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Nutrient enrichment in wadeable urban streams in the Piedmont Ecoregion of the Southeastern United States

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Abstract

The U.S. Geological Survey (USGS) Southeastern Stream Quality Assessment (SESQA) collected weekly samples for nitrogen and phosphorus in 76 wadeable streams in the urbanized Piedmont Ecoregion of the Southeastern United States, during April–June 2014. Total nitrogen (TN) concentrations in excess of U.S. Environmental Protection Agency (EPA) guidelines and statistically greater than at reference locations indicated nitrogen-nutrient enrichment in streams draining poultry confined animal feeding operations (CAFO) or urban centers. Nitrate plus nitrite ($\text{NO}_3 + \text{NO}_2$) dominated TN species in urban/CAFO-influenced streams. Streams that drained poultry CAFO and Washington DC had statistically higher $\text{NO}_3 + \text{NO}_2$ concentrations than streams draining Atlanta, Charlotte, Greenville, or Raleigh. In contrast, total phosphorus (TP) concentrations in Atlanta and

Washington DC streams statistically were comparable to and lower than, respectively, reference stream concentrations. Over 50% of TP concentrations in Greenville, Charlotte, Raleigh and CAFO-influenced streams exceeded the EPA guideline and reference-location mean concentrations, indicating phosphorus-nutrient enrichment. Urban land use, permitted point sources, and soil infiltration metrics best predicted TN exceedances. Elevated TN and $\text{NO}_3 + \text{NO}_2$ concentrations in urban streams during low flow were consistent with reduced in-stream dilution of point-source or groundwater contributions. Urban land use, permitted point sources, and surface runoff metrics best predicted TP exceedances. Elevated TP in CAFO and urban streams during high flow were consistent with non-point sources and particulate transport.

Keyword: Environmental science

1. Introduction

Anthropogenic changes in nitrogen and phosphorus speciation and concentrations can lead to hypoxic fish kills, increased frequency and severity of nuisance/harmful algal blooms, and degraded ecological condition and function in fluvial habitats [1, 2, 3, 4]. The Piedmont Level III Ecoregion in the Southeastern United States is characterized by highly diverse aquatic biota [5], but over the past decade 51 native fish species and about 65% of native mollusk populations in the Southeast have been identified as imperiled due to habitat degradation and water-quality impairment [6, 7, 8, 9]. To aid in the protection of these sensitive species and to prevent further degradation of aquatic resources, states in the Southeastern U.S. have advanced numerical nutrient criteria for rivers and streams based on 3 general approaches. Initial guidance recommended criteria based on stream nutrient concentrations at the 25th percentile of all sites or at the 75th percentile of reference sites in the region [10] (Table 1). Others have suggested criteria based on models of reference or background nutrient concentrations that assume no anthropogenic watershed influences [11]. More recently, nutrient criteria based on diatom, benthic-macroinvertebrate, and fish-community data have been promulgated [12, 13, 14, 15].

Piedmont region surface-water nutrient management plans primarily focus on wastewater-discharge and agricultural non-point-runoff sources in specific urban centers or watersheds. Management efforts in the Chesapeake Bay, Neuse River, and Cape Fear River basins, for example, target reductions in TP and TN loadings [16, 17, 18], with varying results [19]. Decadal declines in stream TP concentrations in the Atlanta metropolitan area have been ascribed to wastewater phosphorus controls [20, 21]. However, regional impacts of atmospheric nitrogen deposition are growing concerns for Piedmont streams [22, 23], with increasing ammonia (NH_3) wet deposition observed at the majority (68%) of US National Atmospheric

Table 1. Comparison between existing percentile-based and modeled guidelines and corresponding percentiles in 2014 Southeastern Regional Stream Quality Assessment (SESQA) study for nutrient concentrations in streams in the Piedmont region of the eastern United States. [TN, total nitrogen; TP, total phosphorus; NO₃+NO₂, nitrate plus nitrite; mg/L, milligrams per liter].

| Water quality standard/ benchmark/guideline | TN (mg/L) | TP (mg/L) | NO ₃ +NO ₂ (mg/L) | Statistic | Reference |
|---|-----------|--|--|------------------------------|---------------------|
| Drinking water | — | — | 10 | MCL standard | USEPA, 2015 |
| Nutrient Ecoregion IX | 0.69 | 0.037 | — | 25th percentile guideline | USEPA, 2000 |
| Piedmont level III Ecoregion | 0.62 | 0.03 | 0.18 | 25th percentile guideline | USEPA, 2000 |
| Nutrient Ecoregion IX reference condition | 0.25 | 0.045 | — | Model/median guideline | Smith et al. 2003 |
| Connecticut streams for (a) reduction in and (b) loss of sensitive diatoms | — — | ^a 0.040 ^b 0.065 | — — | Ecological response model | Smucker et al. 2013 |
| 75th Percentile of data from reference sites | 0.57 | 0.045 | 0.16 | 75th percentile guideline | This study |
| 25th Percentile of data from all sites | 0.63 | 0.03 | 0.29 | 25th percentile guideline | This study |

Deposition Program network sites [24] and 78% of modeled NH₃ emissions attributed to domestic anthropogenic sources [25]. Soil nitrification has been identified as a primary driver of nitrate in some Piedmont streams [26].

