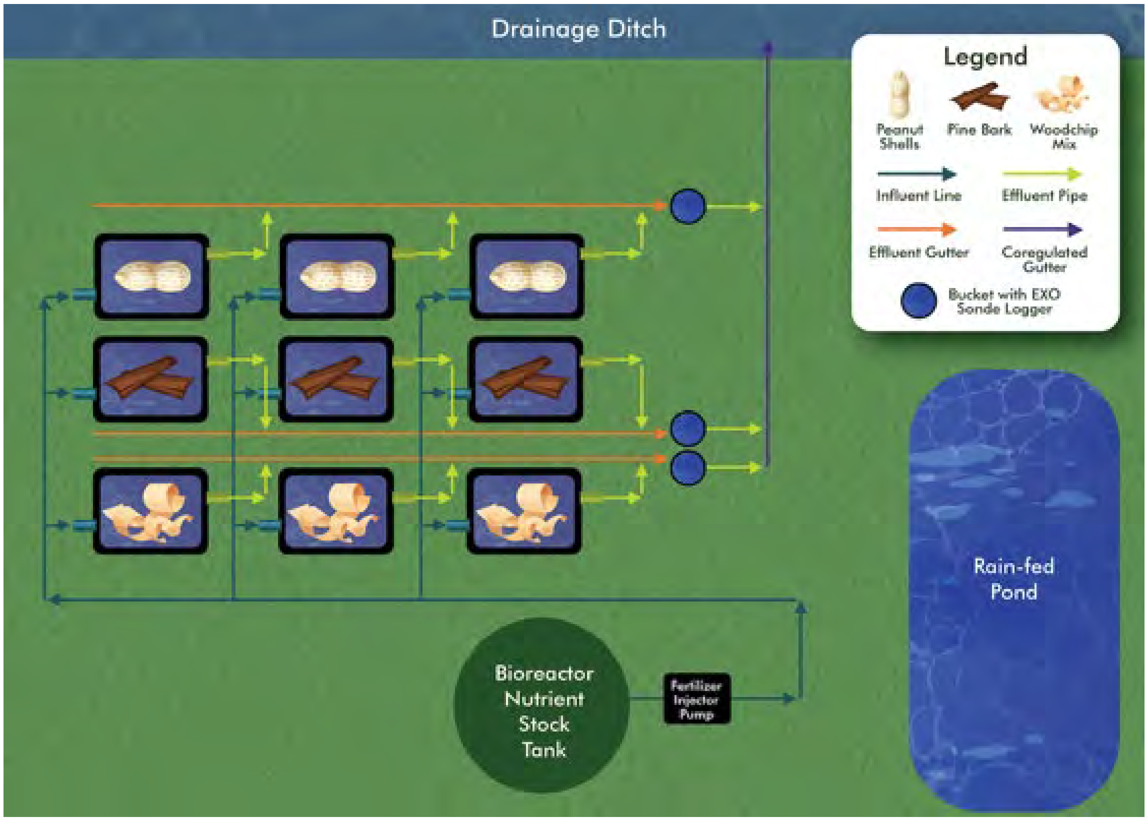
Watershed Name	County	Septic System (#)	Area (ha)	Septic System Density (system ha^{-1})	Latitude	Longitude
Durant	Wake	229	229.48	1.00	35.91	-78.61
Victory Church	Wake	740	458.12	1.62	35.94	-78.72
November	Durham	345	268.91	1.28	36.07	-78.96
Appaloosa	Granville	54	48.91	1.10	36.09	-78.57
Donlin	Franklin	160	79.88	2.00	36.02	-78.54
Jenkins	Wake	110	64.27	1.71	36.00	-78.54
Woody	Person	43	40.04	1.07	36.26	-78.93
Barclay	Durham	150	125.55	1.19	36.10	-78.90
Harold	Durham	43	32.03	1.34	36.12	-78.92
Green Bay	Durham	162	159.14	1.02	36.10	-78.92
Asbury	Durham	17	8.51	2.00	35.97	-78.78
Macon	Wake	272	152.44	1.78	35.92	-78.70
Park Ridge	Wake	72	46.21	1.56	35.94	-78.66
Brookfield	Wake	119	52.60	2.26	35.92	-78.68
Tacketts Pond 1	Wake	31	16.74	1.85	35.98	-78.68
Tacketts Pond 2	Wake	39	28.62	1.36	35.98	-78.68
Green Downs 1	Wake	112	59.31	1.89	35.95	-78.70084
Green Downs 2	Wake	19	13.45	1.41	35.95	-78.70
Green Downs 3	Wake	52	23.64	2.20	35.95	-78.70
Appaloosa Run E	Wake	89	47.10	1.89	35.96	-78.70
Ethan	Wake	46	43.80	1.05	35.94	-78.74
Indigo Moon Way	Wake	101	66.80	1.51	35.93	-78.73
Bushveld	Wake	95	72.62	1.31	35.92	-78.72
Cranesbill	Wake	270	189.69	1.42	35.93	-78.69
Liatris	Wake	53	37.27	1.42	35.93	-78.70
Old Creedmoor	Wake	69	34.71	1.99	35.93	-78.68999
Kinsdale 1	Wake	76	45.10	1.69	35.93	-78.68
Kinsdale 2	Wake	65	37.49	1.73	35.93	-78.67
Leslie 1	Wake	58	29.59	1.96	35.93	-78.66
Coachmans Way	Wake	108	53.60	2.01	35.93	-78.64

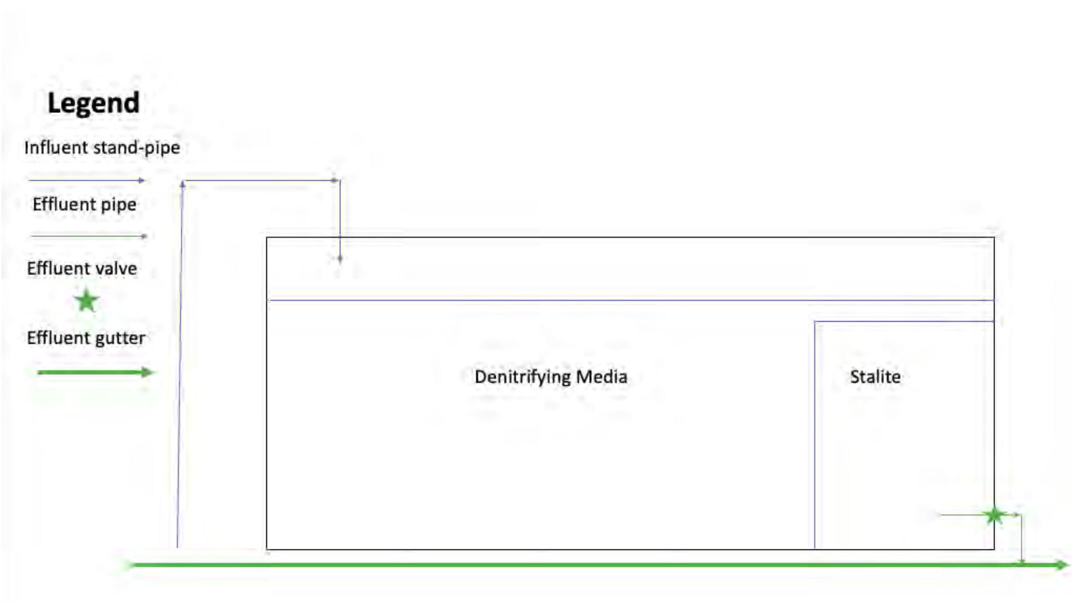

State Permitted Septic
County Permitted Septic
Falls Lake
Stream
Watershed
City Limits
8 km


Sub-Watershed Septic System Density (#/ha)
1.00 - 1.10
1.11 - 1.34
1.35 - 1.51
1.52 - 1.73
1.74 - 1.96
1.97 - 2.26
Falls Lake
Stream
Watershed
City Limits
8 km

Water Quality Assessment: Two sampling events occurred in December 2020 and February 2021 to evaluate water quality in sub-watersheds (Fig. 2) with elevated septic system densities (> 1 system ha^{-1}). During each sampling event, a multiprobe sonde was used to measure water temperature, pH, dissolved oxygen, conductivity, and oxidation-reduction potential. Water samples were collected into clean 500 mL polypropylene bottles, stored on ice, and transported to East Carolina University where they were either immediately frozen or analyzed in the Environmental Research Laboratory. A *Unity SmartChem* autoanalyzer was used to analyze samples for nitrate, ammonium, total Kjeldahl nitrogen (TKN), particulate nitrogen (PN), total phosphorus (TP), total dissolved phosphorus (TDP), phosphate, particulate phosphorus (PP). The difference between TKN and ammonium was calculated and used to report dissolved organic nitrogen (DON). A *Shimadzu TOC/TN* analyzer was used to measure dissolved organic carbon and total dissolved nitrogen (TDN). Both TN and TP were calculated by summing the total dissolved concentration by the particulate concentration, thus TN= TDN + PN and TP= TDP + PP. A subset of samples was taken and shipped to the Stable Isotope Facility at the University of California at Davis to analyze the isotopic fractionation of $^{15}N/^{14}N$ and $^{18}O/^{16}O$. Nutrient speciation data were used to analyze differences in dominant nitrogen and phosphorus species to provide further insights on sites for potential in-stream bioreactors.

Bioreactor Pilot Study: This ongoing pilot-scale experiment is designed to shed light on the use of denitrifying bioreactors paired with phosphate sorbents, and focuses on three major goals to: 1) compare the efficacy of three different substrates- roasted peanut shells, pine bark, and wood chips of mixed species; 2) compare the efficacy of three different hydraulic retention times (HRTs): 30 minutes, 1 hour, and 2 hours; and 3) quantify the phosphate sorption capacity of an expanded slate aggregate. The pilot scale denitrifying bioreactors consist of nine 150-gallon troughs (Figs. 3 and 4). Approximately two-thirds of the upstream portion of the troughs (50 gallons) were filled with one of the three denitrifying substrates and the remaining third (28 gallons) was filled with an expanded slate aggregate to adsorb phosphate (Fig. 4). The carbonaceous substrates and the phosphate sorbents are separated by a pouch of landscape fabric encasing the phosphate sorbents. Planning and design efforts, materials purchasing, construction, and installation of the outdoor experimental bioreactor system was initiated in Fall 2020 and continued through Spring 2021. Due to COVID-related personnel and supply chain issues, the experimental portion of the project was delayed, with data collection commencing in June 2021 and continuing through November 2021.





Samples were collected weekly over the course of six months (June-November 2021). This sampling period can be divided into 8 three-week trials, in which each HRT is tested once per trial. Bioreactors remain saturated between sampling days to capture water quality dynamics when water is left stagnant, which is typical of these systems.

On sampling days, water with a concentration of 20 mg/L NO_3-N and 1 mg/L PO_4-P is pumped through the bioreactors at a constant rate depending on the HRT that is being tested. Samples are collected upon the initial flush of pore water (PV 0), and then once every pore volume (PV) for five total pore volumes (PV 1-5) to capture the reduction in nitrate-N at steady state. Christianson [18] has reported that bioreactors reach steady state at greater than one to two pore volumes. Temperature, pH, dissolved oxygen, turbidity, specific conductance, oxidation-reduction potential, and dissolved organic matter were continuously monitored during each experimental run using YSI EXO2 multiparameter sondes (YSI Inc./Xylem Inc., Yellow Springs, OH) in buckets of pooled effluent for each of the three substrates. Water was filtered through 0.45 μm syringe filters prior to all lab analyses. Dissolved organic carbon (DOC) was extracted with the Hach TOC Test Kits and measured on a DR6000 spectrophotometer (Hach Company, Loveland, CO). Nitrate + nitrite, ammonium, phosphate, TKN, and total Kjeldahl phosphorus (TKP) were analyzed on the Lachat Quikchem 8500 flow injection analysis system (Hach Company, Loveland, CO). DON was calculated by taking the difference of TKN and ammonium.

During the 14-day start up period, the bioreactors were dosed with micronutrients (4.0 mM CaCl2, 2.0 mM KH2PO4, 1.0 mM K2SO4, 1.0 mM MgSO4, 15 μM H2BO3, 2.0 μM MnSO4, 2.0 μM ZnSO4, 0.5 μM CuSO4, 0.5 μM Na2MoO4) [19] at a HRT of 4 days. Due to a dosing error, influent waters contained greater phosphate concentrations than the initially intended 1 mg/l PO_4-P. Bioreactor inflow contained ~25 mg/L PO_4-P during start up and the phosphate capacity of the expanded slate was reached before regular sampling began. This over-dose changes the dynamics of the phosphate sorption side of the experiment. While it may no longer be possible to quantify the phosphate adsorption capacity of expanded slate over the course of a six-month period, this experiment can provide useful data on phosphate desorption behavior once an adsorbent has already reached capacity in an environment with intermittent periods of flow. Very little research has been done on phosphate desorption of expanded slate when paired with denitrifying bioreactors.

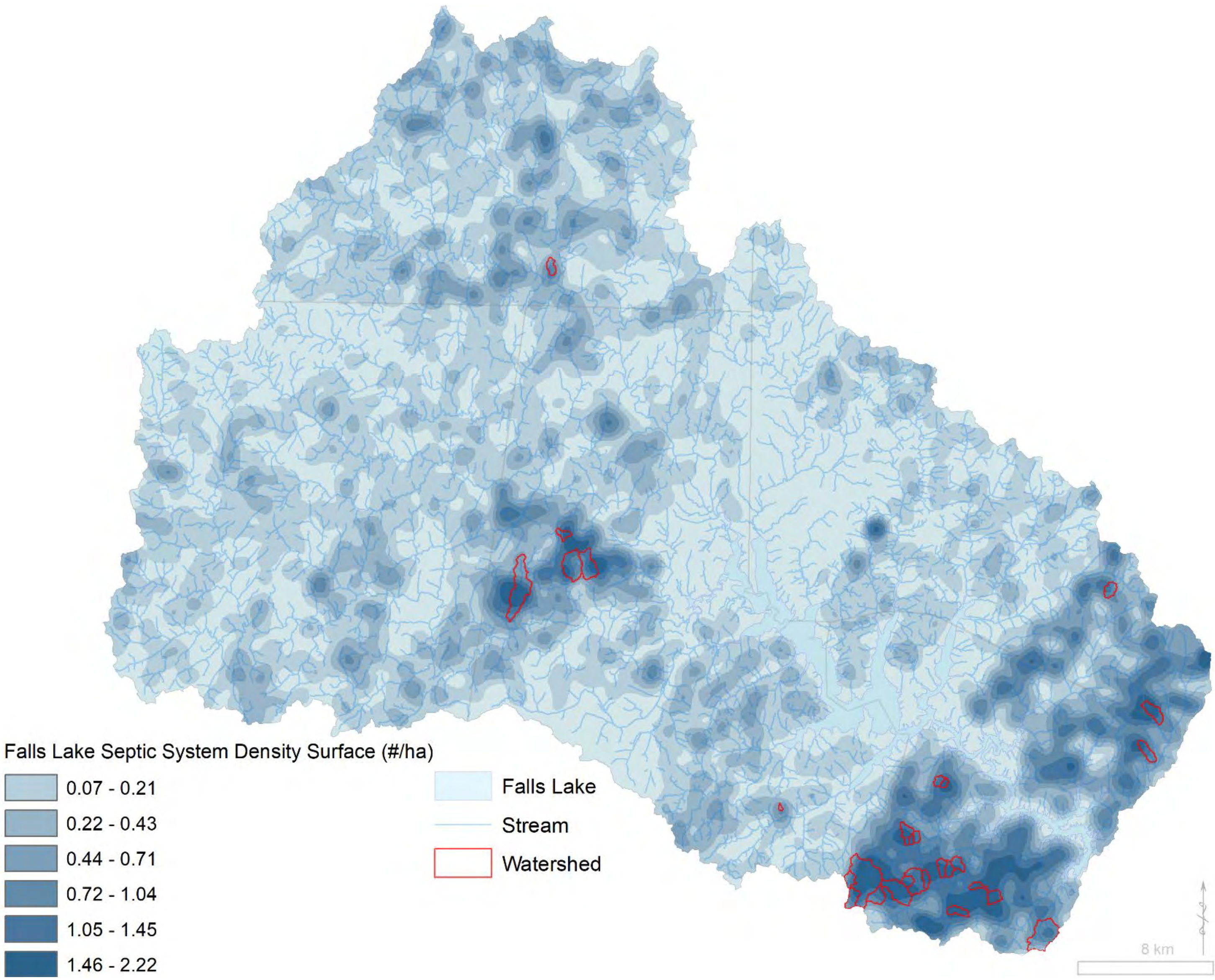
After trial 1, some of the capabilities of the Lachat Quikchem 8500 were compromised. The laboratory is actively working to bring the instrument fully back online. Samples were filtered and immediately frozen until troubleshooting of the Lachat Quikchem 8500 has completed. Prior to freezing, samples were analyzed for analytes that can be analyzed on the instrument, which included DOC, TKN and TKP. Currently, there are DOC data for trials 1-4 and TKN/TP data for trials 1-3.

Initial Findings

Which stream reaches in the Falls Lake watershed are most vulnerable to excess nutrient loading from onsite wastewater treatment system inputs?

Past research suggested that there are numerous variables that affect nutrient transport from septic systems to adjacent streams. These factors include: soil type and hydrogeologic setting, system loading rates and characteristics of wastewater, density of septic systems within watersheds, presence and condition of riparian buffers, individual system characteristics (design, type, size, and age), biomat formation, greywater, garbage disposals, system maintenance, and system functionality (i.e. malfunctioning or functioning as designed) [17, 20-25]. Past research has suggested that septic system density may be an important factor that can aid in identification of watersheds with stream reaches affected by septic systems in Piedmont settings [5, 15]. Furthermore, soil type may also play a significant role in affecting nutrient transport from septic systems to adjacent streams. Results from the 2019 – 2020 NC Policy Collaboratory study [17] showed that watersheds in sandier soils (> 30% sandy loam) were more likely to have elevated nutrient exports relative to soils with 70% or more clay or silt loam.

The highest septic system densities were observed in the Wake, Durham, and Franklin County portions of the Falls Lake Watershed (Fig. 5). Numerous clusters of high septic system densities were found in the southeastern portion of the Falls Lake Watershed where extensive suburban and rural development has occurred, primarily in Wake County. Similar clusters of high system densities were also observed in the central portion of Durham County near the border between the City of Durham and Durham County (city limits viewable on Figs. 1 and 2). Previous research has shown that nutrient concentrations in stream reaches increase proportionally to watershed septic system density after exceeding 1 system ha^{-1} [5]. Another study by Iverson et al. [15] found that concentrations and mass exports between high-density watersheds (> 1 system ha^{-1}) differ significantly from watersheds served by low-density (< 1 system ha^{-1}) and control watersheds. Estimates of septic system density across the Falls Lake Watershed and the estimated septic system density for each sub-watershed suggest that these watersheds are vulnerable to nutrients from septic systems.


Falls Lake Septic System Density Surface (#/ha)
0.07 - 0.21
0.22 - 0.43
0.44 - 0.71
0.72 - 1.04
1.05 - 1.45
1.46 - 2.22
Falls Lake
Stream
Watershed
8 km

In addition to septic system density, hydrologic soil group can also provide insight in assessing vulnerability of stream reaches to septic-derived nutrients (Fig. 6). The northwestern portion of the Falls Lake Watershed is dominated by soils with a hydrologic soil group of B. These soils typically contain moderately low runoff potential when thoroughly wet and water transmission is unimpeded [26]. The southeastern portion of the watershed contains mostly group A or A/D soils (Fig. 6). Group A soils have low runoff potential when thoroughly wet and contain little clay (< 10%), which results in higher hydraulic conductivities than other hydrologic soil groups [26]. The central portion of the watershed consists of group C/D and D soils (Fig. 6). These soils have a moderately high (C/D) to high (D) runoff potential when thoroughly wet. Group C soils typically contain 20 – 40% clay, whereas group D soils contain > 40% clay. In the Triassic Basin, which underlies the central portion of the Falls Lake Watershed, some of these clays have high potential for shrink-swell, which further restricts water transmission in the subsurface. Soils with dual hydrologic groups are placed in group D based on the presence of a water table within 60 cm (24 in) of the surface, even if saturated hydrologic conductivity is favorable for water transmission. The first letter refers to the hydrologic soil group of the drained portion, whereas the second letter refers to the undrained portion [26]. Hydrologic soil group provides a general understanding of the soil texture differences across the watershed, which can play an important role in treatment potential.

Soils belonging to the A and B hydrologic soil groups contain higher hydraulic conductivities, thus these soils can accept higher wastewater loading rates. However, these soils have greater potential to transport nutrients to downgradient water resources, especially in locations with shallow groundwater tables. The increased clay content present in group C and D soils reduces hydraulic conductivity thereby increasing residence time in the vadose zone of the soil. Furthermore, soils with increased clay content have been shown to improve nutrient removal from septic systems because clayey soils have increased reactive surface area [21, 27]. In the Falls Lake Watershed, the Triassic Basin soils are predominantly White Store and Chewacla Series [13], which are expansive clay soils with slow to very slow internal drainage [28]. The typical profile for these series contains clay beginning at approximately 10 and 19 inches for White Store and Chewacla, respectively. Furthermore, perched water tables may be found at 12 – 18 inches during the winter and spring seasons for White Store [28]. These soil characteristics can restrict siting of conventional septic systems because these features inhibit or prevent adequate subsurface treatment of wastewater. Due to these limiting soil features, numerous surface discharging sand filter type systems were permitted in the Triassic Basin, which can discharge elevated concentrations of nutrients [15]. Additionally, previous research has suggested that stream reaches draining watersheds with a high density of septic systems located in the Triassic Basin have elevated nutrients [15, 29] and pathogens [30, 31]. This is thought to be caused by a combination of inhibited pollutant removal and/or septic system malfunction due to soil properties coupled with elevated septic system densities in some watersheds.

Collectively, septic system density and soil type are important factors that affect nutrient transport from septic systems to streams. In the next subsection, we compare the nutrient concentrations and exports of the field study component of this research.

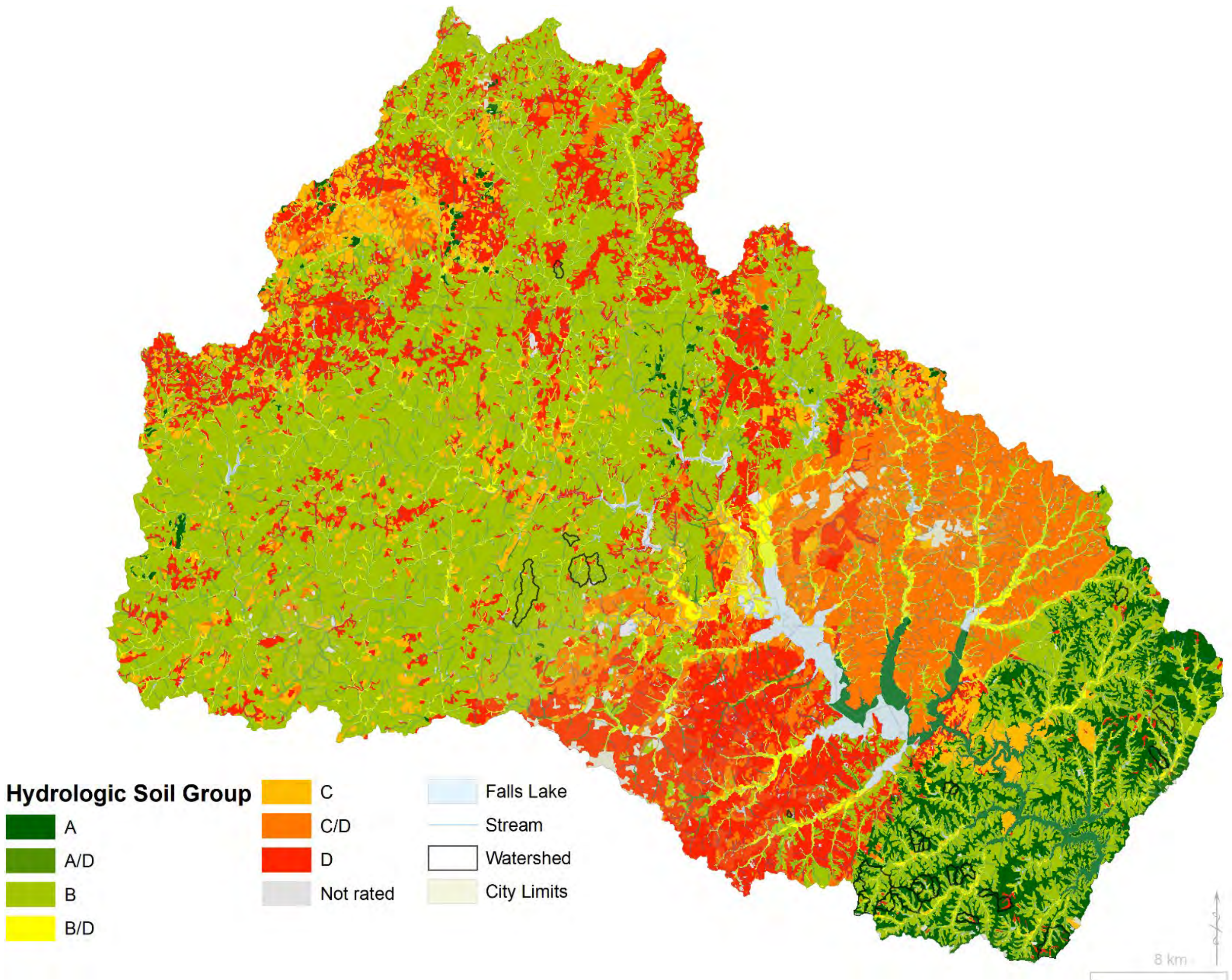


Figure 6. Soil map classified by hydrologic soil group for the Falls Lake Watershed. Group A soils are sandier soils with higher hydraulic conductivities whereas Group D soils contain more clay/silt content and have lower hydraulic conductivities.

Which sub-watersheds in the Falls Lake Watershed that have elevated septic system densities (> 1.5 systems ha^{-1}) have elevated baseflow nutrient concentrations?

Most of the studied sub-watersheds contained elevated concentrations of TN and TDN (Figs. 7 and 8). The median sub-watershed had a median value of 1.75 and 1.69 mg L^{-1} for TN and TDN, respectively. Concentrations of TN and TDN ranged from approximately 1.5 to 10 mg L^{-1}. There are several empirical methods of establishing regional targets of maximum contaminant levels for nutrients. For TN, the target tends to range from approximately 0.5 – 1.2 mg L^{-1} [32], but another study suggests eutrophic conditions are met once TN exceeds 1.5 mg L^{-1} [33]. The study area overlaps with the US EPA Ecoregion IX (southeastern temperate forested plains and hills), which contains a TN reference concentration (based on the 25th percentile of streams) that ranges from 0.07 – 1.0 mg L^{-1} [34]. All the studied sub-watersheds were greater than ecoregion reference conditions and 1.2 mg L^{-1}. There were 5 watersheds (17% of studied sub-watersheds) that contained median TN concentrations between 1.20 and 1.50 mg L^{-1}, potentially indicating that they were not in eutrophic conditions depending on how this threshold is established. In this study, sub-watersheds were compared to the more conservative eutrophic concentration of 1.5 mg L^{-1} suggested by [33]; however, it is possible that eutrophication could occur at lower concentrations as previously discussed [32]. The remaining 25 sub-watersheds exceeded 1.5 mg L^{-1}, suggesting that these stream reaches contained elevated nutrients. Of these 25 sub-watersheds, 8 (27% of all studied sub-watersheds) contained a median TN concentration > 2 mg L^{-1}. Septic system density in these sub-watersheds tended to exceed 1.5 systems ha^{-1} (6 out of 8 sub-watersheds). The remaining 2 sub-watersheds had septic system densities of 1.19 and 1.07 systems ha^{-1}, which are still considered high density by recent studies [5, 15, 30, 31]. One of these sub-watersheds (Park Ridge) contained TN concentrations > 8 mg L^{-1} and had a septic system density of 1.56 systems ha^{-1}. It is also likely that other sources (e.g., domestic animal and wildlife wastes, residential fertilizer use, agriculture, legacy pollutants) are playing a role in the nitrogen cycling at these sub-watersheds, especially at the Park Ridge sub-watershed. This sub-watershed contains substantially greater concentrations of TN and TDN relative to other sub-watersheds. Furthermore, Park Ridge contains a septic system density of 1.56 systems ha^{-1}, which is similar to the median density across all sub-watersheds (1.59 systems ha^{-1}). If TN/TDN inputs from Park Ridge were septic systems alone, one might expect Park Ridge to have a substantially greater density of septic systems or for a large portion of septic systems in this sub-watershed to be experiencing malfunction. These findings suggest that septic system density can be an effective tool to identify sub-watersheds with stream reaches that contain elevated concentrations of TN and TDN relative to reference conditions, and potentially to nitrogen maximum contaminant levels for nutrient-sensitive watersheds depending on how those thresholds are established.

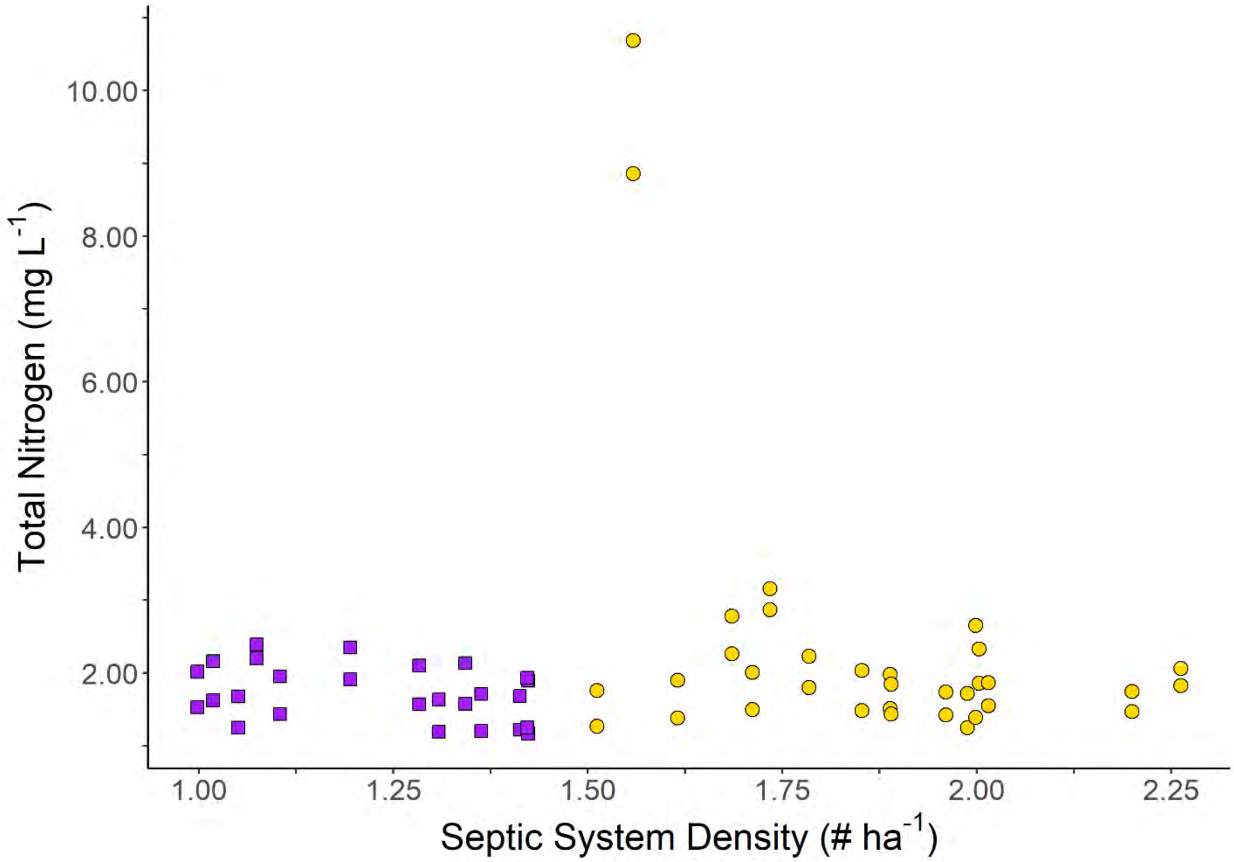


Figure 7. Total nitrogen concentrations compared to watershed septic system density. The concentration for both the December 2020 and February 2021 sampling events are plotted for each sub-watershed grouped by density.

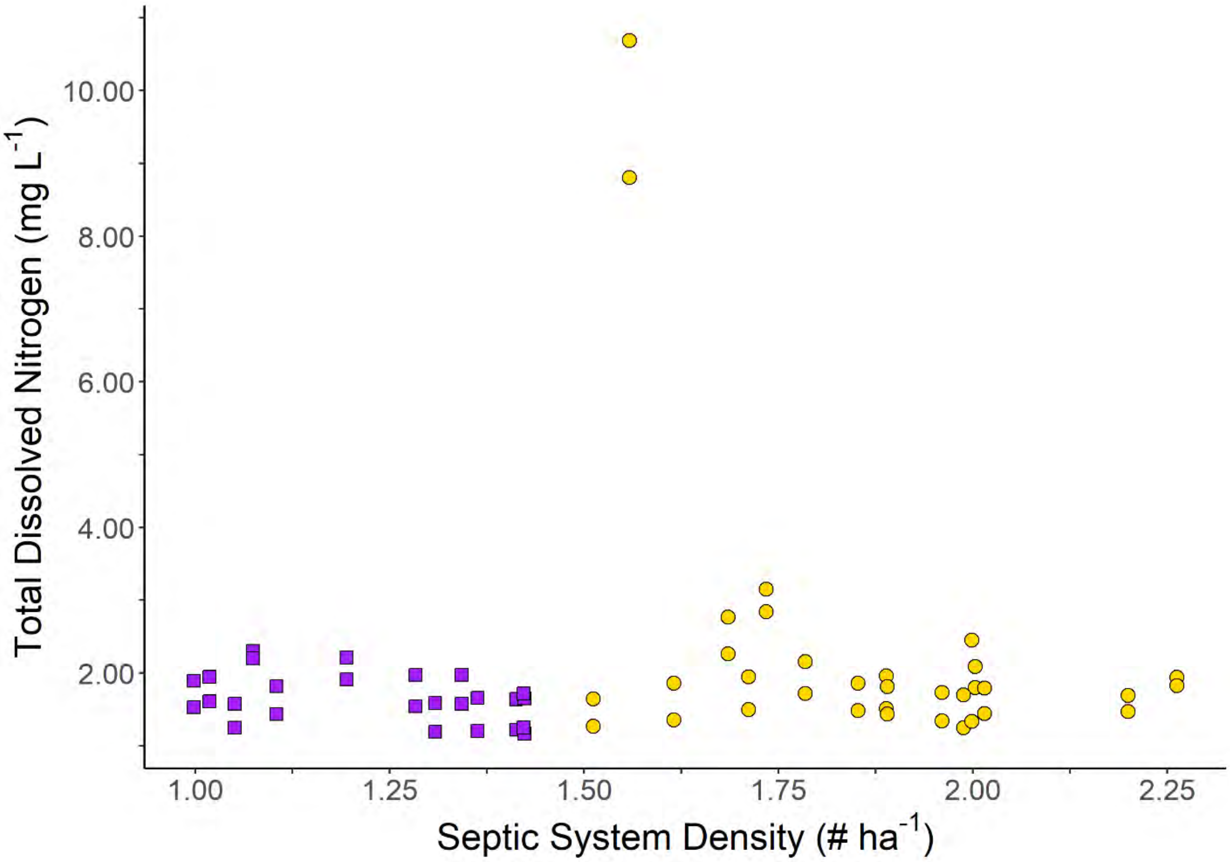
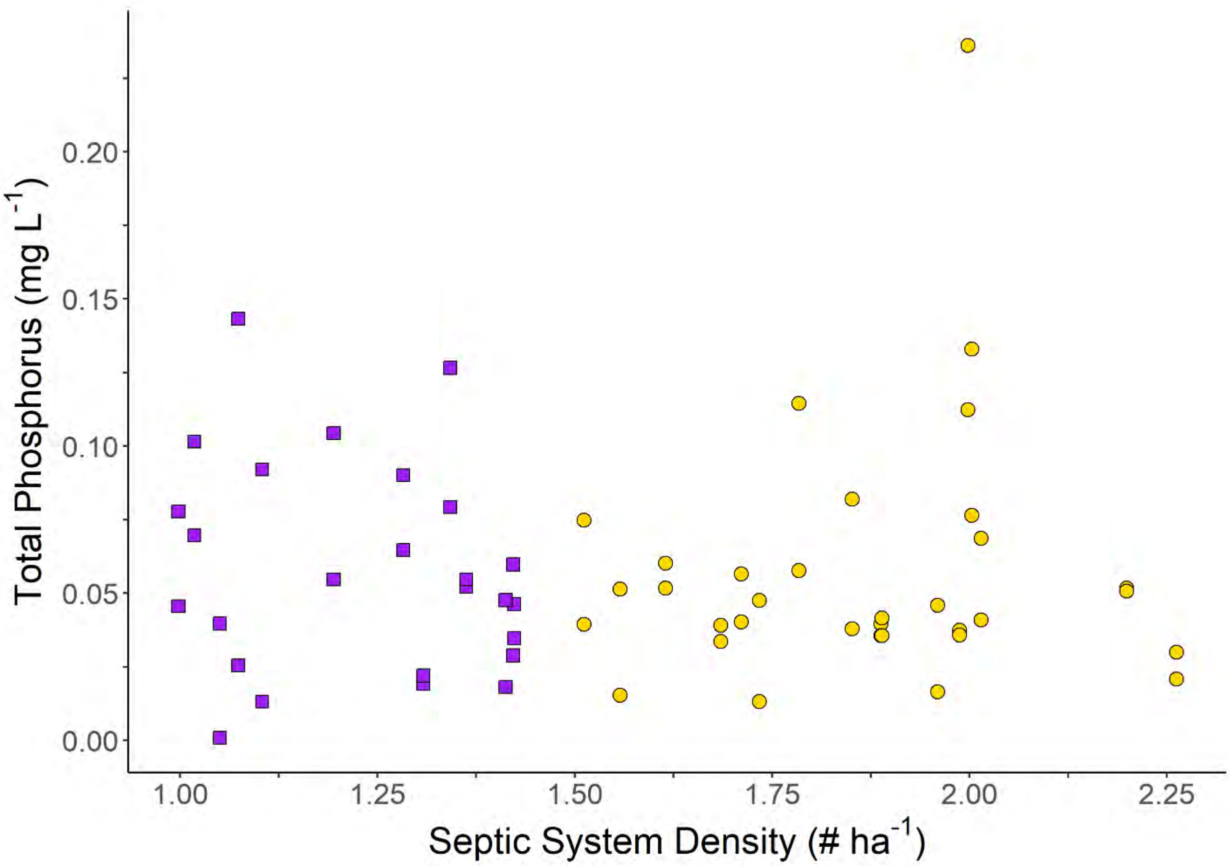
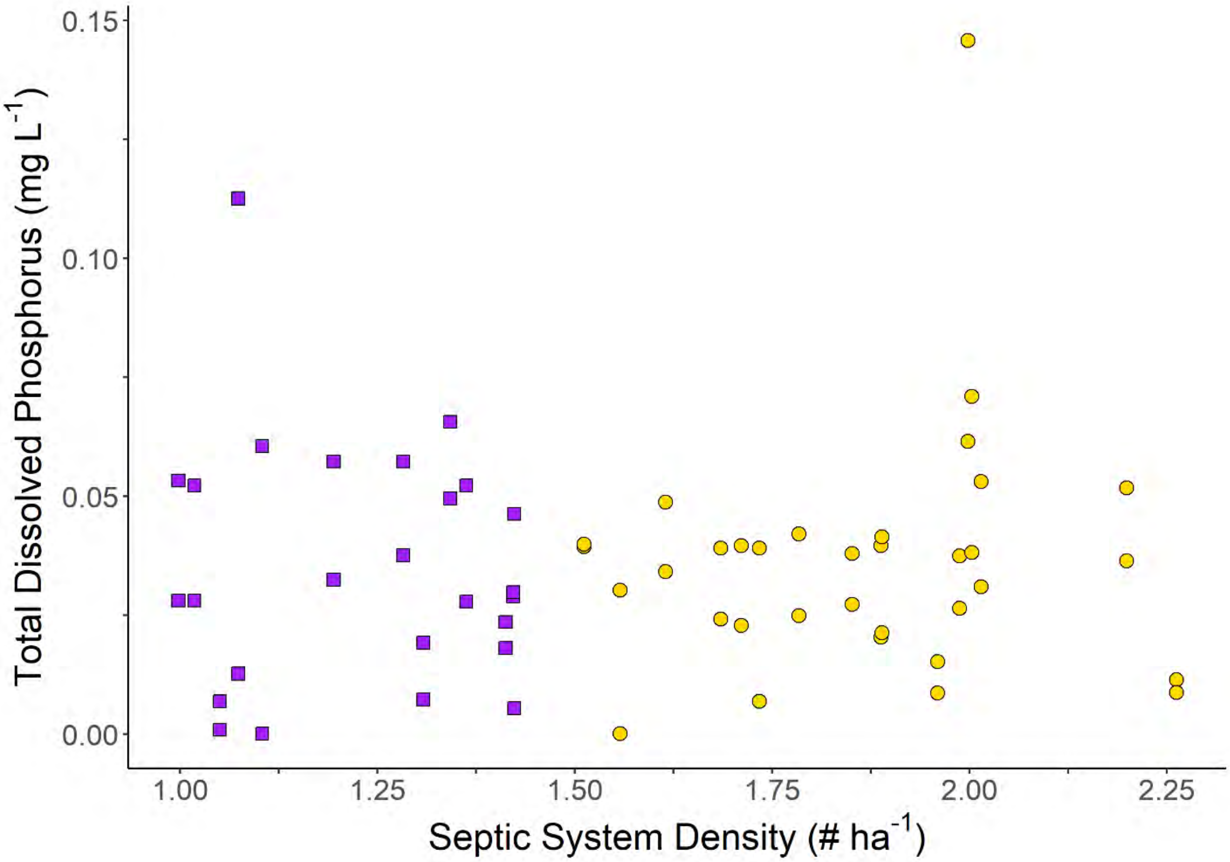


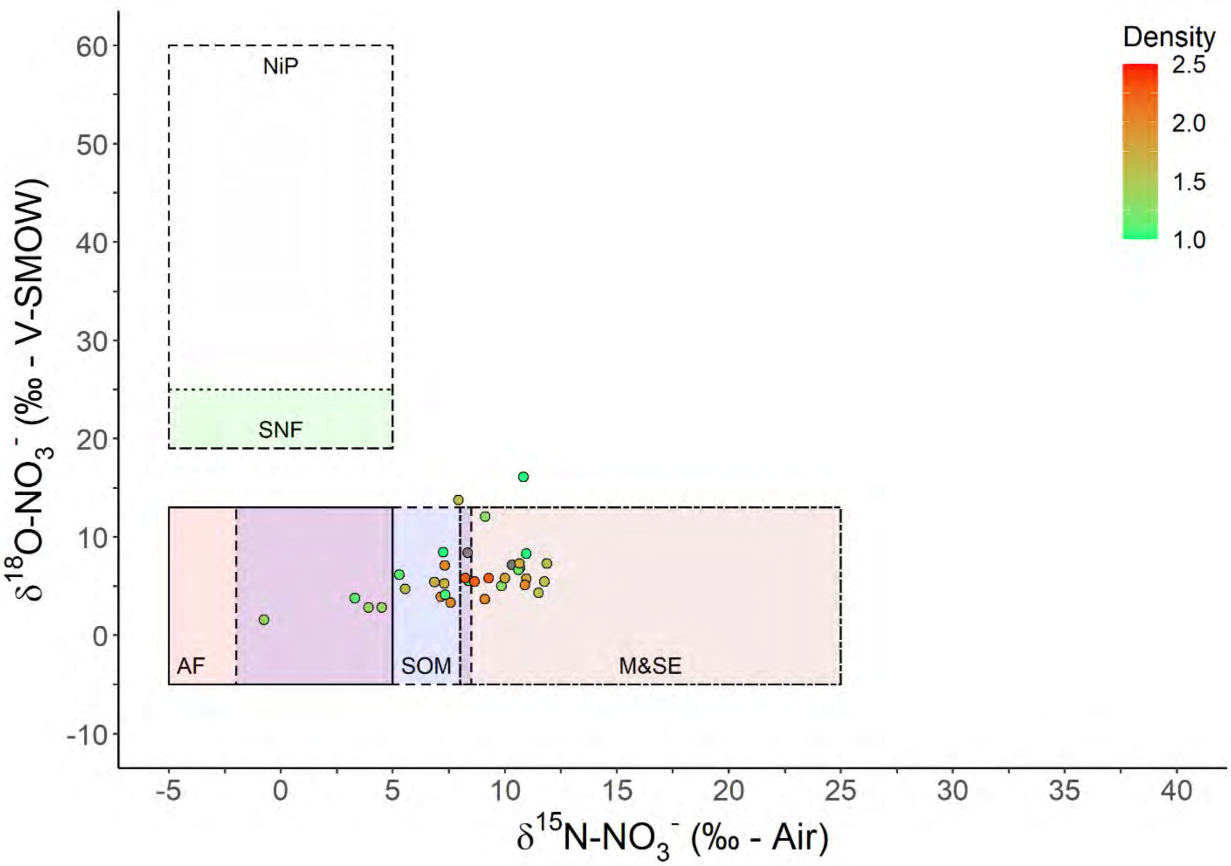
Figure 8. Total dissolved nitrogen concentrations compared to watershed septic system density. The concentration for both the December 2020 and February 2021 sampling events are plotted for each sub-watershed grouped by density.

concentrations to also be elevated. These findings confer with TN results that suggest that septic system density can be an effective tool at locating stream reaches with elevated nutrient concentrations during baseflow conditions. In this study we identified sub-watersheds that contained lower septic system densities than other Piedmont studies (i.e., Hoghooghi et al. [5]). Thus, future work could utilize similar GIS techniques to identify other sub-watersheds that may have a higher density than the range in this study.