As part of the 2014 Southeast Stream Quality Assessment (SESQA) [27] of multiple water-quality stressors, the U.S. Geological Survey (USGS) assessed nitrogen and phosphorus concentrations in 76 wadeable streams in the highly-urbanized Piedmont [Level III Ecoregion; 28, 29] region to address the general lack of Ecoregion-wide information on fluvial nutrient status and the potential importance of in-stream nutrient alteration as a stream-quality driver. Identification of potential watershed predictors of in-stream nutrient concentrations based on correlations with readily available geographic information system (GIS) land-use land-cover (LULC) metrics was a second objective.

2. Materials and methods

2.1. Study area

The SESQA Piedmont study area included 76 watersheds in Alabama, Georgia, South Carolina, North Carolina, and Virginia (Fig. 1, Table S1) [27]. Thirteen low-impact (reference) watersheds included predominately-forested, protected areas like National Parks, State Parks, and wildlife refuges (less than 1.1 % urban development with median of 0.23 %). Four rural watersheds were predominantly forested

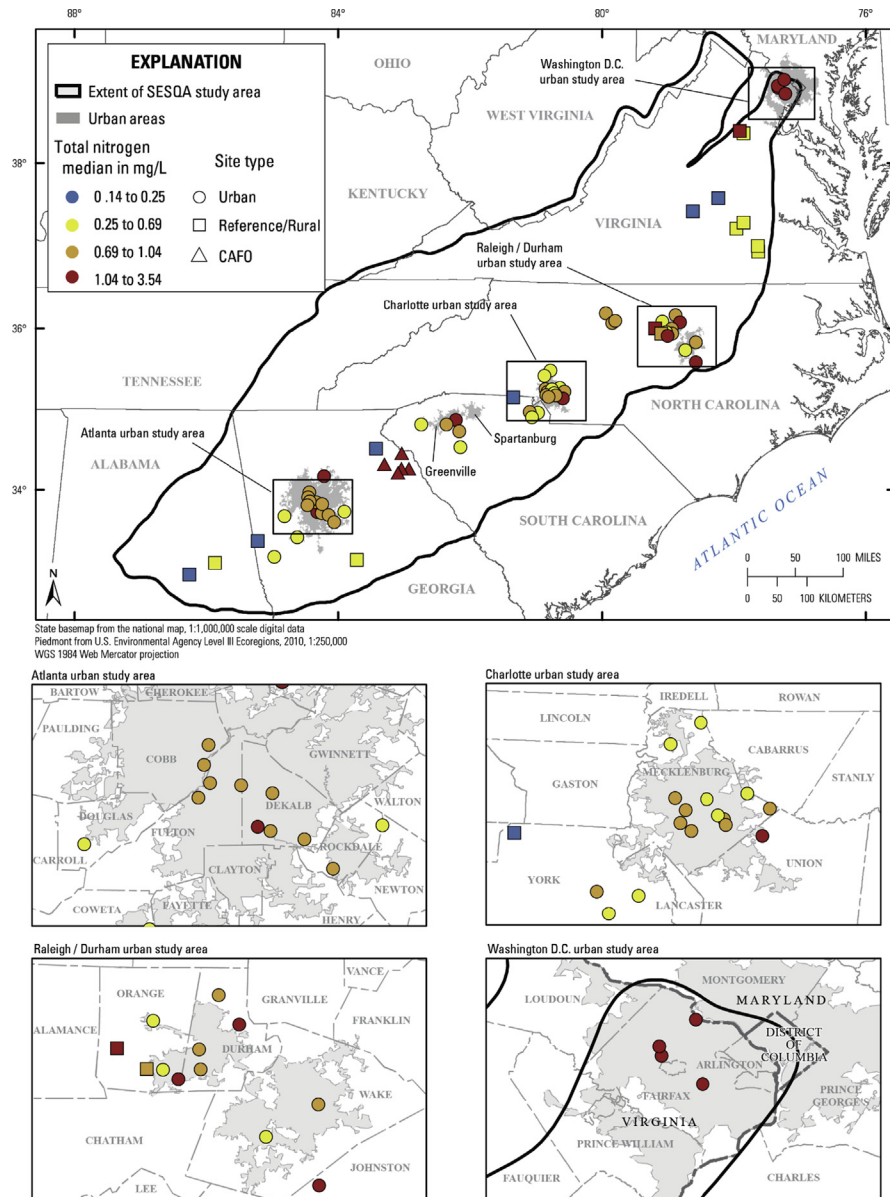


Fig. 1. Spatial distribution of median total nitrogen (TN) concentrations in SESQA study area, April–June 2014. Orange and red symbols indicate median concentrations above existing guidelines [respectively, 10, 11].

with less than 2 % urban development, some pasture or hay, but no row-crop agriculture. The majority of the US poultry industry is concentrated in the Southeastern region [30, 31]. Although agricultural land use (largely associated with livestock production) represented a comparatively small percentage of the SESQA study area, 5 rural, northeast-Georgia watersheds containing multiple poultry confined animal feeding operations (CAFO) were included due to the potential importance of these operations as local stream-quality drivers. The remaining 54 watersheds

represented the region-wide gradient in percent urban land use and were located in or near 5 Piedmont urban centers (Washington, DC; Raleigh-Durham-Greensboro, NC; Charlotte, NC; Greenville-Spartanburg, SC; Atlanta, GA) (Table S1). Percent urban land use was estimated as the sum of 2011 National Land Cover Database (NLCD) categories of Developed, Open; Developed, Low; Developed, Medium; and Developed, High (Table S1).