Another objective of this report was to identify potential sources of nitrate within the studied sub-watersheds. Isotopic fractionation of nitrate-nitrogen has been used by past studies to identify potential sources of nitrate in waters [35, 36]. These sources include nitrate in precipitation, synthetic nitrate fertilizers, ammonia fertilizers, soil organic matter, and manure and septic effluent. Fractionation of nitrate alone cannot distinguish human wastewaters sources from animal waste signatures. However, Tamborski et al. [37] documented that use of boron isotopes can be successful in differentiating human and animal waste signatures, but saltwater systems may complicate analyses since sea spray contains orders of magnitude more boron than various contaminant sources. Isotopic analysis suggested that the watersheds with a higher density (2 – 2.25 systems ha^{-1}) were more likely to contain nitrate originating from a human and/or animal waste signature (Fig. 11). The sub-watersheds that contained < 2 systems ha^{-1} density also contained $\delta^{15}N$ and $\delta^{18}O$ values of nitrate-nitrogen that indicated human and/or animal waste signatures. Some of these sub-watersheds also showed ammonia fertilizers and soil organic matter (natural sources) as probable sources of nitrate. These results show that septic systems can be a source of nitrate at the sub-watershed scale, thus other species of nutrients may be reaching sub-watershed outlets and increasing nutrient loads to Falls Lake.



Sub-watershed exports of nutrients were also calculated, and the results can be found in Appendix A. Physicochemical data that were collected in the field can also be accessed in Appendix B. These data were not required to address this question; however, we collected these data to aid water quality management efforts since these data are often valuable in watershed nutrient management.

Does the published literature suggest that in-stream bioreactors can reduce nutrient inputs to Falls Lake, and if so what types of systems are most likely to be effective and what are the potential reductions?

In-stream bioreactors are BMPs designed similarly as denitrifying bioreactors. Denitrifying bioreactors (also referred to as permeable reactive barriers and denitrification walls) are BMPs that contain carbon-rich media that enhances nitrogen treatment by reducing nitrate compounds into dinitrogen gas. More recently, similar practices have been adopted to treat elevated nutrients from human [38] and animal [39] waste sources. Past studies have found that denitrifying bioreactors can reduce nitrate-N concentrations by 14 – 98% and mass removal rates are just as variable (Table 2). Some of these BMPs have also incorporated adsorptive media designed to create reactive sites for phosphate sorption, thereby facilitating dual purpose removal of nitrate and phosphate. Phosphate reductions from dual-purpose bioreactors is also highly variable ranging from 10 – 96% (Table 3). Most of the research on denitrifying bioreactors have focused on subsurface installations or laboratory mesocosms and few studies have quantified the efficacy of in-stream bioreactors.

Table 2. Denitrifying bioreactors summarizing nitrogen (N) concentration reductions (Red) and mass removal (rem). HRT= hydraulic retention time; IBR= in-stream bioreactor; BR= bioreactor; WC= woodchips; MS= mixed species; PS= peanut shells; PG= pea gravel; E= eucalyptus; HW= hardwood.

Reference	Setting	Media	Flow (L min^{-1})	HRT	Inflow (mg L^{-1})	N Red (%)	Nitrate Mass Rem
Robertson & Merkley [40]	Field; IBR	WC	24	N/A	4.8	78	6 g m^{-2} d^{-1} (up to 360 g d^{-1})
Iverson [41]	Field; IBR	WC (MS)	26	N/A	0.9	78	0 – 12 g m^{-2} d^{-1}
Bell et al. [42]	Field; BR	WC (MS)	5.8-23.3	2-8 h	<0.1- 17	20-98	11.6 g m^{-3} d^{-1}
Christianson [18]	Pilot; BRs	WC (pine)	N/A	2-15 h	7.7-35.6	14-37	2.1-6.7 g m^{-3} d^{-1}
Ramirez-Godinez et al. [43]	Lab; BR	PS	N/A	3 d	50	95	N/A
Lynn et al. [44]	Lab[a]	2:1 PG; WC (E)	Not specified[a]	1 – 9 h[a]	4	Up to 98	N/A
Hoover et al. [45]	Lab; BR	WC (MS, HW)	N/A	2-24 h	11.5-35.1	39	15.6 g m^{-3} d^{-1}

[a]= Antecedent dry conditions between treatments; designed with stepwise flow rate decrease to simulate storm conditions; HRT in sequence 1 hr, 1 hr, 2 hr, 3 hr, 4 hr, 6 hr, 9 hr to simulate storm conditions
[b]= Studies that quantified bioreactor efficacy downstream of septic systems

Table 3. Denitrifying bioreactors paired with adsorbent media to facilitate phosphate (P) sorption. HRT= hydraulic retention time; Red= reduction; IBR= in-stream bioreactor; PB= pine bark; ESA= expanded slate aggregate.

Reference	Setting	Media	Flow ($L\ min^{-1}$)	HRT (h)	Inflow ($mg\ L^{-1}$)	P Red (%)	P Mass Rem
Goodwin et al. [46]	Lab	Steel turnings	0.002-0.089	1-3	4.36	95.6	N/A
Thapa [47]	Field	Steel chips and turnings	500-1500	0.66	2.59	10-90	49 $g\ m^{-3}\ d^{-1}$
Iverson [41]	IBR	ESA	26	N/A	0.23	74	0 - 200 $g\ d^{-1}$

There are several factors that can affect nutrient treatment efficiency in denitrifying bioreactors. The primary treatment factors that affect the efficacy of denitrifying bioreactors are water temperature, hydraulic retention time (HRT), and carbon substrate [48]. In a field-scale in-stream bioreactor study, Robertson and Merkley [40] reported higher nitrate-N removal rates during warm-season operation than during the cold-season operation. HRT is a function of flow rate: the faster water moves through a system (high flows), the less time it spends in the system (low HRT). Hoover et al. [45] reported higher nitrate-N removal and load reductions in bioreactors exposed to higher HRTs for column-scale, denitrifying bioreactors with HRTs ranging from 2-24 hours. Bell et al. [42] reported higher nitrate-N percent load reductions in bioreactors exposed to lower HRTs for field-scale denitrification trenches with HRTs ranging from 2-8 hours. HRTs that are too short may not allow for a sufficient reduction of DO to promote denitrifying microbial processes, but HRTs that are too long may cause sulfate reduction or mercury methylation [48]. The USDA [49] recommends HRTs ranging from 4-8 hours, but many of the low-order streams in the Falls Lake watershed have flow rates that may correspond to lower HRTs in denitrification trenches.

Another treatment factor controlling bioreactor performance is media type. Christianson et al. [48] recommends that substrate be chosen based on C:N ratio, porosity, cost, and longevity, as these physical properties influence bioreactor hydraulics and can degrade over time. High C:N ratios are important because materials with low C:N ratios experience higher rates of mass degradation and flushing losses [48]. Woodchips are commonly used as a carbon substrate [48], but Christianson [18] incorporated pine chips into pilot-scale denitrifying bioreactors and reported a nitrate reduction of 14-37%. Ramirez-Godinez et al. [43] tested the denitrifying efficacy of pulverized and sieved peanut hulls in lab-scale batch-reactors and reported a 95% nitrate-N reduction. Other studies using rice straw [50], lodgepole pine needles, barley straw, and cardboard [51] were found to be effective carbon media to facilitate denitrification. These studies found nitrate reductions as high as 99% in laboratory mesocosms, thus it is possible that use of carbon media other than woodchips could improve nitrate reductions in field trials.

Phosphate sorbents can be paired with a denitrifying bioreactor to attenuate both nitrates and phosphates from water. Metal oxidizing materials high in aluminum, iron, or calcium provide the cation to bond with dissolved phosphorus to form insoluble compounds [52]. Phosphate sorbents have been paired with denitrifying bioreactors in a variety of studies. Thapa [47] quantified the phosphate reduction in a phosphate adsorption bed installed downstream of a denitrifying bioreactor and reported an average dissolved phosphorus reduction of 45%. Goodwin et al. [46] found that adding a filter of steel turnings after woodchips reduced phosphate concentrations by approximately 96%. Steel byproducts such as chips, slag, and turnings are commonly used as phosphate sorbents, but few studies have investigated the phosphate sorbing capacity of rotary-kiln expanded slate. Future research is needed to identify optimal media for both denitrification and phosphate adsorption, especially in in-stream bioreactors.

In-stream bioreactors are installed in the streambed of small drainage ways, tile drains, or shallow, low-order streams to facilitate denitrification by providing a carbon-rich media (often woodchips). At the time of the current study, in-stream bioreactors have not been widely used. There have been two published studies on in-stream bioreactors conducted in an agricultural drainage way in Canada [40] and in an unnamed tributary to Lick Creek (and ultimately Falls Lake) draining an area with a high density of septic systems [41]. Both studies found that nitrate concentrations were reduced by 78% between inflow and outflow. Inflow nitrate concentrations in the Canada and Lick Creek studies were an average value of 4.8 and a median value of 0.89 mg L^{-1}, respectively. Thus, outflow concentrations were 1.04 and 0.20 mg L^{-1} for the Canada and Lick Creek studies, respectively [40, 41]. Mass removal of nitrate was also variable ranging from 0 to 750 g-N day^{-1}, but that factor may vary substantially between BMPs as Robertson et al. [40] found a maximum mass removal of nitrate at approximately 330 – 360 g-N day^{-1}. Temperature and flow rate can be significant factors affecting treatment efficiency of in-stream bioreactors. Temperature appears to be more of a significant factor if the BMP is installed in a location with harsher winters (e.g., Canada) [40]. The carbon media used in both BMPs were 100% woodchips. The in-stream bioreactor installed in the Falls Lake Watershed also contained a layer of *Stalite*, which is a rotary kiln expanded slate lightweight aggregate, overlain atop the woodchips to create reactive sites for phosphate adsorption. Median concentrations of phosphate in the inflow were 0.23 mg L^{-1}, which were reduced by 74% to a median concentration of 0.06 mg L^{-1} in BMP outflow [41].

Past studies suggest that in-stream bioreactors or other denitrifying bioreactors can be an effective means to reduce septic-derived or other nonpoint sources of nitrate and phosphate in shallow, low-order streams in the Falls Lake Watershed. While these studies indicate promise, there is still future work needed to evaluate the efficacy of these practices. Additional research is needed to evaluate the efficacy of in-stream or stream adjacent bioreactors in Piedmont settings, especially in stream reaches that contain predominantly nitrate at elevated levels that may stimulate eutrophication.

What bioreactor porous media are most effective at reducing onsite nutrient transport?

Initial findings from the ongoing pilot study are presented by summarizing the reductions for nitrate and releases for phosphate (desorption), ammonium, DOC, TKN, and TP.

<u>Nitrate Reduction from Start-Up Through Trial 1</u>

Nitrate data from Trial 1 suggest that the pine bark media most effectively reduces nitrate concentrations, peanut hulls are the second most effective, and woodchips are the least effective of the materials trialed, with mean percent reductions of 72%, 51%, and 34%, respectively (Fig. 12). These data also shows that even at low HRTs, nitrate reduction was observed after the system has reached steady state (two pore volumes). Additionally, the pine bark bioreactor was estimated to have the capacity to reduce approximately 9 pounds of nitrate per cubic ft of bioreactor each year. Though this data is from early in the sampling period, it already challenges the general bioreactors guidelines of using woodchips as carbon media [49, 53] and HRTs of >4 hours [49]. Furthermore, preliminary findings suggest that pilot bioreactors are finding similar treatment efficiencies for nitrate concentrations as summarized in Table 2. Results from the pine bark bioreactor were similar to findings by Robertson and Merkley [40] and Iverson [41]. Both studies reported a 78% nitrate reduction, which may indicate that pine bark media could be an effective media in nutrient-sensitive stream reaches of the Falls Lake Watershed.

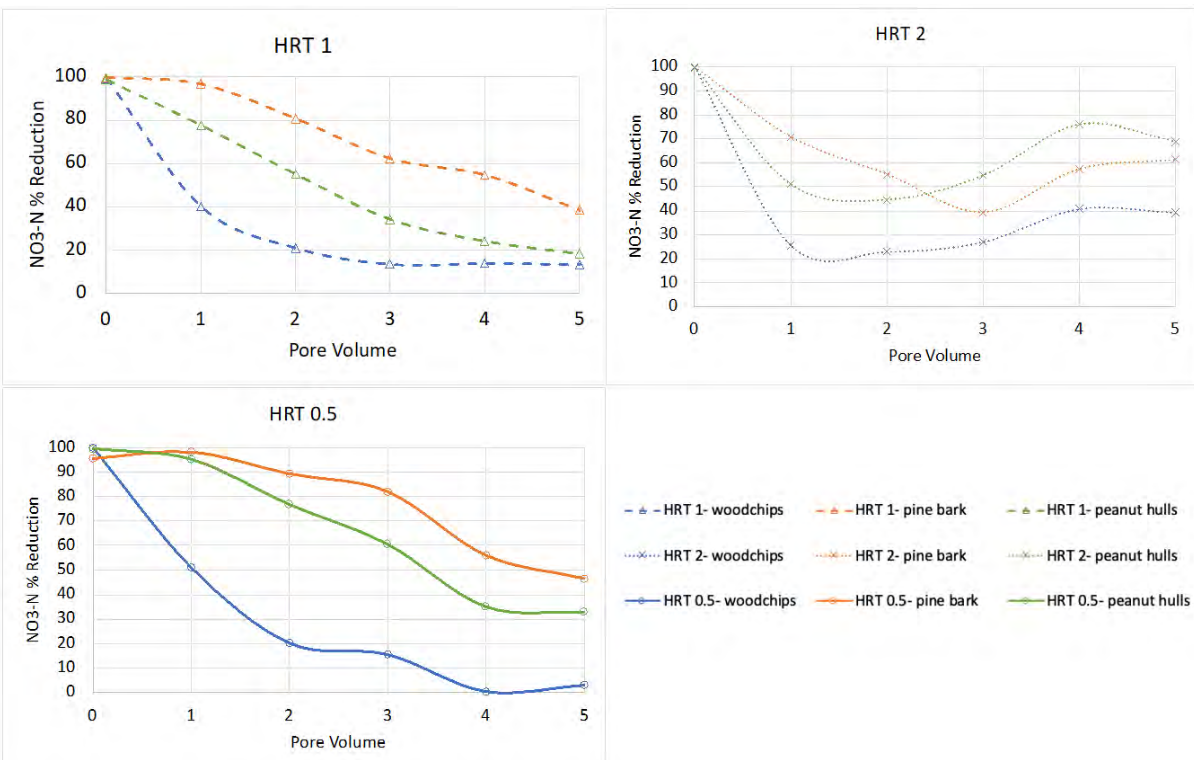


Figure 12. Nitrate reductions during Trial 1 for HRT of 1 hour (top left), 2 hours (top right), 0.5 hours (bottom). HRT= hydraulic retention time.

Phosphate Desorption in Trial 1

The data shows that directly after start-up, phosphate effluent concentrations were significantly higher than the influent phosphate concentration of 1 mg/L (Fig. 13). This can most likely be attributed to the desorption of phosphate that occurs during the reducing conditions in between periods of flow [54]. However, these high concentrations decrease over the first three weeks of sampling. More data needs to be collected before definitive conclusions can be drawn about desorption behaviors in expanded slate.

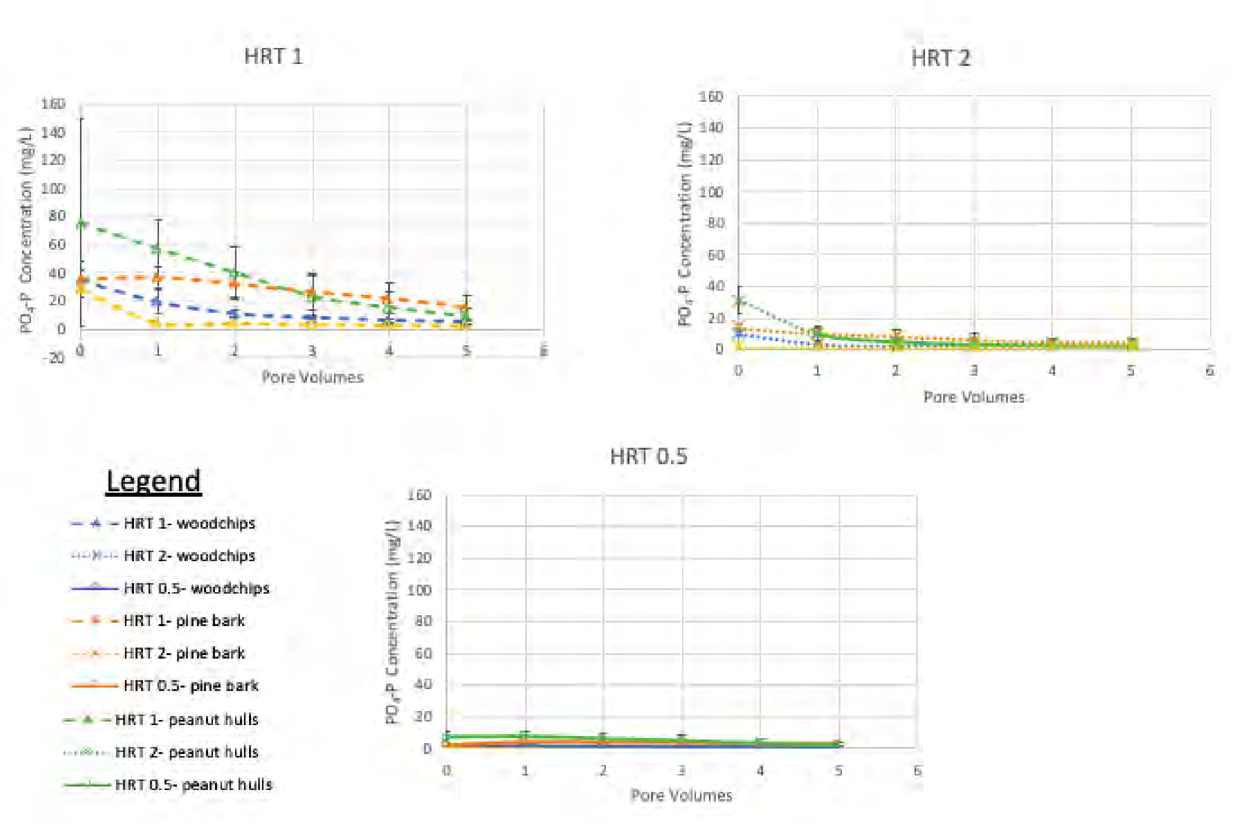


Figure 13. Phosphate concentrations amongst the influent (shown in yellow) and effluent of all three substrates for HRT of 1 hour (upper left), 2 hours (upper right), and 0.5 hours (bottom middle). HRT= hydraulic retention time.

Ammonium Release in Trial 1

Peanut hulls were found to release the most ammonium during Trial 1 (Fig. 14). Both the pine bark and woodchips contained effluent concentrations of ammonium that were similar to influent concentrations (<1 mg/L). The first sampling day after the start up period (HRT 1) yielded the highest concentrations of ammonium, most likely because of the degradation of the carbonaceous substrate that had taken place during start up.

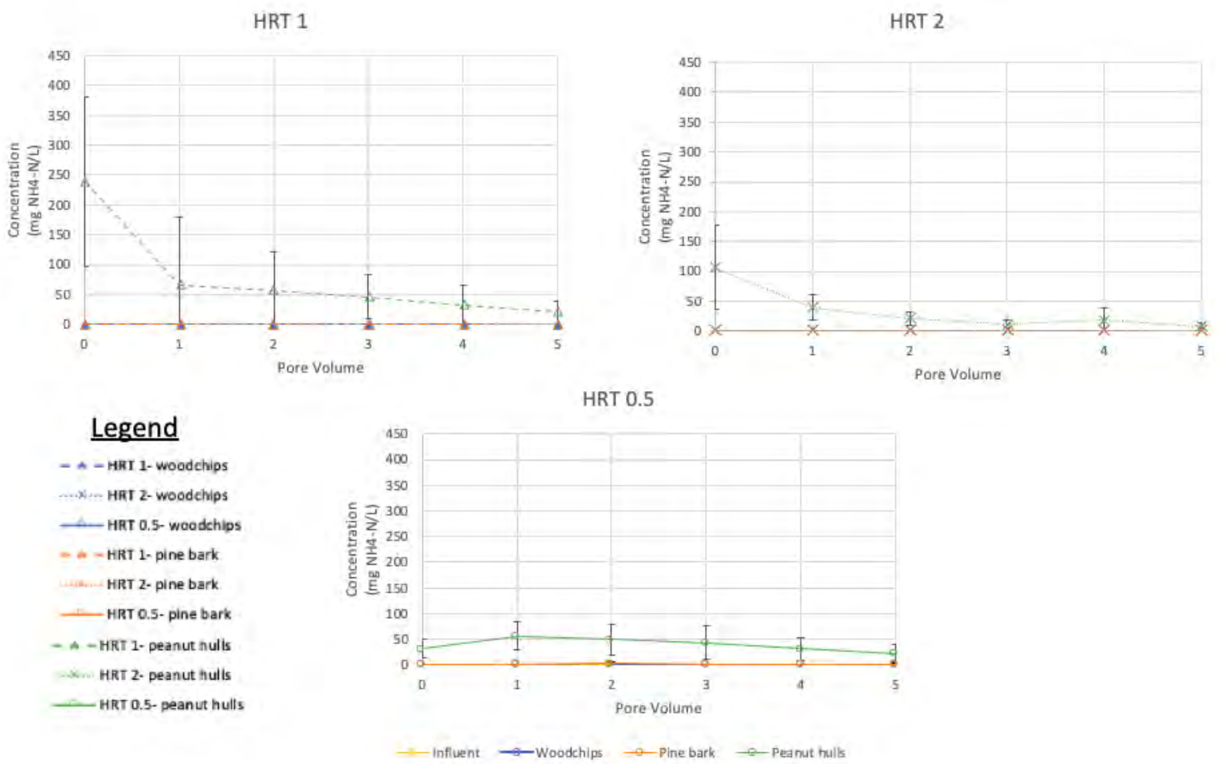


Figure 14. Ammonium concentrations in inflow and outflow of all three substrates at HRTs of 1 hour (upper left), 2 hours (upper right), and 0.5 hours (bottom middle). HRT= hydraulic retention time.

<u>Release of DOC, TKN, and TKP from Start Up through Trial 4</u>

The DOC data shows that the highest flush of DOC occurred during the 14-day start-up period and continued for approximately six more weeks into Trial 2 before decreasing by almost half (Fig. 15). DOC concentrations are highest in the initial flush of pore water (PV 0) after the ~7-day dormant period. Lynn [44] reported that in between periods of flow, DOC concentrations increased in pore water due to the dissolution of the woodchips. Peanut hulls were found to release the highest concentrations of DOC compared to the other substrates.

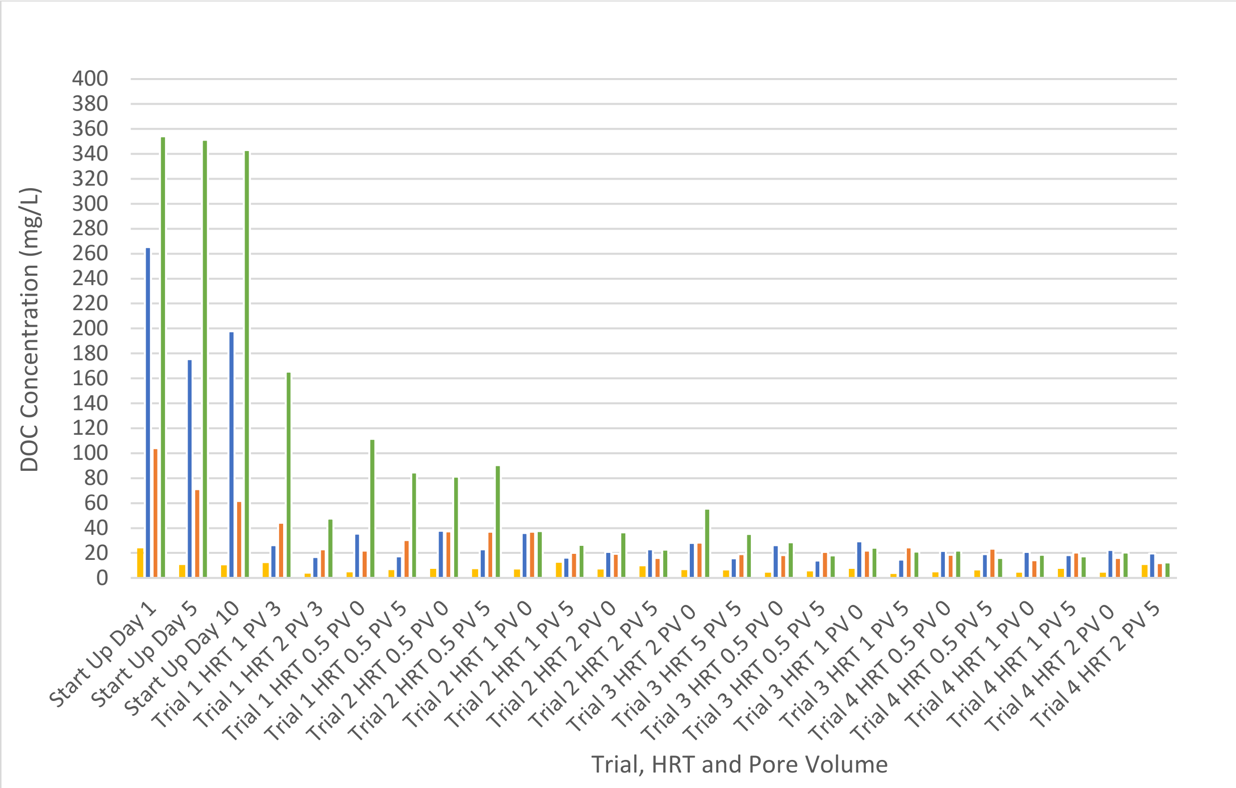


Figure 15. DOC concentrations over the course of the sampling period from June to September 2021, with yellow indicating influent, blue indicating woodchips, orange indicating pine bark, and green indicating peanut hulls. HRT= hydraulic retention time; PV= pore volume; DOC= dissolved organic carbon.

TKN concentrations were highest in the beginning of the sampling period and decrease over time (Fig. 16). Similar to DOC, peanut hulls released substantially more TKN than the other substrates. Additionally, the greatest concentrations of TKN were observed in the initial flush of pore water (PV 0). Lynn [44] reported a decrease in TKN as flow rates decreased, suggesting that the flush of TKN may be due to biofilm scouring in response to high flow rates. This could mean that the TKN found in the initial flush of pore water is present as the microbial biofilm that is being flushed away at the beginning of the run by high flow rates. Schmidt and Clark [55] reported high levels of TKN downgradient of a denitrification wall, they note that the changes in TKN do not correlate to changes in DOC, suggesting that TKN in pore water may be attributed to net microbial mineralization or ammonification (the process through which organic N is broken down into ammonium). Iverson [41] also found that ammonium concentrations downgradient of an in-stream bioreactor were nearly double that of upstream concentrations, and he attributed that increase to ammonification within the in-stream bioreactor. Ammonification generally often occurs in bioreactors when the C:N ratios of a substrate fall below 100 [55]. The bioreactors with peanut shells have a C:N ratio of 33:1, while the pine bark and woodchips both have C:N ratios over 100:1. Thus, the higher TKN values observed in the peanut shell media may be related to ammonification.

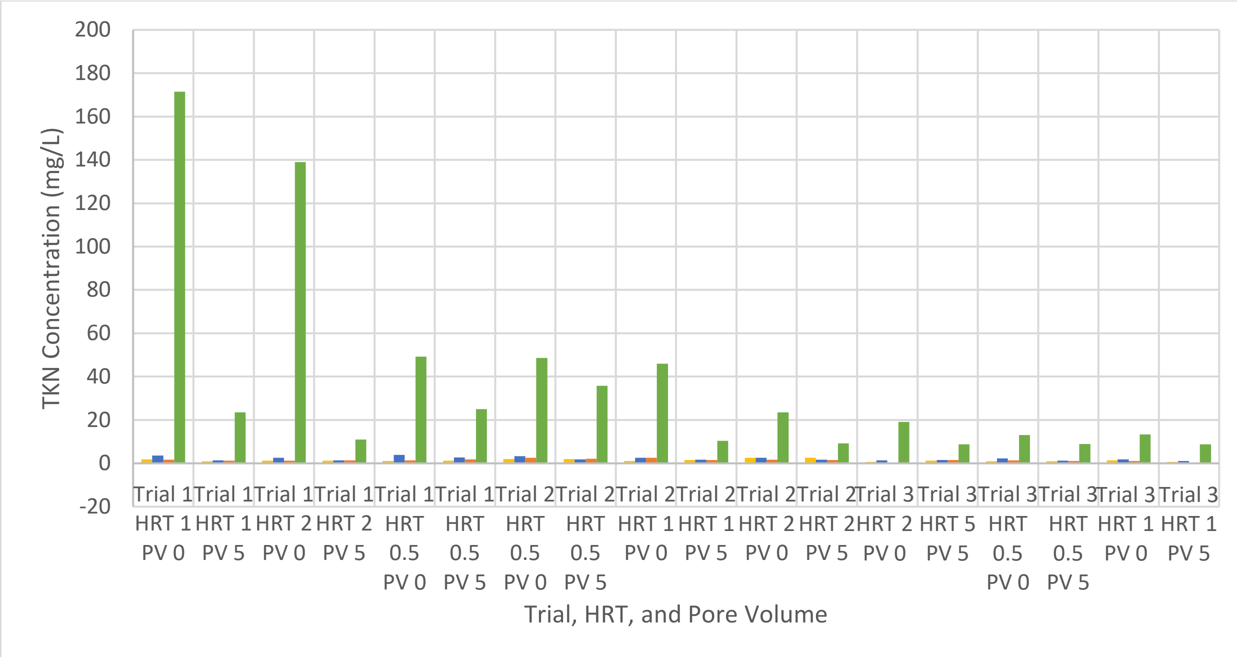


Figure 16. TKN concentrations from June to September 2021, with yellow indicating influent, blue indicating woodchips, orange indicating pine bark, and green indicating peanut hulls. HRT= hydraulic retention time; PV= pore volume.

Concentrations of TP are nearly the same as concentrations of phosphate, thus most of the TP occurred as inorganic phosphate. Similar to both DOC and TKN, peanut hulls released the highest concentrations of TP relative to pine bark and woodchips (Fig. 17). Ramirez-Godinez et al. [43] reported that peanut hulls released higher quantities of TP than woodchips throughout the course of the sampling period (woodchips: negligible amounts; peanut hulls: 0.13 mg TP per g substrate).

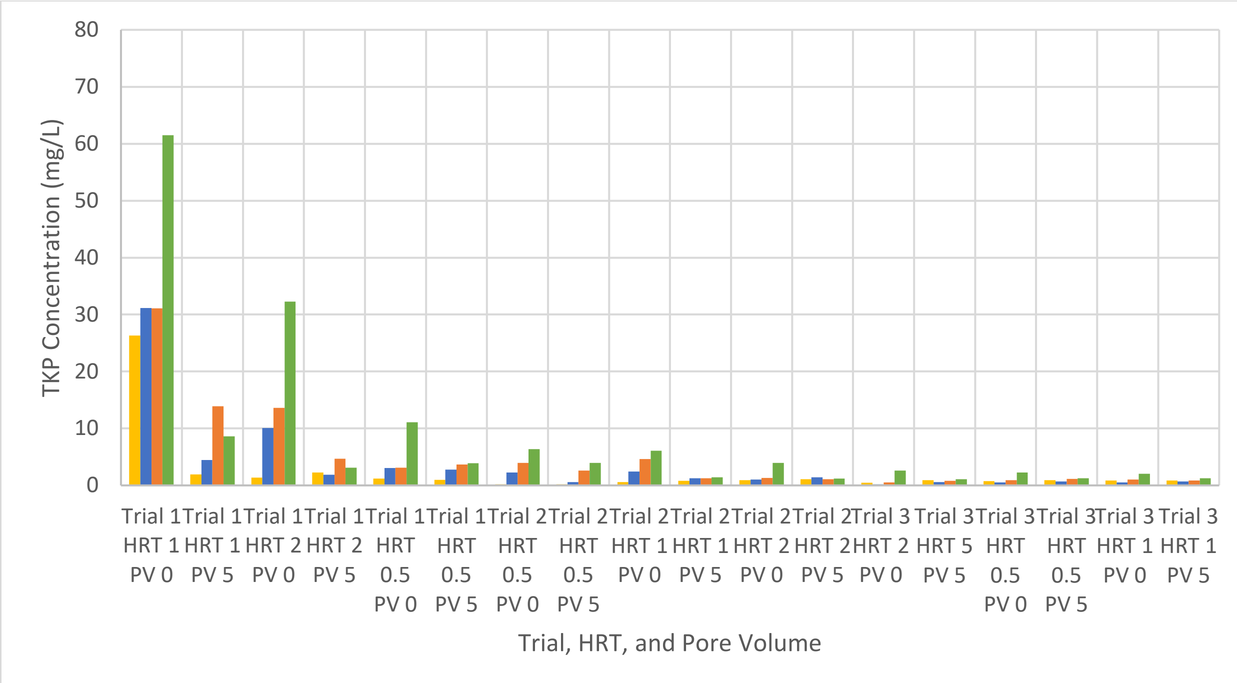


Figure 17. TP concentrations over the course of the sampling period from June to September 2021, with yellow indicating influent, blue indicating woodchips, orange indicating pine bark, and green indicating peanut hulls. HRT= hydraulic retention time; PV= pore volume.

Initial Conclusions from Ongoing Pilot Study

This on-going, pilot-scale study will continue to quantify and compare the denitrifying efficacy of a woodchip mix (waste-wood mix), pine bark, and peanut hulls to determine which media has the greatest potential to reduce plant available nitrogen and phosphorous in bioreactors. These data will help provide guidance about optimal bioreactor designs that could be installed in or adjacent to low-order streams in the Falls Lake Watershed. The waste-wood mix was chosen to represent the most cost-effective option with an intermediate C:N ratio (Table 4). The woodchip mix is of assorted species, but most likely consists of some of the most common trees in the region: *Pinus* spp. (pine), *Quercus* spp. (oak), and *Liquidambar* spp. Pine bark is particularly useful to this study because *Pinus taeda* (loblolly pine) is the most common tree in the Piedmont of North Carolina [56], so it would be an available and cost-effective carbon media source for potential denitrifying bioreactors in North Carolina streams. Furthermore, early results suggest that the pine bark is also excellent at nitrate attenuation while also releasing lower concentrations of ammonium, DOC, TKN, and TKP. *Arachis hypogaea* (peanut) hulls were chosen to use as a substrate for this experiment because, while they have low C:N ratios, limited research is available on their denitrifying efficacy. Additionally, these are an agricultural waste product that could possibly be repurposed for environmental engineering purposes. This study will contribute to filling the gap in knowledge on the performance of un-sieved peanut hulls in denitrifying bioreactors. This study will also quantify the denitrifying efficacy of bioreactors with intermittent, high flow rates, similar to streams in the Fall’s Lake watershed that are affected by stormwater runoff.

Table 4. Porosity and carbon-to-nitrogen (C:N) ratios of each substrate.

Substrate	Porosity	C:N
Woodchip mix	0.534	114:1
Pine bark	0.634	199:1
Peanut hulls	0.8	31:1

Preliminary results indicate that pine bark appears to be the best substrate for promoting denitrification and the HRT of 2 hours appears to have the highest potential for nitrate reduction (between 15-100%). These data suggest that at an HRT of 2 hours, pine bark can reduce annual nitrate masses by up to 15.6 pounds per cubic feet of bioreactor. Though the phosphate adsorbents had reached their full adsorption capacity before the end of the start-up period, phosphate concentrations in the effluent significantly decreased over the first three weeks of sampling, most likely because of the desorption that commonly occurs under reducing conditions during the dormant periods. These data imply that after expanded slate has reached its sorption capacity, desorption of phosphates may occur thereby releasing phosphate into the water under dormant flow conditions.

The full data set will be reported in Ann Marie Lindley’s Master’s thesis by Spring of 2022 and will be incorporated into a manuscript for submission to a peer-reviewed journal. She will also present the results of her work at the 2022 NC WRRI conference.

What are the optimal locations for bioreactors along low-order streams to reduce nutrient inputs to Falls Lake?

There are several factors to consider when identifying potential sites for bioreactors. Some of these factors include land cover, nutrient concentration and speciation, size of the stream, distance to Falls Lake, bioreactor design, installation and maintenance costs, and site access and permission. Land cover, size of stream, and distance to Falls Lake can be analyzed using geospatial techniques (e.g., analyzing the watershed using ArcMap, ArcGIS Pro, or another geospatial information system). The Falls Lake Watershed is mostly forested with large areal extents of developed and agricultural land cover classes (Fig. 18). Development is most dense on the south to west side of Falls Lake that include portions of the Cities of Durham and Raleigh. These areas also contain the greatest density of septic systems in the watershed (Fig. 5). Developed land classes can be further subdivided into 4 sub-categories of developed land and septic system densities were highest in the “Developed, open space” and “Developed, low intensity” classes. Septic systems were also found in the “forest” and “agriculture” land cover classifications. These results were expected since all classes overlap with suburban to rural land uses where septic systems are most used. As previously discussed, stream reaches that drain watersheds with elevated septic system densities (> 1 system ha^{-1}) may contain elevated nutrient concentrations.

In addition to septic densities, soil type and geology should be considered. The Triassic Basin region of the Falls Lake Watershed would be a good area to focus BMP efforts given their propensity for elevated nutrients in stream reaches [29, 57]. Additionally, sub-watersheds of Falls Lake that contain a high number or density of sand filter type septic systems should also be considered as a candidate for in-stream bioreactors. Sand filters can discharge elevated nutrient concentrations compared to reference conditions [15]. Additionally, the design of sand filter systems are highly compatible with retrofitting BMPs engineered to enhance nutrient processing. Based on these results and land cover analyses, the studied sub-watersheds would be good candidates for bioreactors. However, nutrient concentration and speciation and flow conditions must also be considered in selecting optimal locations for bioreactors.

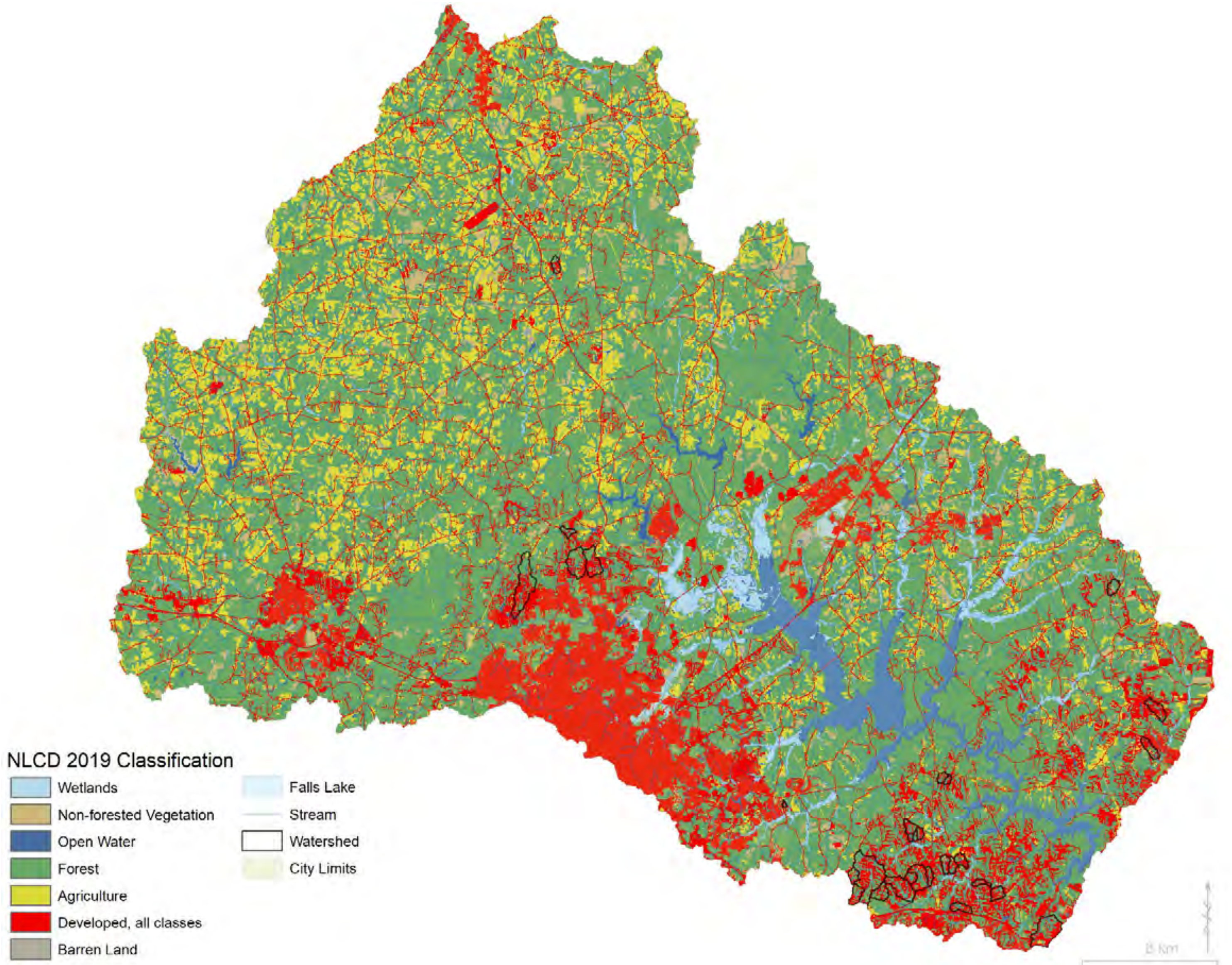


Figure 18. National land cover database (NLCD) classification for the Falls Lake Watershed in 2019. Detailed land cover data for each sub-watershed is summarized in Appendix C.