2.2. Existing nutrient guidelines

The SESQA study area lies within the EPA Aggregated Nutrient Ecoregion IX, wherein non-regulatory guidelines of 0.69 mg/L and 0.037 mg/L have been promulgated for total nitrogen (TN) and total phosphorus (TP), respectively (Table 1) [10]. Piedmont-specific Level III Ecoregion streams have 25th percentile guidelines of 0.62 mg/L and 0.030 mg/L for TN and TP, respectively [10]. Reference conditions in streams from the EPA Ecoregion IX have been modeled as 0.25 mg/L and 0.045 mg/L for TN and TP, respectively [11].

2.3. Sampling and analysis

Ten weekly water samples were collected at urban and CAFO stream sites from April 7 to June 19, 2014 using equal-width-increment procedures and clean sample processing methods [27, 32, 33]. Reference and rural sites were sampled weekly during the last four weeks of the sampling period. The water-sampling period culminated with a survey of ecological conditions at all sites. Samples for nutrients were analyzed by the USGS National Water Quality Laboratory in Denver, CO. Concentrations of TP were determined by colorimetry according to EPA method 365.1 [34]. Dissolved ammonia (NH₃), nitrite (NO₂), and orthophosphate (PO₄) concentrations [35] and dissolved nitrate-plus-nitrite (NO₃ + NO₂) concentrations [36] were determined by colorimetric analyses. To provide information on potential nitrate sources, nitrogen (δN^{15}) and oxygen (δO^{18}) isotope water samples were collected during the 3rd and 9th week of sampling and analyzed by continuous-flow isotope-ratio mass spectrometry at the USGS Reston Stable Isotope Laboratory in Reston, VA, as described [37]. Nutrient concentration and streamflow data are available as a USGS data release [38] and streamflow data are downloadable from the USGS National Water Information System [39].

2.4. Quality assurance

Quality control/quality assurance samples included field blanks, matrix spikes, and replicates [32, 33, 40, 41]. Dissolved NH₃ was detected in 11 of the 27 (41 %) field blanks

at levels (range: 0.010–0.015 mg/L) near the laboratory reporting level (LRL = 0.010 mg/L). Accordingly, the LRL for environmental NH₃ data was raised to the 90th percentile of field blank concentrations (<0.015 mg/L at 94 percent confidence) prior to data analysis. Total organic nitrogen (TON) concentrations were calculated as the difference between TN and dissolved inorganic nitrogen (NH₃ and NO₃ + NO₂) concentrations. Zero substitution for censored NH₃ or NO₃ + NO₂ data created a potential overestimation (median = 5%) of TON concentrations.

2.5. Statistical analyses

The statistical significance ($\alpha = 0.05$) of differences in nutrient concentrations among various groups of stream sites (e.g., urban centers, site type) was tested by nonparametric, univariate statistical analysis of individual nutrient species concentrations from all weekly sampling events [42, 43, 44], using routines in the ‘**coin**’ [45, 46] R package (<https://cran.r-project.org/index.html>) for permutation one-way analysis of variance (ANOVA) followed by nonparametric Wilcoxon pairwise multiple comparison (with p-value adjustment for multiple testing [47]) to identify statistically-different groups (Table S2). Permutation-based hypothesis tests are not influenced by data distribution or symmetry and generally have more power than the Kruskal-Wallis nonparametric test on ranks [48, 49, 50, 51]. Significant ($\alpha = 0.05$) monotonic associations between statistical metrics (median, 25th and 75th percentiles) of the individual nutrients and land-use, soil, hydrologic, and geologic characteristics (Tables S3 and S4) were assessed by nonparametric Spearman rho correlation analysis [44] (Table S5).

Statistically significant site groupings of median nutrient concentrations were identified using nonparametric, constrained, divisive cluster analysis (LINKTREE routine in PRIMER 7.0; [52, 53, 54]). The Global R test statistic (R) computed by the Analysis of Similarity (ANOSIM; PRIMER 7.0 routine, 100 permutations, minimum p -value = 0.001; alpha level = 0.05) method [52, 53, 54] was used to determine differences in the pattern of multiple nutrient species and metrics for the 76 sites grouped by selected categorical factors [5 urban centers, site type (reference, rural, CAFO, and urban); 6 Land-Use classes [55]] (Table S6).

Significant relations between the pattern (resemblance matrix) of nutrient metrics and the corresponding patterns of land-use and hydrological characteristics of each site were assessed using the nonparametric RELATE routine of PRIMER 7.0 (Table S6). Watershed-scale land-cover characteristics were aggregated using a combination of medium-resolution (1:100,000-scale) hydrography from the National Hydrography Version 2 Dataset (NHDPlus) and selected Geographic Information System (GIS) parameters summarized for site watersheds [56]. Wet deposition data from annual grids generated by the National Atmospheric Deposition Program

[57] were used as input to the USGS tool “FeatureStatisticsToTable” [58] to calculate the mean wet deposition of ammonia for each watershed.

Watershed characteristics that correlated significantly with in-stream nutrients in bivariate (Tables S5, S7, and S8) and/or multivariate space (Table S6) were candidate input variables for multiple logistic regression [MLR; 43, 44] analysis to determine which combinations of watershed characteristics best predicted the likelihood that median TN and TP exceeded the EPA recommended guidelines of 0.69 and 0.037 mg/L, respectively (Table 3; Table S9). Low Wald and high Variance Inflation Factor (VIF) statistics were used to identify and remove redundant co-variables from the model (Table S9). The Pearson Chi-square (minimize p-values), Likelihood Ratio test (minimize p-values), and Hosmer-Lemeshow (maximize p-values) statistics were used to evaluate equation goodness of fit. For each site, streamflow at the time of sampling was normalized to stream basin drainage area to compute unit area discharge (cubic meters per second per square kilometer or $\text{m}^3/\text{s}\cdot\text{km}^2$) for direct comparison of streamflow among sites of differing basin size. Basin drainage areas in this study ranged two orders of magnitude, from 5 to 681 km^2 (Table S1). Tables S1, S2, S3, S4, S5, S6, S7, S8, S9 are provided in the file Supplementary_Information.xlsx and are downloadable from USGS ScienceBase [38].

3. Results and discussion

3.1. Spatial variation in stream nutrients

Permutation one-way ANOVA results indicated that CAFO and Washington, DC urban streams had statistically higher TN and $\text{NO}_3 + \text{NO}_2$ concentrations than streams in other urban-center, rural, or reference groups (Fig. 2A and C; Table 2 and Table S2). CAFO sites also had statistically higher NH_3 concentrations than all other groups, followed by sites in Atlanta (Table S2). Reference sites had mean concentrations of TN, $\text{NO}_3 + \text{NO}_2$, and NH_3 that were consistently and statistically lower than all other groups. Mean $\text{NO}_3 + \text{NO}_2$ concentrations in minimally developed, rural watersheds were lower than in urban and CAFO groups but higher than in reference group (Fig. 2A). However, mean concentrations of TN and NH_3 in the rural group were similar to and greater than, respectively, some urban center groups (Fig. 2C; Table S2). Of the nitrogen species, concentrations of TON appeared to be the least sensitive to watershed land-use differences (Table S2).

The overall range in TN concentrations in the SESQA stream sites was 0.09–7.17 mg/L during the study period (April 7 to June 19, 2014) (Table 1). The median TN concentration of the reference group was 0.28 mg/L, consistent with the predicted background TN concentration of 0.25 mg/L modeled by Smith et al [11]. The 25th percentile of TN concentrations measured at all SESQA sites was 0.63 mg/L, within the range of corresponding EPA guideline conditions of 0.62 and 0.69 mg/