Forested land cover classifications constitute a large portion of the Falls Lake Watershed (Fig. 18). For individual sub-watersheds, developed and forested land classes were the most dominant land cover types (Table 5). Of the 30 studied sub-watersheds, the average sub-watershed was approximately 52% developed land and 44% forested. These two land cover classes constituted more than 95% of the average watershed area. Most of the developed lands were classified as other “developed, open space” and “developed, low intensity” which is common for suburban and rural watersheds served by septic systems. Natural vegetated lands (e.g., forests and wetlands) can provide substantial reductions of nutrient concentrations and loads. Forested land was common in the individual sub-watersheds (Table 5). However, wetlands were not as abundant totaling approximately 6 ha in all 30 sub-watersheds combined. Most of the wetlands in the Falls Lake Watershed are found adjacent to the reservoir itself and along some of the major tributaries to the reservoir (Fig. 18). Furthermore, when analyzing land cover along stream reaches, model estimates suggest that most of the forested land cover is not found within 50 ft of streams. The percentage of forested land cover found within the riparian buffers of streams ranged from approximately 2 – 18% (Table 5). These model estimates suggest that most of the forested land is in upland areas or farther from stream reaches, which could inhibit natural processing of nutrients. While the model indicated that most of wetlands were within riparian buffers of streams, there were limited total area of wetlands, which also reduces potential nutrient reductions.

The 2019 National Land Cover Database uses a 900 m^2 resolution (30m by 30m) in classifying land cover and the dominant land cover within each cell grid is applied to the entire cell. Therefore, some stream-adjacent residences may be classified as “developed”, suggesting the absence of a riparian buffer or wetland. However, the resolution of the dataset may be too coarse to differentiate subtle changes in land cover at the site and/or sub-watershed scale. Therefore, additional work studying orthogonal imagery and/or repeating these analyses with a finer resolution (e.g., 225 m^2) would improve GIS-based reconnaissance in identifying optimal locations for in-stream bioreactors. With the current resolution, GIS methods can be employed to generate a list of potential candidates for further evaluation. Therefore, coupling GIS data with nutrient concentration and speciation data can further constrain optimal locations for in-stream bioreactors.

Table 5. Total sub-watershed area and area (ha) for forested, wetlands, and developed land classes. Percentages show the percent of forest, wetlands, and developed land classes located within 50 ft of the stream reach. Forest, wetlands, and developed land classes are aggregated together with all sub-classes.

Watershed	Watershed Area	Forest		Wetlands		Developed	
	(ha)	Area	Buffer	Area	Buffer	Area	Buffer
Durant	229.48	99.16	11.5%			119.90	2.0%
Victory Church	458.12	219.16	7.5%	3.51	53.6%	219.96	2.4%
November	268.91	129.21	5.4%			127.07	3.1%
Appaloosa	48.91	28.77	5.3%			14.72	0.5%
Donlin	79.88	19.90	14.3%			55.99	1.5%
Jenkins	64.27	24.01	3.9%			35.35	0.9%
Woody	40.04	18.29	3.1%			18.17	2.7%
Barclay	125.55	46.17	1.8%	0.09	1.5%	70.41	3.8%
Harold	32.03	18.49	9.9%			13.16	4.3%
Green Bay	159.14	82.17	4.6%			70.56	4.4%
Asbury	8.51	2.26	17.6%			3.75	6.2%
Macon	152.44	65.51	12.2%	1.98	55.0%	84.74	0.9%
Park Ridge	46.21	10.80	5.8%			29.58	6.8%
Brookfield	52.60	22.76	6.1%			27.45	1.3%
Tacketts Pond 1	16.74	7.42	10.9%			9.32	3.0%
Tacketts Pond 2	28.62	14.66	5.9%			12.03	0.8%
Green Downs 1	59.31	26.01	8.4%			32.14	4.7%
Green Downs 2	13.45	8.22	10.8%			5.23	6.8%
Green Downs 3	23.64	10.09	4.0%			13.55	5.8%
Appaloosa Run E	47.10	21.41	7.0%			24.98	3.4%
Ethan	43.80	21.41	9.4%			17.27	1.1%
Indigo Moon Way	66.80	27.12	6.6%			36.31	1.5%
Bushveld	72.62	39.68	5.8%			32.16	2.5%
Cranesbill	189.69	77.49	8.0%			102.55	1.8%
Liatris	37.27	14.33	10.0%			20.55	1.1%
Old Creedmoor	34.71	14.31	7.8%			20.19	6.0%
Kinsdale 1	45.10	21.27	11.4%			20.76	2.1%
Kinsdale 2	37.49	14.78	9.4%			21.06	2.4%
Leslie 1	29.59	9.00	11.5%			20.59	4.0%
Coachmans Way	53.60	22.46	6.1%			30.49	0.2%

Nutrient concentration and speciation are also important considerations in determining optimal locations for bioreactors. The simplest bioreactor design involves supplying a natural environment with a carbon-rich media to facilitate denitrification via denitrifying microorganisms and/or a phosphate-sorbing media to encourage phosphate adsorption. Thus, the most optimal location would be a stream reach that contains elevated nutrients, primarily nitrate and phosphate. When compiling the 8 sub-watersheds with the most TN, the median sub-watershed contained 53% nitrate and was the most dominant nitrogen species of TN (Fig. 19). In all but 2 of these sub-watersheds, nitrate was the most dominant nitrogen species and comprised 43 – 97% of the TN. When TN exceeded 2.50 mg L^{-1}, nitrate was >75% of TN. In the Macon and Donlin sub-watersheds, nitrate comprised 42% and 38% of TN, respectively, second only to DON (Table 6). In the remaining 22 sub-watersheds, DON tended to be the dominant species of TN. The median percent of TN as nitrate in these sub-watersheds was 27% (range: 11 – 47%), thus some of these sub-watersheds also contained elevated nitrate (Table 6). Based on these results, Park Ridge, Kinsdale 1 and 2, Woody, Barclay, and Asbury would be excellent candidates for in-stream bioreactors given that nitrate was the dominant species. Additionally, Greens Down 1 and 3, Appaloosa Run East, Tacketts Pond 1, Jenkins, Leslie 1, and Brookfield could also be considered since nitrate comprised > 30% of TN.

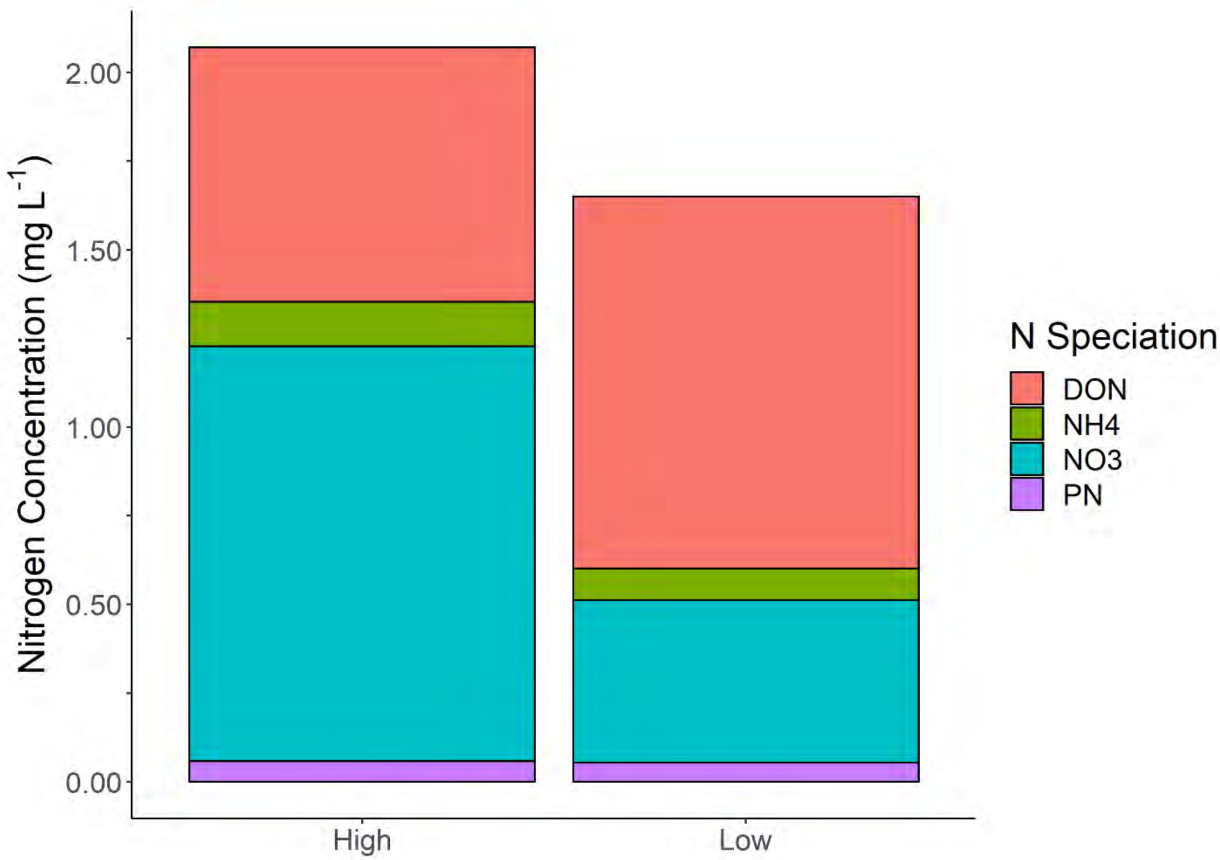


Figure 19. Nitrogen speciation for the median sub-watershed with "high" and "low" total nitrogen concentrations at the outlet. This threshold is defined at 2.00 mg L^{-1} total nitrogen. There were 8 sub-watersheds in the high group and 22 in the low.

Table 6. Nutrient speciation (%) of total nitrogen (TN) and total phosphorus (TP). NH4= ammonium, NO3= nitrate, DON= dissolved organic nitrogen, PN= particulate nitrogen, PO4= phosphate, DOP= dissolved organic phosphorus, PP= particulate phosphorus

Watershed	Density	TN Speciation				TN	TP Speciation			TP
	(# ha^{-1})	NH4	NO3	DON	PN	(mg L^{-1})	PO4	DOP	PP	(mg L^{-1})
Durant	1.00	20.1%	24.7%	51.7%	3.5%	1.77	38.1%	27.8%	34.1%	0.06
Brookfield	2.26	5.4%	35.3%	56.2%	3.1%	1.94	35.9%	3.8%	60.4%	0.03
Park Ridge	1.56	2.3%	97.4%	0.0%	0.2%	9.77	1.5%	44.0%	54.5%	0.03
Macon	1.78	5.8%	41.9%	48.7%	3.7%	2.01	24.2%	14.6%	61.1%	0.09
Victory Church	1.62	16.6%	11.5%	69.9%	1.9%	1.64	47.7%	26.5%	25.8%	0.06
Asbury	2.00	18.6%	42.9%	32.1%	6.4%	2.02	59.5%	0.0%	40.5%	0.17
November	1.28	9.1%	27.9%	58.7%	4.3%	1.84	18.8%	42.6%	38.6%	0.08
Green Bay	1.02	10.1%	28.1%	55.8%	6.0%	1.89	46.9%	0.0%	53.1%	0.09
Harold	1.34	15.2%	26.4%	54.0%	4.4%	1.86	40.0%	16.0%	44.0%	0.10
Barclay	1.19	7.1%	45.9%	43.7%	3.2%	2.13	24.5%	31.9%	43.6%	0.08
Woody	1.07	4.6%	59.4%	34.1%	1.9%	2.30	54.5%	19.8%	25.7%	0.08
Appaloosa	1.10	11.5%	19.0%	65.6%	3.9%	1.69	8.9%	48.7%	42.4%	0.05
Donlin	2.00	6.4%	37.6%	48.7%	7.2%	2.10	45.9%	6.2%	47.8%	0.10
Jenkins	1.71	8.9%	36.1%	53.4%	1.6%	1.75	34.5%	30.0%	35.5%	0.05
Tacketts Pond 1	1.85	2.6%	39.8%	52.7%	4.9%	1.76	12.6%	41.9%	45.6%	0.06
Tacketts Pond 2	1.36	0.8%	17.3%	80.0%	1.9%	1.46	9.0%	65.9%	25.1%	0.05
Green Downs 1	1.89	1.7%	46.8%	50.9%	0.6%	1.75	17.6%	62.2%	20.1%	0.04
Green Downs 2	1.41	1.7%	19.5%	77.4%	1.5%	1.45	29.8%	33.5%	36.6%	0.03
Green Downs 3	2.20	2.2%	31.4%	64.9%	1.5%	1.60	31.3%	54.7%	14.0%	0.05
Appaloosa Run E	1.89	15.9%	37.1%	45.8%	1.1%	1.64	8.2%	73.1%	18.7%	0.04
Ethan	1.05	33.2%	10.9%	52.6%	3.4%	1.46	19.1%	0.0%	80.9%	0.02
Indigo Moon Way	1.51	12.2%	16.1%	67.9%	3.8%	1.51	12.6%	56.9%	30.5%	0.06
Bushveld	1.31	4.5%	11.7%	82.1%	1.6%	1.41	64.1%	0.0%	35.9%	0.02
Cranesbill	1.42	4.5%	16.1%	71.5%	8.0%	1.53	23.4%	40.5%	36.1%	0.04
Liatris	1.42	4.6%	21.5%	67.1%	6.7%	1.59	20.1%	46.2%	33.8%	0.04
Old Creedmoor	1.99	4.6%	27.9%	66.9%	0.7%	1.48	5.3%	82.1%	12.7%	0.04
Kinsdale 1	1.69	0.5%	75.8%	23.3%	0.3%	2.52	20.6%	66.4%	13.0%	0.04
Kinsdale 2	1.73	1.1%	86.4%	12.0%	0.5%	3.01	13.6%	62.3%	24.1%	0.03
Leslie 1	1.96	1.0%	33.8%	62.6%	2.7%	1.58	32.4%	5.8%	61.8%	0.03
Coachmans Way	2.01	1.7%	28.0%	65.0%	5.3%	1.71	24.9%	51.8%	23.3%	0.05

Bioreactor design can be modified to include media that serves as reactive surfaces for phosphate, which immobilizes phosphate temporarily or until it mineralizes. Phosphorus speciation data suggest that a bioreactor amended with a sorbent media could further improve nutrient processing (Fig. 20; Table 6). When compiling the 6 sub-watersheds with high TP concentrations, the median sub-watershed contained 46% phosphate, which was similar to the speciation of PP. Phosphate speciation ranged from 24 – 60% in these sub-watersheds. In the other 24 sub-watersheds, phosphate accounted for 20% of TP and speciation ranged from 2 – 64%. While in-stream bioreactors are typically designed to facilitate reductions of plant available nutrient species, these BMPs can be engineered with forebays or other technologies to create sedimentation sites. This would allow PP concentrations to also decline. Based on these results, the Asbury, Green Bay, Harold, Woody, and Donlin sub-watersheds would make excellent candidates for a bioreactor with sorbent reactive media designed to immobilize phosphate. One consideration is that the Green Bay and Harold sub-watersheds do not contain as much nitrate as the other sub-watersheds, thus these locations would not likely see large improvements to nitrogen and phosphorus treatment efficiencies.

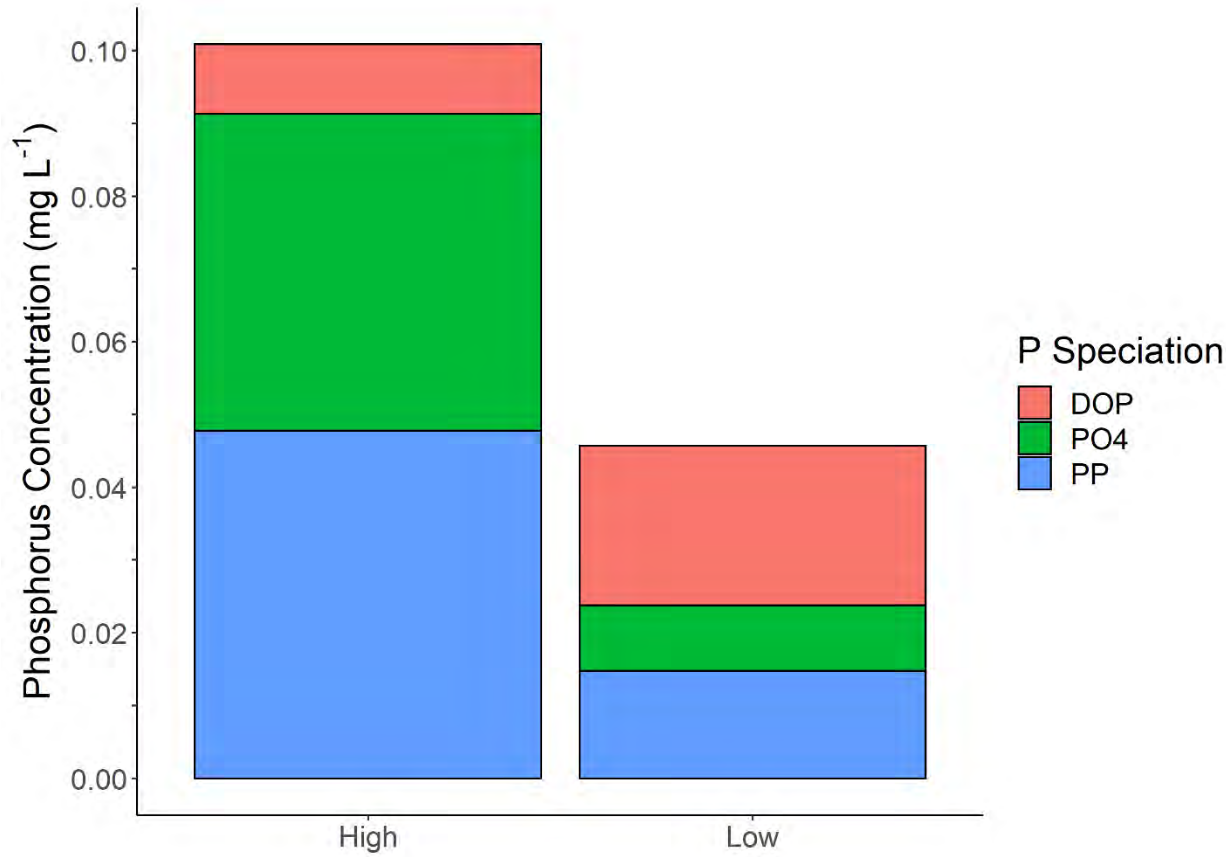


Figure 20. Phosphorus speciation for the median sub-watershed with “high” and “low” total phosphorus concentrations at the outlet. This threshold is defined at 0.08 mg L^{-1} total phosphorus. There were 6 sub-watersheds in the high group and 24 in the low.

Spatial variability of TN, TP, and $\delta^{15}N$ were summarized at the sub-watershed scale in Appendix D.

Management Implications

Using an integrated approach to identify sub-watersheds with elevated nutrients can be an effective tool. Results from this study showed that most sub-watersheds contained elevated nitrogen and phosphorus concentrations relative to reference conditions. When considering BMPs to remediate elevated nutrients, coupling water quality data with GIS analyses can yield successful results. Retrofitting in-stream bioreactors at the aforementioned studied locations could significantly reduce watershed TN and TP values. Previous research has suggested that in-stream bioreactors can reduce nitrate and phosphate concentrations by approximately 78% and 74%, respectively. Assuming similar reductions in nitrate and phosphate occur at the watersheds recommended as "excellent", in-stream processing of TN and TP reductions could increase by 21 – 76% and 1 – 44%, respectively (Table 7). Early results from the bioreactor pilot study suggest that pine bark was an effective media for denitrification and its nitrate reduction efficiency was similar to recent studies on in-stream bioreactors. Additional field-based experiments would improve estimates of nutrient treatment efficiencies from implementation of in-stream bioreactors. Additionally, there are numerous other factors that could affect treatment based on bioreactor design, weather, retention time, intra- and inter-annual temporal effects on local hydrogeology and geochemistry, among others. Additional research is recommended to characterize the efficacy of in-stream or stream adjacent bioreactors to aid in nutrient management strategies, especially in sub-watersheds with elevated nutrient concentrations and septic system densities.

Table 7. Estimated attenuation factors for nitrate, total nitrogen (TN), phosphate, and total phosphorus (TP) based on bioreactor efficiencies from the literature. Before and after columns refer to the concentrations that may be observed if bioreactors were established in those respective watersheds. Concentrations entering theoretical bioreactors were assumed to be the same as concentrations measured at the watershed outlet. NO3= nitrate-nitrogen; Red= reduction; PO4= phosphate-phosphorus

Watershed	NO3 (mg L^{-1})		TN (mg L^{-1})		TN Red	PO4 (mg L^{-1})		TP (mg L^{-1})		TP Red
	In	Out	Before	After	(%)	In	Out	Before	After	(%)
Park Ridge	9.52	2.09	9.77	2.35	76.0%	0.00	0.00	0.03	0.03	1.1%
Asbury	0.87	0.19	2.02	1.35	33.5%	0.10	0.03	0.17	0.10	44.0%
Barclay	0.98	0.22	2.13	1.37	35.8%	0.02	0.01	0.08	0.07	18.1%
Woody	1.36	0.30	2.30	1.23	46.3%	0.05	0.01	0.08	0.05	40.3%
Kinsdale 1	1.91	0.42	2.52	1.03	59.1%	0.01	0.00	0.04	0.03	15.2%
Kinsdale 2	2.60	0.57	3.01	0.98	67.4%	0.00	0.00	0.03	0.03	10.1%
Green Bay	0.53	0.12	1.89	1.48	21.9%	0.04	0.01	0.09	0.06	34.7%
Harold	0.49	0.11	1.86	1.47	20.6%	0.04	0.01	0.10	0.07	29.6%

COVID-19 Impacts: Supply chain disruptions and equipment malfunctions caused substantial delays in completing the pilot bioreactor project. The research team continues to work towards fulfilling these deliverables. These data will serve as the basis for at least one master's thesis in the Department of Geological Sciences. Therefore, these data and findings will be featured in future research products (e.g., thesis, manuscript(s), and/or conference presentation(s).