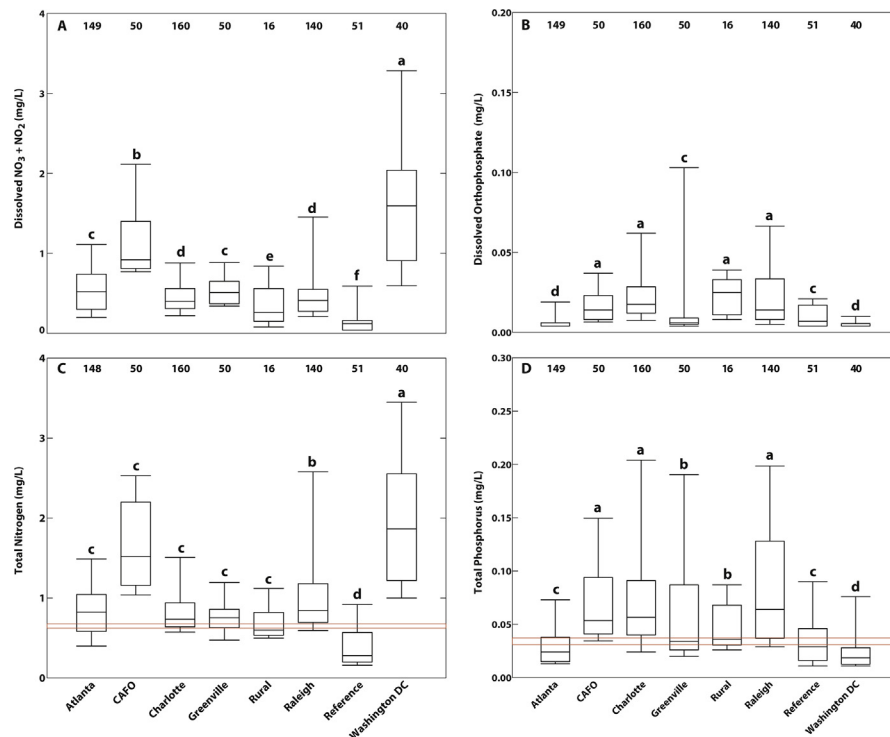


Fig. 2. Truncated (10th to 90th percentile) boxplots of (A) dissolved NO₃+NO₂, (B) dissolved orthophosphate (PO₄), (C) total nitrogen (TN), and (D) total phosphorus (TP) concentrations at sites grouped by urban center, confined animal feeding operations (CAFO), rural, and reference sites. Numbers at top of the plots indicate the number of samples analyzed. Red box represents existing guidelines for concentrations of total nitrogen of 0.69 and 0.62 mg/L (C) and total phosphorus of 0.037 and 0.030 mg/L (D) in streams in nutrient Ecoregion IX and Piedmont Level III Ecoregion, respectively [10]. Within each plot, sites with different lower-case letters are statistically different ($\alpha = 0.05$).

L (Fig. 2C; Table 2). Urban center and CAFO groups had median TN concentrations above (Greenville, 0.75 mg/L; Charlotte, 0.74 mg/L) to well above (Washington, DC, 1.87 mg/L; CAFO, 1.52 mg/L; Raleigh, 0.85 mg/L; Atlanta, 0.83 mg/L) guideline levels (Fig. 2C; Table 2). Inorganic species (NO₃ + NO₂) composed the majority of the TN concentrations at most of the SESQA sites, especially at the urban and CAFO sites (Fig. 2A and C). Thus, median NO₃ + NO₂ concentrations were strongly correlated (Spearman rho (ρ) = 0.911, p -value < 0.001) to total nitrogen concentrations. Overall, NO₃ + NO₂ concentrations ranged from <0.04 to 5.73 mg/L, well below the EPA drinking water standard of 10 mg/L and well below the 41.8 mg/L maximum observed in the Midwestern Stream Quality Assessment in 2013 [59].

Two of 13 (15%) reference and 1 of 4 (25%) rural sites had median TN concentrations at or above the recommended EPA guidelines of 0.69 mg/L (nutrient Ecoregion IX) and 0.62 mg/L (Piedmont Level III Ecoregion): NC_Morgan, Cross (0.69 mg/L), NC_Cane (1.07 mg/L), and VA_Mountain (1.06 mg/L) (Fig. 1; Table 1 and Table S3). In contrast,

Table 2. Summary statistics of concentrations for nutrient data collected at wadeable stream sites in Piedmont ecoregion of the Southeastern United States from April 7 to June 19, 2014 , grouped by location relative to urban center, confined animal feeding operation (CAFO), rural, and reference conditions [mg/L, milligrams per liter; 25th P, 25th percentile; 75th P, 75th percentile; min, minimum; max, maximum].

| Site type or Urban Center | Total nitrogen (mg/L) | | | | | Dissolved ammonia (mg/L) | | | | | Dissolved nitrate plus nitrite (mg/L) | | | | | Total phosphorus (mg/L) | | | | | Dissolved orthophosphate (mg/L) | | | | |
|--------------------------------------|-----------------------|--------|--------|--------|------|--------------------------|--------|--------|--------|-------|---------------------------------------|--------|--------|--------|------|-------------------------|--------|--------|--------|-------|---------------------------------|--------|--------|--------|-------|
| | Min | 25th P | Median | 75th P | Max | Min | 25th P | Median | 75th P | Max | Min | 25th P | Median | 75th P | Max | Min | 25th P | Median | 75th P | Max | Min | 25th P | Median | 75th P | Max |
| Agricultural CAFO | 0.92 | 1.16 | 1.52 | 2.20 | 7.17 | <0.015 | 0.040 | 0.058 | 0.089 | 0.870 | 0.56 | 0.81 | 0.92 | 1.40 | 2.34 | 0.023 | 0.041 | 0.054 | 0.094 | 2.63 | 0.005 | 0.008 | 0.014 | 0.023 | 0.373 |
| Atlanta, Ga., Urban Center | 0.28 | 0.59 | 0.83 | 1.05 | 2.79 | <0.015 | 0.025 | 0.038 | 0.054 | 1.79 | 0.08 | 0.30 | 0.52 | 0.74 | 2.31 | 0.009 | 0.015 | 0.024 | 0.038 | 0.227 | <0.004 | <0.004 | <0.004 | 0.006 | 0.082 |
| Charlotte, N.C., Urban Center | 0.21 | 0.64 | 0.74 | 0.94 | 5.98 | <0.015 | <0.015 | 0.017 | 0.036 | 0.316 | <0.04 | 0.31 | 0.40 | 0.56 | 5.61 | 0.013 | 0.040 | 0.057 | 0.091 | 1.04 | <0.004 | 0.012 | 0.018 | 0.029 | 0.784 |
| Greenville, S.C., Urban Center | 0.44 | 0.63 | 0.75 | 0.86 | 2.03 | <0.015 | <0.015 | 0.018 | 0.027 | 0.085 | 0.28 | 0.37 | 0.51 | 0.65 | 1.33 | 0.015 | 0.026 | 0.034 | 0.087 | 0.581 | <0.004 | 0.005 | 0.006 | 0.009 | 0.196 |
| Raleigh, N.C. Urban Center | 0.38 | 0.70 | 0.85 | 1.18 | 1.51 | <0.015 | <0.015 | 0.031 | 0.054 | 0.044 | <0.04 | 0.28 | 0.41 | 0.55 | 0.88 | 0.021 | 0.037 | 0.064 | 0.128 | 0.094 | <0.004 | 0.008 | 0.014 | 0.034 | 0.040 |
| Washington, D.C. Urban Center | 0.59 | 1.22 | 1.87 | 2.56 | 5.57 | <0.015 | <0.015 | <0.015 | 0.022 | 0.240 | 0.35 | 0.91 | 1.60 | 2.04 | 3.68 | 0.006 | 0.013 | 0.019 | 0.028 | 1.06 | <0.004 | <0.004 | <0.004 | 0.006 | 0.028 |
| Hybrid-rural | 0.41 | 0.54 | 0.60 | 0.82 | 6.66 | <0.015 | 0.021 | 0.029 | 0.033 | 0.253 | 0.06 | 0.15 | 0.26 | 0.56 | 5.73 | 0.024 | 0.031 | 0.036 | 0.068 | 1.05 | 0.008 | 0.011 | 0.025 | 0.033 | 1.06 |
| Reference | 0.09 | 0.20 | 0.28 | 0.57 | 1.86 | <0.015 | <0.015 | <0.015 | <0.015 | 0.041 | <0.04 | <0.04 | 0.12 | 0.16 | 0.92 | 0.008 | 0.016 | 0.029 | 0.046 | 0.139 | <0.004 | <0.004 | 0.007 | 0.017 | 0.034 |