References Cited

1. Naylor, E.; Humphrey, C.; Easter, L.; Iverson, G. Evaluation of Nitrate Concentrations and Potential Sources of Nitrate in Private Water Supply Wells in North Carolina.(ADVANCEMENT OF THE SCIENCE). *J. Environ. Health* **2018**, *80,* 16-23.

2. US Census Bureau. **2019.** North Carolina QuickFacts. Available online: https://www.census.gov/quickfacts/NC (accessed on 24 September 2021).

3. Buetow, W. S. Septic wastewater nitrogen contributions to a shallow aquifer and adjacent stream. Masters thesis, NC State University, Raleigh, NC, **2002.**

4. Iverson, G.; O'Driscoll, M. A.; Humphrey Jr, C. P.; Manda, A. K.; Anderson-Evans, E. Wastewater Nitrogen Contributions to Coastal Plain Watersheds, NC, USA. *Water Air Soil Pollut* **2015**, *226,* 1-17.

5. Hoghooghi, N.; Radcliffe, D. E.; Habteselassie, M. Y.; Clarke, J. S. Confirmation of the Impact of Onsite Wastewater Treatment Systems on Stream Base-Flow Nitrogen Concentrations in Urban Watersheds of Metropolitan Atlanta, GA. *J. Environ. Qual.* **2016**, *45,* 1740-1748.

6. Harman, J.; Robertson, W. D.; Cherry, J. A.; Zanini, L. Impacts on a Sand Aquifer from an Old Septic System: Nitrate and Phosphate. *Groundwater* **1996**, *34,* 1105-1114.

7. Robertson, W. D.; Cherry, J. A. Hydrogeology of an unconfined sand aquifer and its effect on the behavior of nitrogen from a large flux septic system. *Appl. Hydrogeol.* **1992**, *0,* 32-44.

8. Valiela, I.; Collins, G.; Kremer, J.; Lajtha, K.; Geist, M.; Seely, B.; Brawley, J.; Sham, C. H. Nitrogen Loading from Coastal Watersheds to Receiving Estuaries: New Method and Application. *Ecol. Appl.* **1997**, *7,* 358-380.

9. Pradhan, S. S.; Hoover, M. T.; Austin, R. E.; Devine, H. A. Potential nitrogen contributions from on-site wastewater treatment systems to North Carolina's River Basins and Sub-basins. North Carolina Agricultural Research Service, NC State University, Technical Bulletin 324; **2007.**

10. Humphrey, C.; Anderson-Evans, E.; O'Driscoll, M.; Manda, A.; Iverson, G. Comparison of Phosphorus Concentrations in Coastal Plain Watersheds Served by Onsite Wastewater Treatment Systems and a Municipal Sewer Treatment System. *Water Air Soil Pollut* **2015**, *226,* 1-18.

11. Humphrey, C. P.; O'Driscoll, M.; Iverson, G.; Anderson-Evans, E. Is on-site wastewater a significant source of phosphorus to coastal plain streams? *Int. J. Environ. Sci. Technol.* **2020**, *17,* 1199-1210.

12. Robertson, W. D.; Schiff, S. L.; Ptacek, C. J. Review of phosphate mobility and persistence in 10 septic system plumes. *Groundwater* **1998***, 36,* 1000-1010.

13. Humphrey, C.; Jernigan, J.; Iverson, G.; Serozi, B.; O'Driscoll, M.; Pradhan, S.; Bean, E. Field Evaluation of Nitrogen Treatment by Conventional and Single-Pass Sand Filter Onsite Wastewater Systems in the North Carolina Piedmont. *Water Air Soil Pollut* **2016***, 227,* 1-17.

14. Humphrey, C.; Serozi, B.; Iverson, G.; Jernigan, J.; Pradhan, S.; O'Driscoll, M.; Bean, E. Phosphate treatment by onsite wastewater systems in nutrient-sensitive watersheds of North Carolina's Piedmont. *Water Sci. Technol.* **2016***, 74,* 1527-1538.

15. Iverson, G.; Humphrey, C. P.; O'Driscoll, M. A.; Sanderford, C.; Jernigan, J.; Serozi, B. Nutrient exports from watersheds with varying septic system densities in the North Carolina Piedmont. *J. Environ. Manag.* **2018***, 211,* 206-217.

16. Bradshaw, J. K.; Radcliffe, D. E. Nitrogen fate and transport in a conventional onsite wastewater treatment system installed in a clay soil; experimental results. *Vadose Zone J.* **2013***, 12,* 1-20.

17. O'Driscoll, M.; Humphrey, C.; Iverson, G.; Hoben, J. Estimating the Influence of Onsite Wastewater Treatment Systems on Nutrient Loading to Falls Lake Watershed, North Carolina. University of North Carolina at Chapel Hill, NC Policy Collaboratory; **2020.**

18. Christianson, L. E. Design and performance of denitrification bioreactors for agricultural drainage. Doctoral dissertation, Iowa State University, Ames, IA, **2011.**

19. Nadelhoffer, K. J. Microlysimeter for measuring nitrogen mineralization and microbial respiration in aerobic soil incubations. *Soil Sci. Soc. Am. J.* **1990***, 54,* 411-415.

20. Beal, C. D.; Gardner, E. A.; Menzies, N. W. Process, performance, and pollution potential: a review of septic tank-soil absorption systems. *Aus. J. Soil Res.* **2005***, 43,* 781.

21. Humphrey, J., C P; O'Driscoll, M. A.; Zarate, M. A. Controls on groundwater nitrogen contributions from on-site wastewater systems in coastal North Carolina. *Water Sci. Technol.* **2010***, 62,* 1448-1455.

22. O'Driscoll, M. A.; Humphrey, C. P.; Deal, N. E.; Lindbo, D. L.; Zarate-Bermudez, M. A. Meteorological Influences on Nitrogen Dynamics of a Coastal Onsite Wastewater Treatment System. *J. Environ. Qual.* **2014***, 43,* 1873-1885.

23. Withers, P. J.; Jordan, P.; May, L.; Jarvie, H. P.; Deal, N. E. Do septic tank systems pose a hidden threat to water quality? *Front. Ecol. Environ.* **2014**, *12,* 123-130.

24. D'Amato, V. Nutrient Attenuation in Chesapeake Bay Watershed Onsite Wastewater Treatment Systems - Final Report. Tetra Tech, Tetra Tech; **2016.**

25. Lusk, M. G.; Toor, G. S.; Yang, Y.; Mechtensimer, S.; De, M.; Obreza, T. A. A review of the fate and transport of nitrogen, phosphorus, pathogens, and trace organic chemicals in septic systems. *Crit. Rev. Environ. Sci. Technol.* **2017**, *47,* 455-541.

26. USDA. Part 630 Hydrology National Engineering Handbook. National Resources Conservation Service, United States Department of Agriculture; **2007.**

27. Karathanasis, A. D.; Mueller, T. G.; Boone, B.; Thompson, Y. L. Nutrient removal from septic effluents as affected by soil thickness and texture. *J. Water Health* **2006**, *4,* 177-195.

28. USDA. **2021.** Soil Surveys for North Carolina. Available online: https://www.nrcs.usda.gov/wps/portal/nrcs/surveylist/soils/survey/state/?stateId=NC (accessed on 24 September 2021).

29. O'Driscoll, M.; Humphrey, C.; Iverson, G.; Hoben, J.; Bell, N.; Richardson, J.; Lindley, A. M.; Jernigan, J. A Paired-Watershed Approach to Evaluate the Influence of Onsite Wastewater Nutrient Inputs to Falls Lake, NC. East Carolina University, Funded by the NC DEQ 319 Grant Program; **2021.**

30. Humphrey Jr, C.; Sanderford, C.; Iverson, G. Concentrations and Exports of Fecal Indicator Bacteria in Watersheds with Varying Densities of Onsite Wastewater Systems. *Water Air Soil Pollut* **2018**, *229,* 1-16.

31. Iverson, G.; Sanderford, C.; Humphrey, C. P.; Etheridge, J. R.; Kelley, T. Fecal Indicator Bacteria Transport from Watersheds with Differing Wastewater Technologies and Septic System Densities. *Appl. Sci.* **2020**, *10,* 6525.

32. Chambers, P. A.; Vis, C.; Brua, R. B.; Guy, M.; Culp, J. M.; Benoy, G. A. Eutrophication of agricultural streams: defining nutrient concentrations to protect ecological condition. *Water Sci. Technol.* **2008**, *58,* 2203-2210.

33. Dodds, W. K.; Jones, J. R.; Welch, E. B. Suggested classification of stream trophic state: distributions of temperate stream types by chlorophyll, total nitrogen, and phosphorus. *Water Res.* **1998**, *32,* 1455-1462.

34. US EPA. **2000.** Ecoregional Nutrient Criteria for Rivers and Streams. Available online: https://www.epa.gov/nutrient-policy-data/ecoregional-nutrient-criteria-rivers-and-streams (accessed on 26 September 2021).

35. Silva, S. R.; Ging, P. B.; Lee, R. W.; Ebbert, J. C.; Tesoriero, A. J.; Inkpen, E. L. Forensic Applications of Nitrogen and Oxygen Isotopes in Tracing Nitrate Sources in Urban Environments. *Environ. Forensics* **2002**, *3,* 125-130.

36. Kendall, C.; McDonnell, J. J. *Isotope tracers in catchment hydrology*, First Edition; Elsevier Science, B.V.: Amsterdam, **1998;** pp. 538-553.

37. Tamborski, J.; Brown, C.; Bokuniewicz, H.; Cochran, J. K.; Rasbury, E. T. Investigating Boron Isotopes for Identifying Nitrogen Sources Supplied by Submarine Groundwater Discharge to Coastal Waters. *Front. Res. Found.* **2020**, *8,* 1-15.

38. Humphrey, C.; Pradhan, S.; Bean, E.; O□Driscoll, M.; Iverson, G. Preliminary Evaluation of a Permeable Reactive Barrier for Reducing Groundwater Nitrate Transport from a Large Onsite Wastewater System. *Amer. J. Environ. Sci.* **2015**, *11,* 216-226.

39. Long, L. M.; Schipper, L. A.; Bruesewitz, D. A. Long-term nitrate removal in a denitrification wall. *Agri. Ecosyst. Environ.* **2011**, *140,* 514-520.

40. Robertson, W. D.; Merkley, L. C. In-Stream Bioreactor for Agricultural Nitrate Treatment. *J. Environ. Qual.* **2009**, *38,* 230-237.

41. Iverson, G. Nutrient contributions from septic systems in nutrient-sensitive watersheds: Quantifying nutrient inputs, reduction methods, and economic feasibility. Doctoral Dissertation in Coastal Resources Management, East Carolina University, Greenville, NC, March **2019.**

42. Bell, N.; Cooke, R. A. C.; Olsen, T.; David, M. B.; Hudson, R. Characterizing the Performance of Denitrifying Bioreactors during Simulated Subsurface Drainage Events. *J. Environ. Qual.* **2015**, *44,* 1647-1656.

43. Ramírez-Godínez, J.; Beltrán-Hernández, I.; Álvarez-Hernández, A.; Coronel-Olivares, C.; Contreras-López, E.; Quezada-Cruz, M.; Vázquez-Rodríguez, G. Evaluation of Natural Materials as Exogenous Carbon Sources for Biological Treatment of Low Carbon-to-Nitrogen Wastewater. *BioMed. Res. Int.* **2015**, *2015,* 754785-8.

44. Lynn, T. J.; Yeh, D. H.; Ergas, S. J. Performance of Denitrifying Stormwater Biofilters Under Intermittent Conditions. *Environ. Eng. Sci.* **2015**, *32,* 796-805.

45. Hoover, N. L.; Bhandari, A.; Soupir, M. L.; Moorman, T. B. Woodchip Denitrification Bioreactors: Impact of Temperature and Hydraulic Retention Time on Nitrate Removal. *J. Environ. Qual.* **2016**, *45,* 803-812.

46. Goodwin, G. E.; Bhattarai, R.; Cooke, R. Synergism in nitrate and orthophosphate removal in subsurface bioreactors. *Ecol. Eng.* **2015***, 84,* 559-568.

47. Thapa, U. Evaluation of Woodchip Bioreactors and Phosphorus Adsorption Media for Nutrient Removal from Subsurface Drainage Water. Master's thesis, South Dakota State University, Brookings, SD, **2017.**

48. Christianson, L. E.; Bhandari, A.; Helmers, M. J. Practice-oriented Review of Woodchip ioreactors for Subsurface Agricultural Drainage. *Appl. Eng. Agri.* **2012***, 28,* 861-874.

49. USDA. Denitrifying Bioreactors Engineering Field Handbook. Iowa Amendments (IA) Chapter 14, Amendment 68, IA-92(1-5), United States Department of Agriculture; **2016.**

50. Liang, X.; Lin, L.; Ye, Y.; Gu, J.; Wang, Z.; Xu, L.; Jin, Y.; Ru, Q.; Tian, G. Nutrient removal efficiency in a rice-straw denitrifying bioreactor. *Biores. Technol.* **2015***, 198,* 746-754.

51. Healy, M. G.; Barrett, M.; Lanigan, G. J.; João Serrenho, A.; Ibrahim, T. G.; Thornton, S. F.; Rolfe, S. A.; Huang, W. E.; Fenton, O. Optimizing nitrate removal and evaluating pollution swapping trade-offs from laboratory denitrification bioreactors. *Ecol. Eng.* **2015***, 74,* 290-301.

52. Weng, L. P.; Riemsdijk, v., W.H; Hiemstra, T. Factors Controlling Phosphate Interaction with Iron Oxides. *J. Environ. Qual.* **2012***, 41,* 628-635.

53. Christianson, L. E.; Bhandari, A.; Helmers, M.J. **2012.** A Practice-oriented Review of Woodchip Bioreactors for Subsurface Agricultural Drainage. Available online: https://lib.dr.iastate.edu/abe_eng_pubs/297

54. Christianson, L. E.; Lepine, C.; Sibrell, P. L.; Penn, C.; Summerfelt, S. T. Denitrifying woodchip bioreactor and phosphorus filter pairing to minimize pollution swapping. *Water Res.* **2017***, 121,* 129-139.

55. Schmidt, C. A.; Clark, M. W. Efficacy of a denitrification wall to treat continuously high nitrate loads. *Ecol. Eng.* **2012***, 42,* 203-211.

56. Holmes, J. S. Owen, W. L. Common Forest Trees of North Carolina (revised). North Carolina Department of Agriculture and Consumer Services, North Carolina Forest Service; **2012.**

57. O'Driscoll, M.; Humphrey, C.; Iverson, G.; Hoben, J. Estimating the Influence of Onsite Wastewater Treatment Systems on Nutrient Loading to Falls Lake

Watershed, North Carolina. East Carolina University, NC Policy Collaboratory; **2020.**

APPENDIX A: WATERSHED NUTRIENT EXPORTS

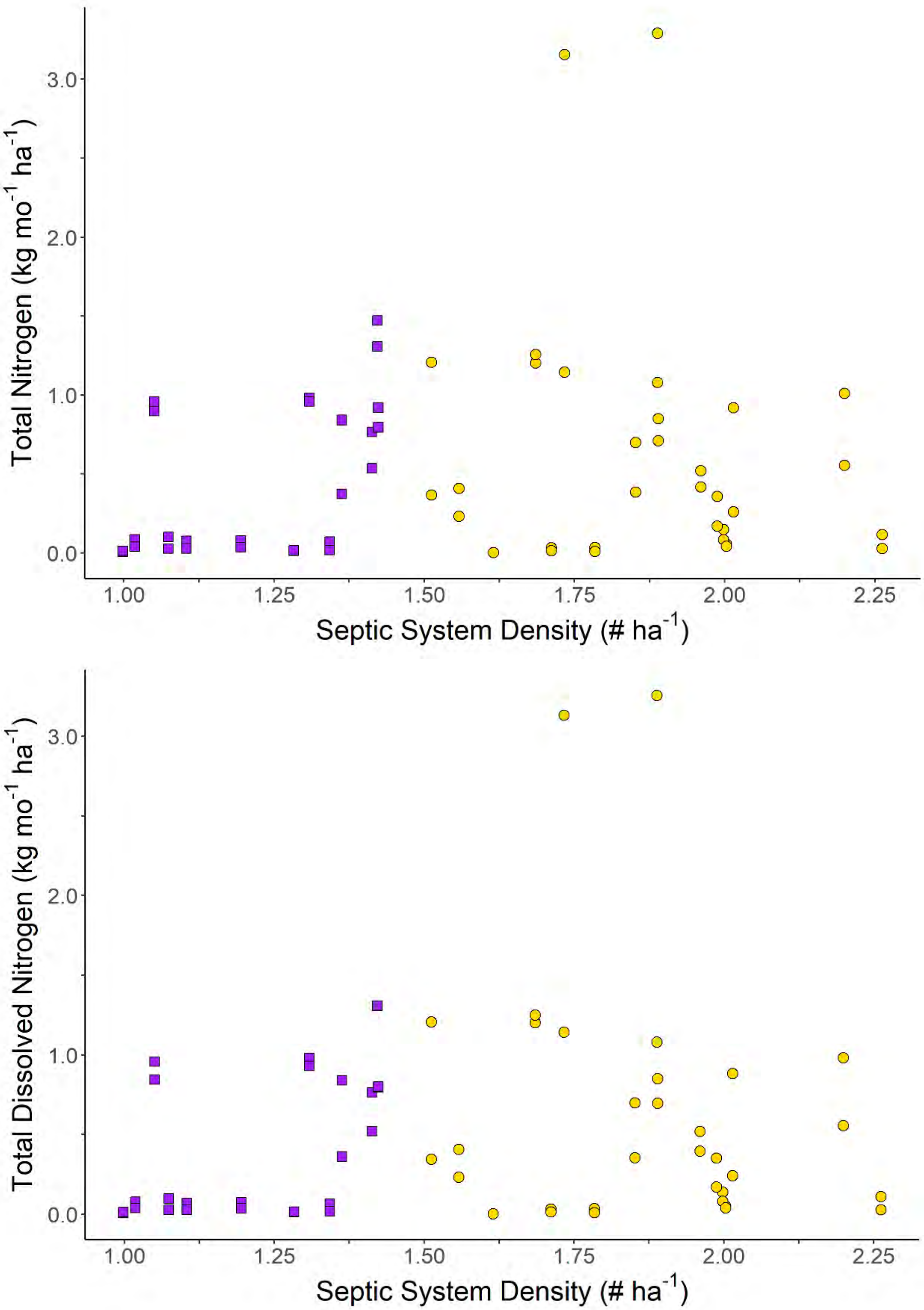


Figure A1. Total nitrogen and total dissolved nitrogen exports normalized by area.

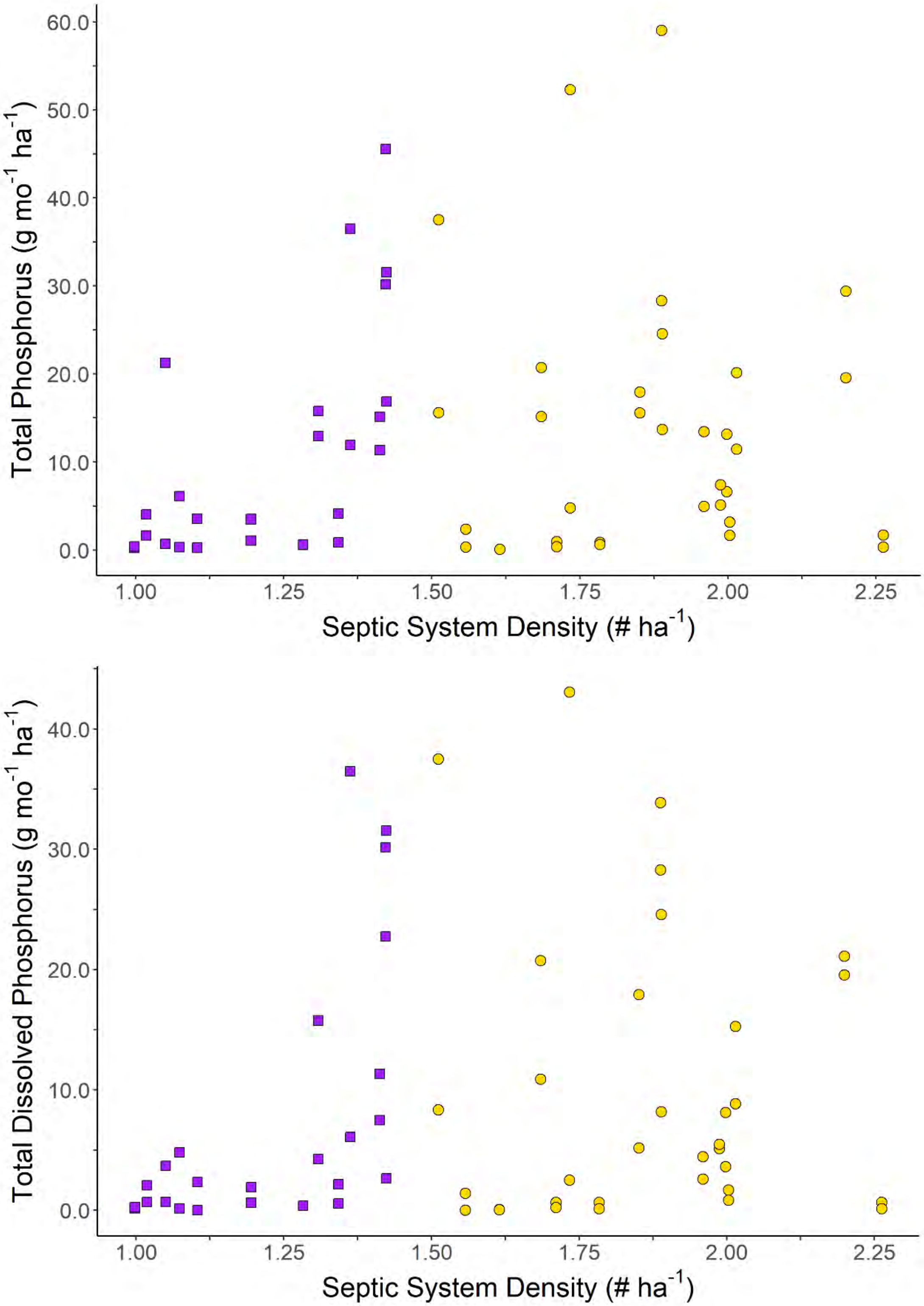


Figure A2. Total phosphorus and total dissolved phosphorus exports normalized by area.