all sampled streams in the Washington, DC and Raleigh urban centers and in CAFO watersheds had median TN concentrations above guidelines. About 73%, 80%, and 88% of the sampled streams in Atlanta, Greenville, and Charlotte, respectively, had median TN concentrations above these guideline levels.

Permutation one-way ANOVA results indicated that CAFO, Charlotte, and Raleigh sites had statistically greater mean TP concentrations than all other groups (Figs. 2D and 3; Table 2 and Table S2). TP concentrations in Greenville and in rural sites were

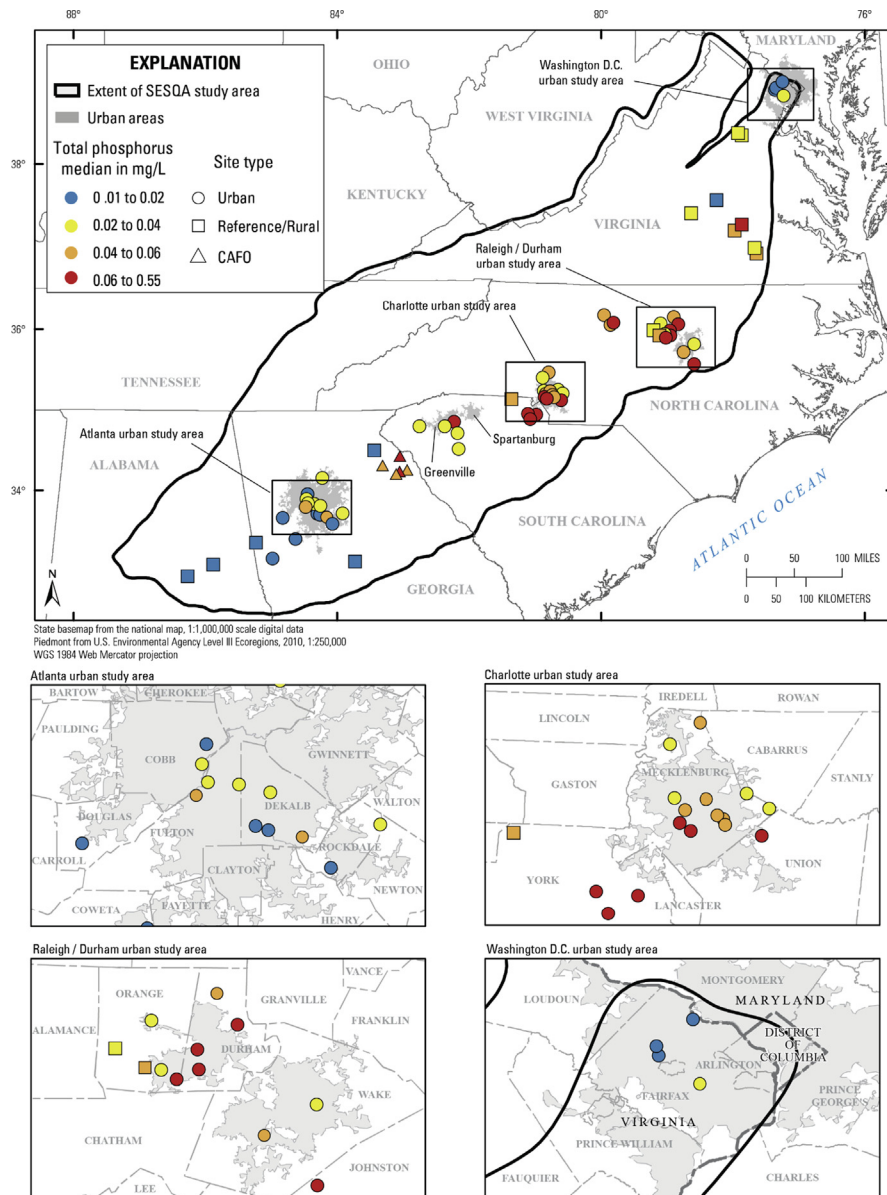


Fig. 3. Spatial distribution of median total phosphorus (TP) concentrations in the SESQA study area, April–June 2014. Orange and red symbols indicate median concentrations above existing guidelines [respectively, 10, 11].

significantly greater than for Atlanta, Washington, DC, or reference sites (Table S2). Washington, DC sites had significantly lower TP concentrations than all other groups including reference. Dissolved PO₄ concentrations were significantly correlated to TP concentrations ($\rho = 0.875$, $p < 0.001$) in the SESQA study area. However, dissolved PO₄ did not consistently explain the elevated TP in urban centers, suggesting particle-bound phosphorus was also an important contributor (Fig. 2B and D; Table 1 and Table S3). CAFO and Charlotte and Raleigh urban site groups had statistically greater PO₄ concentrations than all other groups, except rural sites.

The overall range in TP concentrations in the SESQA study area was 0.006–2.63 mg/L (Table 2). The median TP concentration of reference sites was 0.029 mg/L, which was lower than the predicted background TP of 0.045 mg/L [11] and the EPA recommended guidelines of 0.030 (nutrient Ecoregion IX) and 0.037 mg/L (Piedmont Level III Ecoregion) mg/L [10] (Fig. 2D; Tables 1 and 2). Charlotte and Raleigh urban site groups and CAFO sites had median TP concentrations above the EPA guidelines (0.057, 0.064, and 0.054 mg/L, respectively; Fig. 3; Table 2). Even the 25th percentile TP concentrations for CAFO sites (0.041 mg/L) exceeded the EPA guideline values. In contrast, median, 25th percentile, and 75th percentile TP concentrations of the Washington, DC group were all below the guideline values (0.019, 0.013, and 0.028 mg/L, respectively). Reference and Atlanta groups had 75th percentile TP concentrations within the range of predicted background TP concentration in the Piedmont Level III Ecoregion (0.045 mg/L) [11].

Spatially, only 2 of the 15 (13%) Atlanta and none of the Washington DC stream sites had median TP concentrations exceeding the EPA guideline value of 0.037 mg/L (Fig. 3; Table S3). In contrast, 6 of 17 (35%) reference and rural stream sites exceeded the guideline. Stream sites within Charlotte, Raleigh, and Greenville areas had 88%, 71%, and 40% of median TP concentrations above the EPA guideline, respectively.

3.2. In-stream nutrient concentrations and watershed metrics

TP, TN, and NO₃ + NO₂ concentration statistical metrics (median, 25th percentile, 75th percentile) exhibited strong ($p < 0.004$), positive correlations to urban development, changes in impervious surface, and housing-density metrics (Tables S4 and S5). Additionally, TN and NO₃ + NO₂ metrics had weaker ($p < 0.05$), positive correlations to the change in population density and generally negative correlations with forested land class (Tables S4 and S5). TP and PO₄ concentration statistical metrics did not correlate as strongly as nitrogen with respect to summary GIS classes; however, phosphorus metrics tended to have a stronger negative correlation with forested land class ($p < 0.007$). The weaker correlation between phosphorus metrics and urban land cover variables compared to nitrogen metrics, may reflect differences in phosphorus management strategies between urban centers [16, 20, 21], with

storm-water and particulate-management strategies expected to affect particulate-dominated TP loads more than dissolved-species-dominated TN loads.

The relation between site-specific median, 25th percentile, and 75th percentile nitrogen species concentrations and the estimated 2014 mean wet deposition of NH₃ in kilograms per hectare of watershed was assessed by Spearman rho correlation (Table S7). Strongest, significant, positive correlations were identified between median, 25th percentile, and 75th percentile TON concentrations and mean NH₃ wet deposition across all 76 study sites ($\rho = 0.419$, $p < 0.001$; $\rho = 0.491$, $p < 0.001$; and $\rho = 0.343$, $p = 0.002$, respectively). A statistically significant, negative correlation existed between the 25th percentile NH₃ concentrations in streams and NH₃ wet deposition ($\rho = -0.282$, $p = 0.013$) across all study sites. Positive correlations of TON and NH₃ concentrations with NH₃ wet deposition were observed only in the reference and rural site groups for median and 25th percentile TON concentrations ($\rho = 0.691$, $p = 0.002$; $\rho = 0.699$, $p = 0.002$, respectively) and for median, 25th percentile, and 75th percentile TN concentrations ($\rho = 0.623$, $p = 0.007$; $\rho = 0.625$, $p = 0.007$, and $\rho = 0.669$, $p = 0.017$, respectively). Past studies in forested, headwater streams have reported increased fluxes of soil organic-nitrogen in response to increased watershed inorganic-nitrogen deposition, with NH₃ more strongly adsorbed on soil particle or mineral surfaces than NO₃ [60, 61, 62].