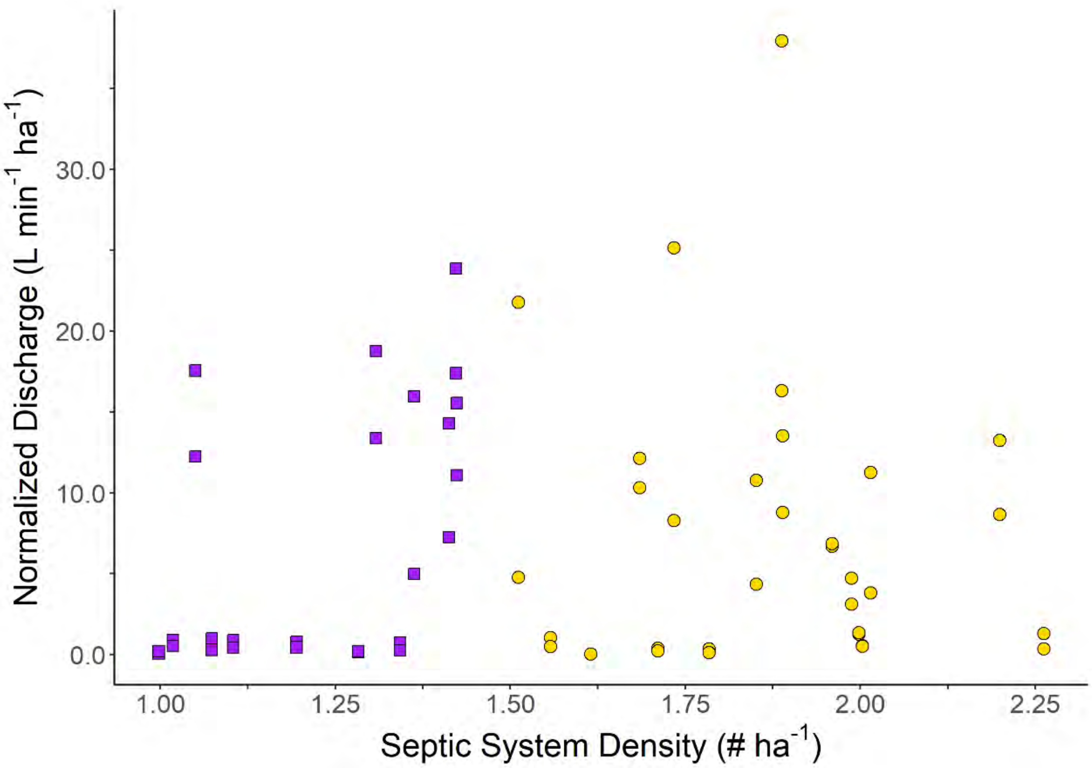


Figure A3. Stream discharge for each sub-watershed normalized by area. Exports are calculated by multiplying the watershed nutrient concentration by the stream discharge.

APPENDIX B: PHYSICOCHEMICAL PARAMETERS

This appendix includes the raw physicochemical data collected during the two sampling events. Temp= temperature; SC= specific conductance; Turb= turbidity; ORP= oxidation reduction potential; DO= dissolved oxygen

Table B1. Raw physicochemical data for the December sampling event.

Date	Watershed Name	Temp (°C)	SC (μS cm^{-1})	Turb (FNU)	pH	ORP (mV)	DO (mg L^{-1})	Discharge (L sec^{-1})
15-Dec-20	Durant	8.1	169.8	14.85	6.62	110	11	0.3
	Brookfield	8.3	175.7	7.3	6.42	122.1	6.75	1.1
	Park Ridge	9	402.7	5.81	6.79	108.4	10.53	0.8
	Macon	8.2	252.3	15.21	6.99	88.6	11.14	0.9
	Victory Church	8.6	181.8	22.9	7.53	101	10.44	0.2
	Asbury	9.9	222.8	49.3	6.97	114.5	10.25	0.2
	November	8.9	208.5	19.06	7.29	114.6	11.36	0.6
	Green Bay	9.7	88.5	30.73	6.65	144.6	10.97	2.4
	Harold	9.4	108.8	57.68	6.4	168.6	9.26	0.4
	Barclay	9.4	130.8	24.63	6.71	148.4	11.1	1.6
	Woody	11.5	83.6	27.01	6.36	216.7	9.55	0.7
	Appaloosa	8.9	92.1	39.79	6.8	146.5	10.82	0.7
	Donlin	9.1	75.2	17.26	6.63	163.9	11.04	0.7
	Jenkins	11.6	74.2	17.51	6.78	139	9.72	0.4
	Tacketts Pond 1	9.08	188	31.2	5.7	103	8.9	3.0
	Tacketts Pond 2	8.11	172	53.9	5.96	124	7.4	7.6
	Green Downs 1	8.3	6	14.2	6.7	73	10.7	16.1
	Green Downs 2	8.5	127	74	6.8	82	7.4	3.2
	Green Downs 3	8.8	88	15.3	6.9	98	9.68	3.4
	Appaloosa Run E	8.9	130	9.6	6.7	105	10.4	10.6
	Ethan	8.1	104	25	6.65	101	10.5	12.8
	Indigo Moon Way	8.4	85	33.8	6.86	107	12.3	24.2
	Bushveld	8.6	91	23	6.6	99	8.8	22.7
	CranesbilL	9.5	98	6.5	6.6	102	9.7	49.1
	Liatris	9.6	113	7	6.6	140	9.3	14.8
	Old Creedmoor	10	112	12.5	6.75	64	9.6	1.8
	Kinsdale 1	9.06	113	14.5	6.9	87	8.2	9.1
	Kinsdale 2	10.8	146	4.2	6.7	98	10.05	15.7
	Leslie 1	9.78	117	8	7.01	89	8	3.3
	Coachmans Way	8.9	30	6.3	6.1	141	8.3	3.4

Sampling was split into two days this sampling even since the team was not able to deploy on the same day.

Table B2. Raw physicochemical data for the February sampling event.

Date	Watershed Name	Temp (°C)	SC (μS cm^{-1})	Turb (FNU)	pH	ORP (mV)	DO (mg L^{-1})	Discharge (L sec^{-1})
22-Feb-21	Durant	5.8	96.5	6.42	6.73	130.6	11.63	0.8
	Brookfield	6.4	99.5	3.22	6.55	108.4	11.24	0.3
	Park Ridge	7.7	223.8	3.16	6.776	149.6	11.01	0.4
	Macon	5.2	113.4	4.35	6.93	122.4	12.2	0.3
	Victory Church	4.9	76.7	11.76	6.98		12.35	0.2
	Asbury	5.8	150.2	19.67	6.72		11.68	0.2
	November	6.7	97.9	19.57	7.20	143.2	12.4	1.0
	Green Bay	7.1	97.4	11.41	6.54	176.4	12.01	1.4
	Harold	7	111.1	17.72	6.35	157.7	11.44	0.1
	Barclay	7.4	138.6	16.92	6.67	161.9	11.93	0.9
	Woody	9.1	67.1	7.55	6.35	139.7	10.55	0.2
	Appaloosa	6.7	96.1	11.64	6.72	147	11.79	0.4
	Donlin	7.2	62.1	19.83	6.51		11.97	0.7
	Jenkins	9.7	73.1	6.4	6.63		10.48	0.2
24-Feb-21	Tacketts Pond 1	12.5	244	4.2	6	194	7.7	1.2
	Tacketts Pond 2	10.5	169	14.6	6.2	197	8.6	2.4
	Green Downs 1	14.1	160	1.1	6.2	189	7.6	37.5
	Green Downs 2	12.5	119	5.6	6.25	202	7.5	1.6
	Green Downs 3	13.8	67	6.2	6.5	191	8.1	5.2
	Appaloosa Run E	13.1	143	4	6.27	207	8.2	6.9
	Ethan	12.2	122	16.8	6.5	198	8.6	8.9
	Indigo Moon Way	13	107	25.2	6.43	197	8.1	5.3
	Bushveld	8.8	91	9.1	7.07	156	8.2	16.2
	Cranesbill	11	102	7.6	6.55	202	8.28	35.0
	Liatris	10.3	109	5.6	6.3	205	7.53	10.8
	Old Creedmoor	10.5	88	5.6	6.47	190	8.5	2.7
	Kinsdale 1	12.1	118	4.2	6.5	218	8.2	7.7
	Kinsdale 2	13.7	155	1	6.5	213	7.7	5.2
	Leslie 1	12.9	125	2.1	6.52	217	7.8	3.4
	Coachmans Way	10.9	51	3	6.4	218	8	10.0

APPENDIX C: LAND COVER DATA

Land use was summarized for the sub-watersheds in the next two tables. The next table shows the estimated area (in hectares [ha]) for each of the 2019 National Land Cover Database classifications. In the table following, it shows the same estimates but presented as a percentage of the total watershed area. Blank data cells denote that this land cover feature is at 0 ha or 0% of the sub-watershed. If the value in the second table shows “0.0%” it denotes that the total percentage is < 0.1% of the sub-watershed. OW= open water; D,OS= developed, open space; D,LI= developed, low-intensity; D,MI= developed, medium intensity; D,HI= developed, high-intensity; BL= barren land; DF= deciduous forest; EF= evergreen forest; MF= mixed forest; SHR= shrub/scrub; HERB= herbaceous/grassland; CROP= cultivated crops; HAY= hay/pasture; WDWET= woody wetlands.

Watershed	OW	D,OS	D,LI	D,MI	D,HI	BL	DF	EF	MF	SHR	HERB	CROP	HAY	WDWET
Durant	1.17	65.40	39.32	13.15	2.04	0.06	17.66	32.41	49.09	0.07	0.81	0.00	6.26	
Victory Church	1.80	152.38	58.10	8.86	0.62	0.53	58.84	60.52	99.80		0.96		10.38	3.51
November	3.06	121.23	5.25	0.59			58.74	26.41	44.06	0.06	0.49		9.01	
Appaloosa		8.27	5.48	0.95	0.02		10.88	10.20	7.69	0.00	4.05		1.37	
Donlin	0.45	35.12	19.06	1.81			9.80	5.18	4.92		0.00	0.18	3.35	
Jenkins		23.89	9.98	1.44	0.04		6.24	8.24	9.53	0.96	0.18		3.78	
Woody		12.88	4.94	0.36			5.93	1.01	11.35				3.58	
Barclay		49.20	13.05	6.82	1.34		21.91	6.63	17.63	0.18	0.51		8.20	0.09
Harold		12.74	0.42				13.64	1.38	3.47		0.09		0.29	
Green Bay	1.35	54.81	7.90	7.31	0.55		48.82	8.44	24.91	1.60	0.54		2.92	
Asbury		2.46	1.16	0.13			0.16	0.45	1.65				0.45	
Macon	0.09	70.81	13.17	0.75	0.02	0.11	15.78	22.53	27.20					1.98
Park Ridge	1.35	20.20	7.80	1.41	0.18		0.72	6.56	3.52		0.09		4.39	
Brookfield	1.35	22.44	4.82	0.10	0.09		5.47	10.99	6.30				1.05	
Tacketts Pond 1		4.67	4.11	0.54			0.62	2.48	4.33					
Tacketts Pond 2		6.17	4.85	1.01			1.63	9.40	3.64				1.93	
Green Downs 1		28.42	3.63	0.09			12.95	4.20	8.86	0.65	0.45		0.06	
Green Downs 2		4.57	0.55	0.12			3.04	2.53	2.65					
Green Downs 3		12.36	1.19	0.00			6.13	0.95	3.01					
Appaloosa Run E		22.05	2.84	0.09			11.12	2.50	7.78	0.65			0.06	
Ethan		10.74	5.02	1.51			6.82	4.42	10.17				4.56	
Indigo Moon Way	0.45	22.66	9.30	3.91	0.44	0.36	6.56	9.11	11.46		0.30		1.22	
Bushveld	0.63	28.37	3.37	0.25	0.18		7.64	14.87	17.17		0.14			
Cranesbill	5.31	60.87	35.56	5.77	0.34		15.10	38.16	24.23	0.18	2.36		1.80	
Liatris	1.53	11.73	8.01	0.81			5.13	6.32	2.88		0.73		0.14	
Old Creedmoor		15.06	4.52	0.62			0.85	9.65	3.82	0.18	0.02			
Kinsdale 1		16.07	4.31	0.39			2.60	9.71	8.95		3.07			
Kinsdale 2		17.04	3.77	0.25			2.62	5.46	6.70		1.65			
Leslie 1		17.43	2.71	0.36	0.09		0.94	3.84	4.22					
Coachmans Way		20.15	9.79	0.56			1.44	14.02	7.01		0.18		0.47	

Watershed	OW	D,OS	D,LI	D,MI	D,HI	BL	DF	EF	MF	SHR	HERB	CROP	HAY	WDWET
Durant	0.5%	28.8%	17.3%	5.8%	0.9%	0.0%	7.8%	14.3%	21.6%	0.0%	0.4%	0.0%	2.8%	
Victory Church	0.4%	33.4%	12.7%	1.9%	0.1%	0.1%	12.9%	13.3%	21.9%		0.2%		2.3%	0.8%
November	1.1%	45.1%	2.0%	0.2%			21.8%	9.8%	16.4%	0.0%	0.2%		3.3%	
Appaloosa		16.9%	11.2%	1.9%	0.0%		22.2%	20.9%	15.7%	0.0%	8.3%		2.8%	
Donlin	0.6%	44.0%	23.9%	2.3%			12.3%	6.5%	6.2%		0.0%	0.2%	4.2%	
Jenkins		37.2%	15.5%	2.2%	0.1%		9.7%	12.8%	14.8%	1.5%	0.3%		5.9%	
Woody		32.2%	12.3%	0.9%			14.8%	2.5%	28.4%				8.9%	
Barclay		39.2%	10.4%	5.4%	1.1%		17.4%	5.3%	14.0%	0.1%	0.4%		6.5%	0.1%
Harold		39.8%	1.3%				42.6%	4.3%	10.8%		0.3%		0.9%	
Green Bay	0.8%	34.4%	5.0%	4.6%	0.3%		30.7%	5.3%	15.7%	1.0%	0.3%		1.8%	
Asbury		38.2%	18.0%	2.0%			2.5%	6.9%	25.5%				6.9%	
Macon	0.1%	46.5%	8.6%	0.5%	0.0%	0.1%	10.4%	14.8%	17.8%					1.3%
Park Ridge	2.9%	43.7%	16.9%	3.1%	0.4%		1.6%	14.2%	7.6%		0.2%		9.5%	
Brookfield	2.6%	42.7%	9.2%	0.2%	0.2%		10.4%	20.9%	12.0%				2.0%	
Tacketts Pond 1		27.9%	24.6%	3.2%			3.7%	14.8%	25.8%					
Tacketts Pond 2		21.6%	16.9%	3.5%			5.7%	32.8%	12.7%				6.7%	
Green Downs 1		47.9%	6.1%	0.2%			21.8%	7.1%	14.9%	1.1%	0.8%		0.1%	
Green Downs 2		33.9%	4.1%	0.9%			22.6%	18.8%	19.7%					
Green Downs 3		52.3%	5.0%	0.0%			25.9%	4.0%	12.7%					
Appaloosa Run E		46.8%	6.0%	0.2%			23.6%	5.3%	16.5%	1.4%			0.1%	
Ethan		24.8%	11.6%	3.5%			15.8%	10.2%	23.5%				10.6%	
Indigo Moon Way	0.7%	34.5%	14.1%	5.9%	0.7%	0.5%	10.0%	13.8%	17.4%		0.4%		1.9%	
Bushveld	0.9%	39.1%	4.6%	0.3%	0.3%		10.5%	20.5%	23.6%		0.2%			
Cranesbill	2.8%	32.1%	18.7%	3.0%	0.2%		8.0%	20.1%	12.8%	0.1%	1.2%		0.9%	
Liatris	4.1%	31.5%	21.5%	2.2%			13.8%	17.0%	7.7%		2.0%		0.4%	
Old Creedmoor		43.4%	13.0%	1.8%			2.4%	27.8%	11.0%	0.5%	0.1%			
Kinsdale 1		35.6%	9.5%	0.9%			5.8%	21.5%	19.8%		6.8%			
Kinsdale 2		45.5%	10.1%	0.7%			7.0%	14.6%	17.9%		4.4%			
Leslie 1		58.9%	9.2%	1.2%	0.3%		3.2%	13.0%	14.3%					
Coachmans Way		37.6%	18.3%	1.0%			2.7%	26.1%	13.1%		0.3%		0.9%	

APPENDIX D: SPATIAL EXTENT OF NUTRIENT SPECIATION AND N-15 VALUES

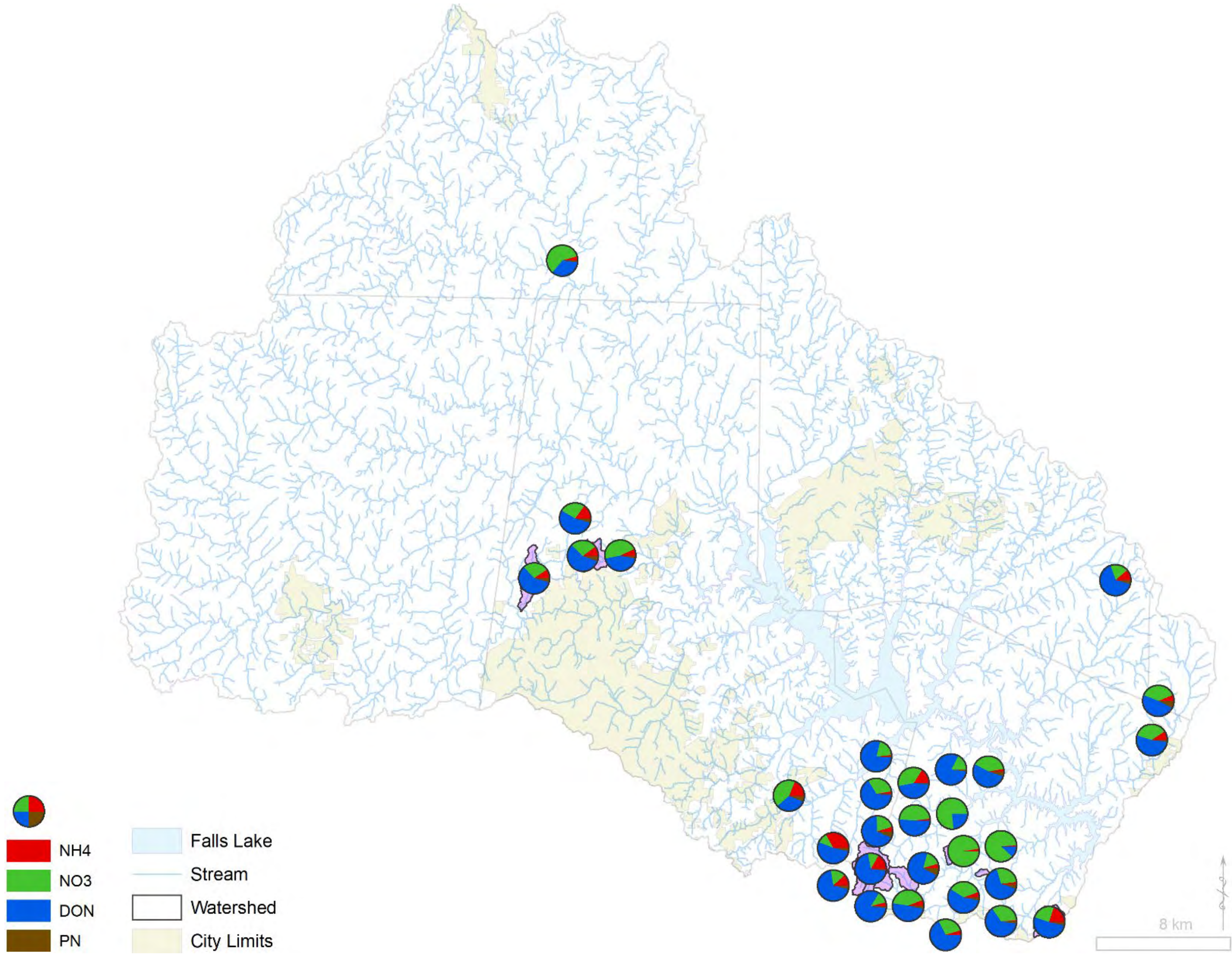


Figure D1. Nitrogen speciation of total nitrogen across the Falls Lake Watershed. The Wake County portion of Falls Lake is summarized in Figure D3. NH4= ammonium; NO3= nitrate; DON= dissolved organic nitrogen; PN= particulate nitrogen.

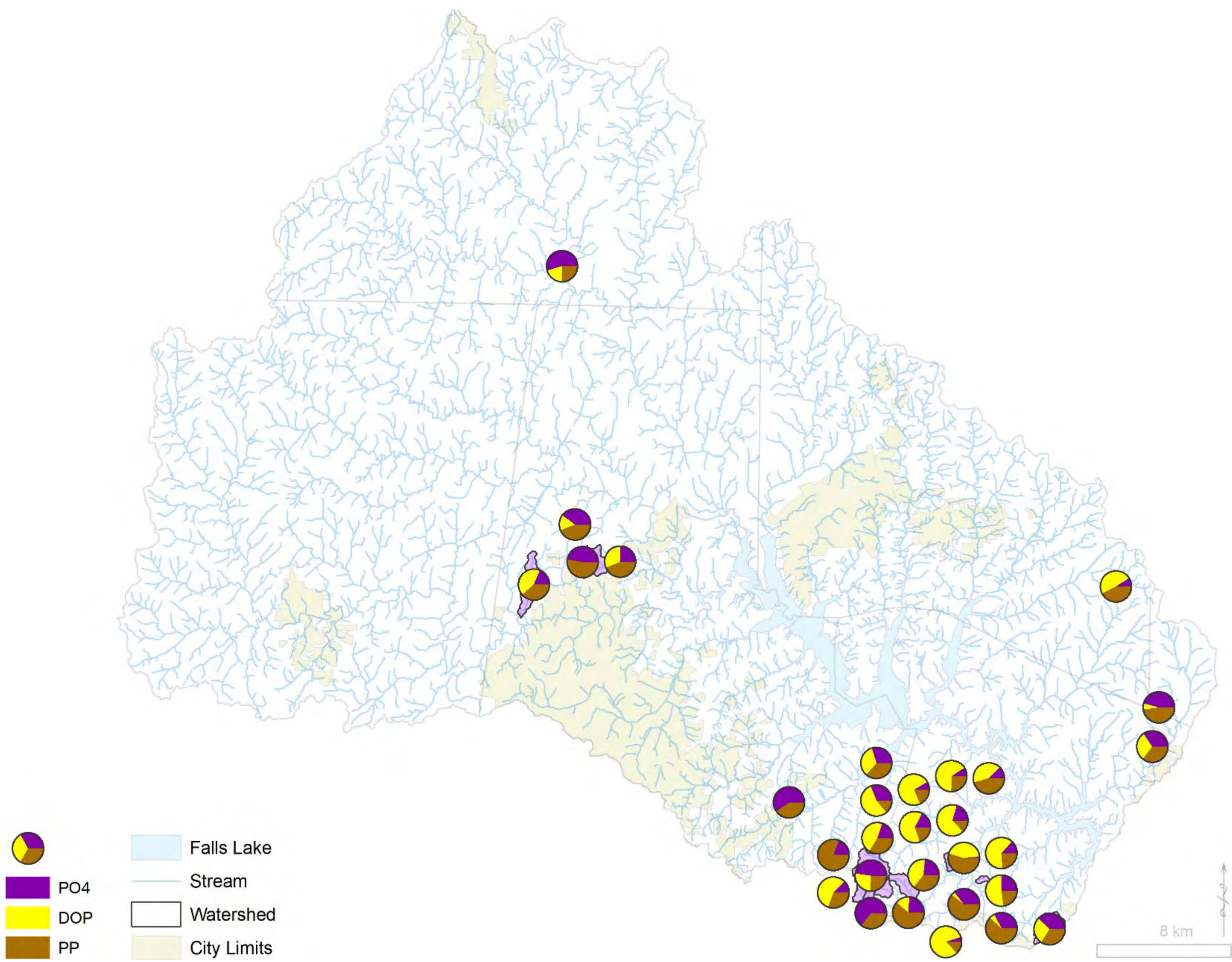


Figure D2. Phosphorus speciation of total phosphorus across the Falls Lake Watershed. The Wake County portion of Falls Lake is summarized in Figure D4. PO4= phosphate; DOP= dissolved organic phosphorus; PP= particulate phosphorus.

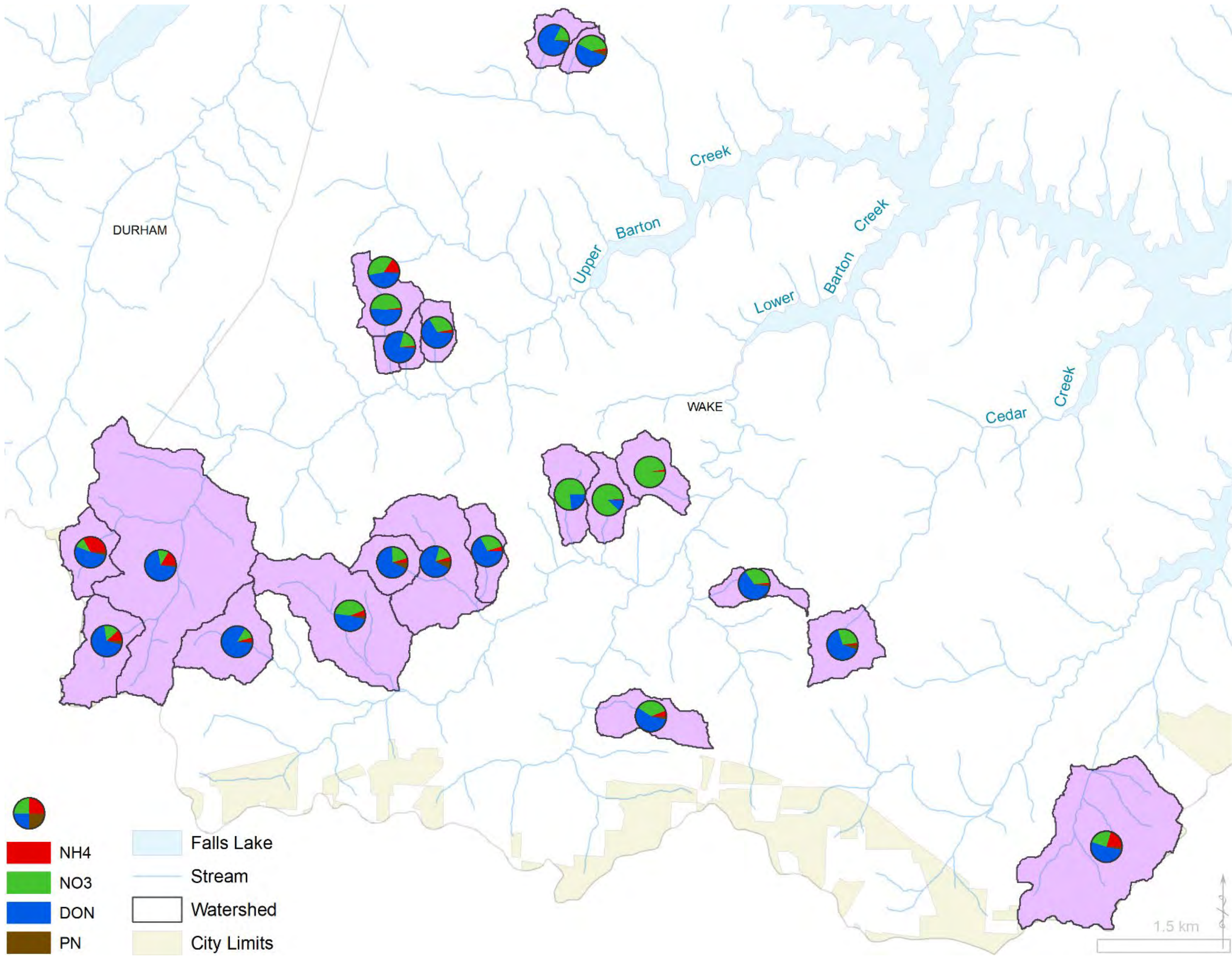


Figure D3. Nitrogen speciation of total nitrogen in sub-watersheds located in Wake County. NH4= ammonium; NO3= nitrate; DON= dissolved organic nitrogen; PN= particulate nitrogen.

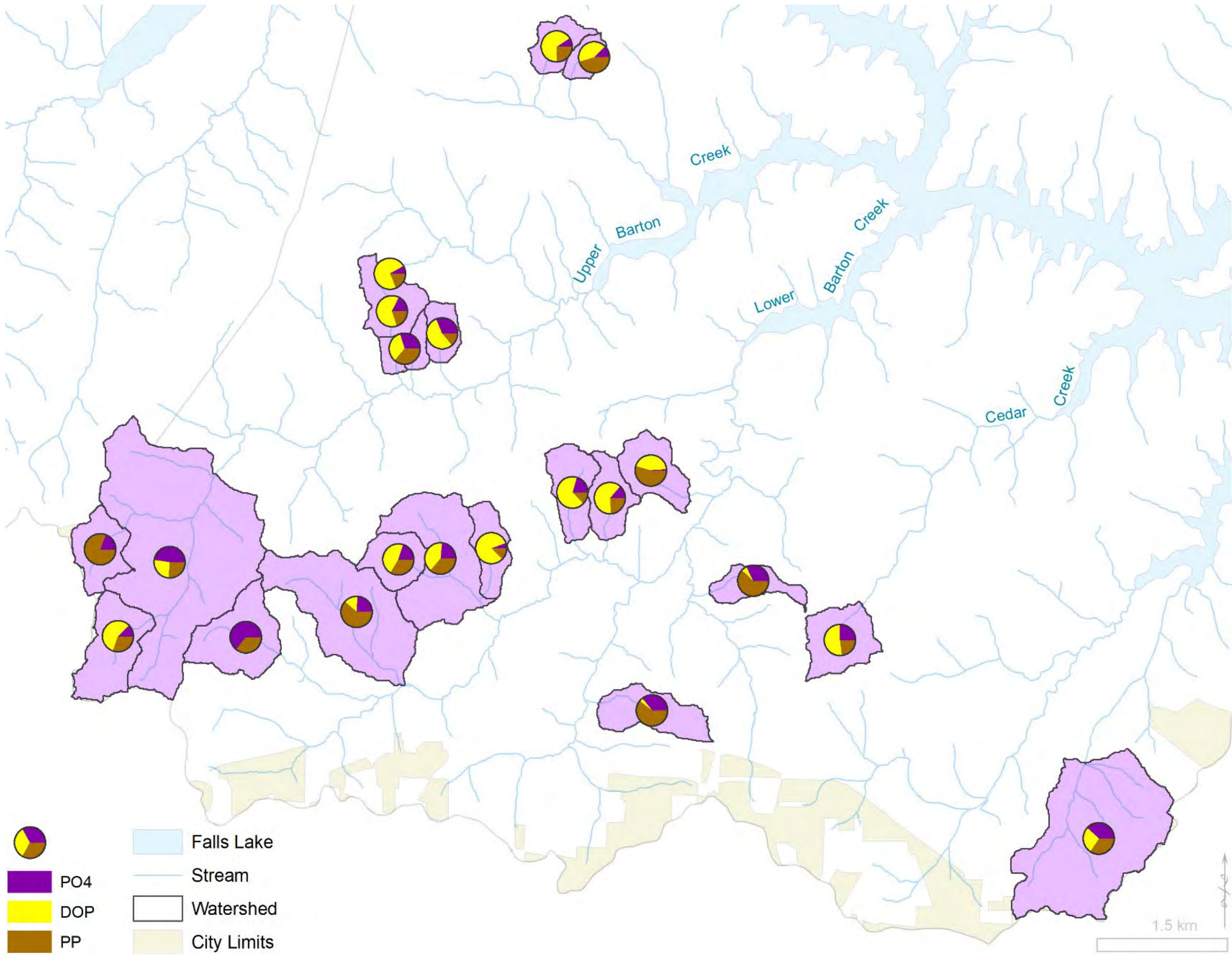


Figure D4. Phosphorus speciation of total phosphorus in sub-watersheds located in Wake County. PO4= phosphate; DOP= dissolved organic phosphorus; PP= particulate phosphorus.

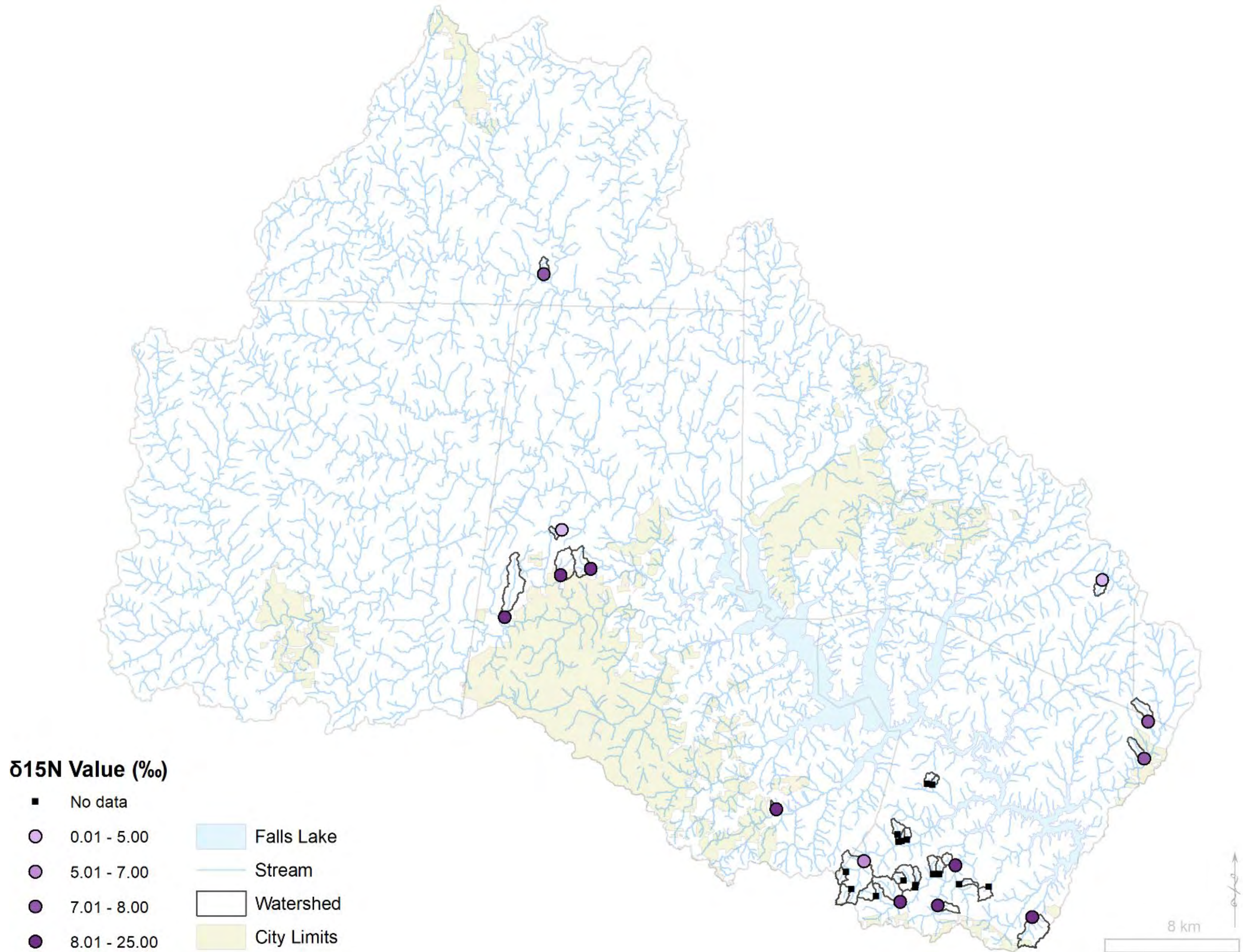


Figure D5. Values of $\delta^{15}N$**21** from sub-watersheds that were sampled for isotopic fractionation of nitrate. Values that contain >7‰ of $\delta^{15}N$ indicate potential sources of human and/or animal waste. Choropleth ranges were selected based on $\delta^{15}N$ values (‰) that overlap with fertilizers or precipitation (-5 to +5‰), soil organic matter (+5 to +8‰), and manure and septic effluent (+8 to +25‰) derived from Silva et al. [35] and Kendall [36].

APPENDIX E: PROJECT PHOTOS

Photographs are compiled by watershed sampling (Figures E1-E6) and then the bioreactor pilot study.

Figure E1. Jen Richardson sampling a tributary in Durham County, NC on Dec. 15, 2020.

Figure E2. Jenifer Richardson (graduate student in foreground) and De'Monnie Williams (undergraduate student in the middle) perform nitrate analysis of surface water samples while Ann Marie Lindley (graduate student at desk) analyzes field data.

Figure E3. Ann Marie Lindley and Jen Richardson (ECU Graduate Students) sampling a tributary in Person County, NC.

Figure E4. Jen Richardson sampling a tributary in Durham County, NC.

Figure E5. Jen Richardson and Ann Marie Lindley estimating streamflow along a tributary in Orange County, NC.

Figure E6. Jen Richardson collecting a water sample along a tributary in Granville County, NC.

Figure E7. Bioreactor pilot study setup showing the nutrient stock tanks (large black cisterns), pilot-scale bioreactors (smaller black tubs), and piping materials.

Figure E8. Close-up of the bioreactor mesocosms and the pipe network inflow mechanisms that deliver nutrient-rich water into the top of the bioreactor.

Figure E9. Graduate student Ann Marie Lindley (Geological Sciences) and undergraduate student Mikayla Dixon (Geological Sciences) shovel carbon media into bioreactor mesocosms.

Figure E10. Mikayla Dixon (undergraduate student in Geological Sciences) pours media into a bioreactor. The water conveyance network can be seen between adjacent bioreactors. Water is composited across all 3 bioreactors and fills the Lowe's buckets in the foreground to monitor water quality using YSI EXO2 sondes.

Figure E11. Close-up of a pine bark bioreactor. The geotextile shows the location of the expanded slate aggregate.

Figure E12. Close-up of a woodchip bioreactor. The geotextile shows the location of the expanded slate aggregate.

Figure E12. Close-up of a peanut shell bioreactor. The geotextile shows the location of the expanded slate aggregate.

Figure E13. Photo showing the completed mesocosm set-up with 9 total bioreactors with each carbon media. The left row shows the 3 woodchip bioreactors, the middle row shows the 3 pine bark bioreactors, the right row shows the 3 peanut shells.

Figure E14. Close-up of the exfiltration system showing the deployed EXO2 sondes collecting high-frequency water quality data.

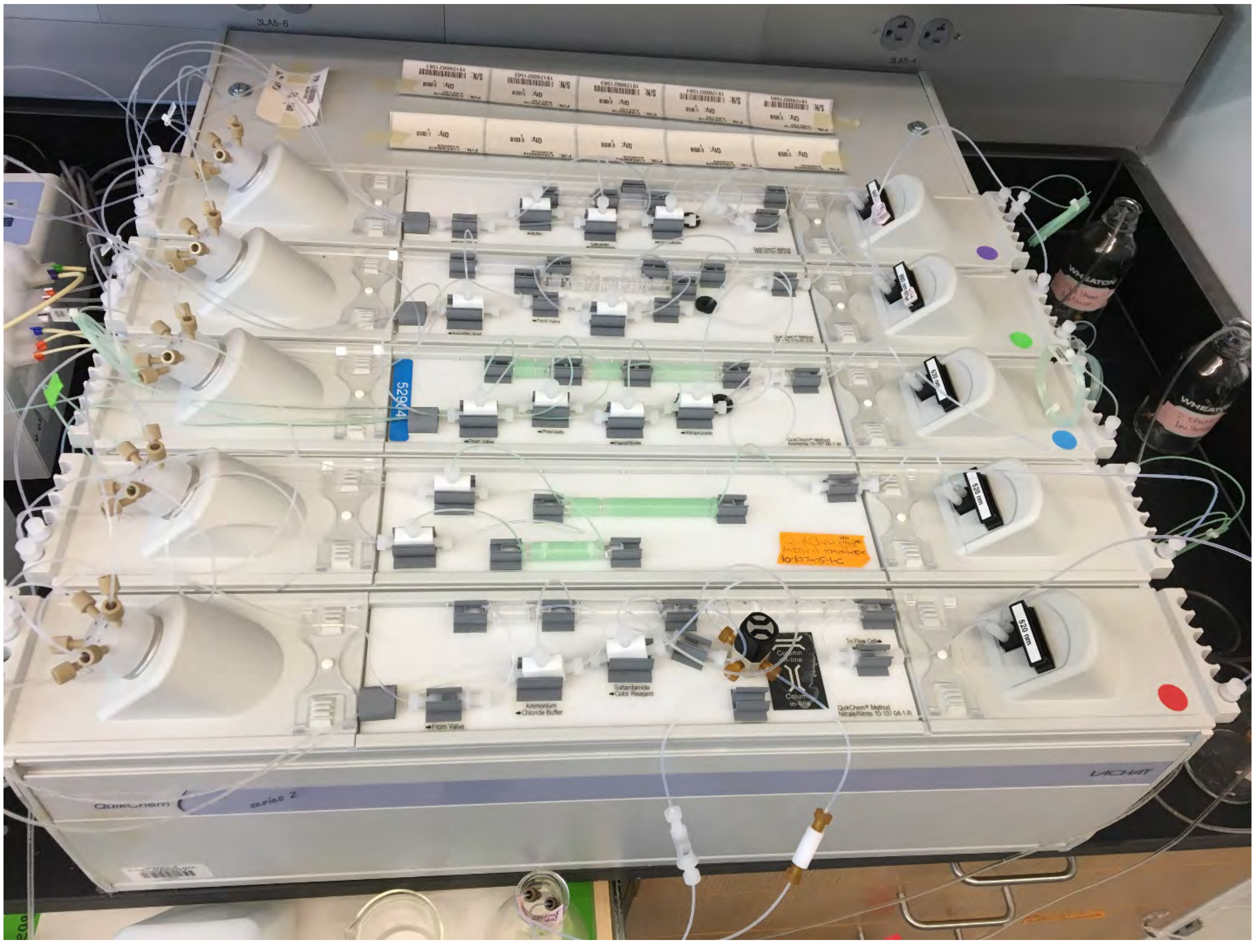


Figure E12. Close-up of the Lachat Quikchem analyzer.