Consistent with the multiple comparison results, multivariate analysis of similarity (ANOSIM) indicated that the strongest difference among the pattern of concentrations of combined nutrient (N and P) species existed between urban centers ($R = 0.257$, $p = 0.001$). When evaluating N and P species separately, a stronger relation was observed between nitrogen species ($R = 0.301$, $p = 0.001$) than for phosphorus ($R = 0.133$, $p = 0.003$) (Table S6). The RELATE multi-variate analysis indicated that a distance matrix based on individual GIS characteristics better explained the variation in the nutrient concentrations matrix than matrices based on grouped GIS characteristics. A reduced GIS dataset (not including U.S. Department of Agriculture 1997 National Resources Inventory [63], PRISM monthly rainfall, U.S. Army Corps of Engineers National Inventory of Dams, and NHD stream characteristic data) provided the strongest relation to all nutrient species data. Therefore, GIS characteristics within the reduced GIS dataset were assessed by regression analysis.

Multiple logistic regression analysis was utilized to test the hypothesis that increased watershed urbanization was associated with increased likelihood of nutrient concentrations above concern levels. CAFO sites were excluded from the analysis. Due to the inherent autocorrelation in NLCD variables, the sum of developed land (open, low, medium, and high density) was selected as the primary urbanization metric. The MLR equation that best predicted the likelihood of median TN to be above the 0.69 mg/L EPA guideline included NLCD 2011 developed land, number of 2002 National Pollutant Discharge Elimination System (NPDES) permitted major

Table 3. Diagnostics and coefficients for best multiple logistic regression (MLR) models for the likelihood of median total nitrogen and total phosphorus concentrations exceeding USEPA recommended nutrient guidelines at 71 Wadeable Urban, Rural, and Reference Stream sites in Piedmont ecoregion of the Southeastern United States from April 7 to June 19, 2014.

| ^a Analyzed nutrient metric relative to above or below EPA recommended guideline | ^b Specificity | ^c Sensitivity | ^d -2*LOG (Likelihood) | ^e Hosmer-Lemeshow | | ^f Pearson chi-squared | | ^g Likelihood ratio test | | Constant | ^h Land-use, soil, and hydrologic variables (transformed by natural logarithm of (value + 1)) | | | | | |
|--|--------------------------|--------------------------|----------------------------------|------------------------------|---------|----------------------------------|---------|------------------------------------|---------|----------|---|---|-------------------------------|-------------------------|---------------------------|--|
| | | | | Statistic | p-value | Statistic | p-value | Statistic | p-value | | NLCD 2011 developed land (Open, Low, Med., High) | 2002 Number of NPDES permitted major facilities | STATSGO Soil hydrologic group | Depth to water table CD | Topographic wetness index | |
| Median total nitrogen | 76 | 89 | 42.6 | 4.250 | 0.834 | 42.596 | 0.986 | 49.571 | <0.001 | 44.341 | 1.310 | 24.787 | -1.037 | -25.704 | - | |
| Median total phosphorus | 83 | 78 | 77.7 | 15.302 | 0.054 | 75.997 | 0.253 | 20.753 | <0.001 | -50.289 | 0.444 | 2.299 | - | - | 20.253 | |

^a EPA guidelines for total nitrogen and phosphorus in streams in the aggregated nutrient ecoregion IX are 0.69 mg/L and 0.037 mg/L, respectively (U.S. Environmental Protection Agency, 2000).

^b Specificity is the ability of the regression equation to predictive streams with nutrient metric below the EPA recommended guideline and value is the percent of correct predictions.

^c Sensitivity is the ability of the regression equation to predictive streams with nutrient metric above the EPA recommended guidelines and value is the percentage of correct predictions.

^d -2*LOG (Likelihood) is the goodness of fit test of observed versus predicted values of the dependent variable. The smaller the statistic the better the fit of the model.

^e Hosmer-Lemeshow (p-value) tests whether the equation fits the data by comparing the number of individuals with each outcome (above or below) with the number expected (large p-values indicate good fit between equation and data).

^f Pearson Chi-Squared (p-value) tests how well the equation fits the data by summing the squares of the Pearson residuals (smaller Pearson Chi-squared statistics indicates good agreement).

^g Likelihood Ratio Test (p-value) tests how well the equation fits the data by summing the squares of the deviance residuals (low p-values indicates good fit). The larger the ratio the better explanatory power of the independent variables for the given dependent variable.

wastewater facilities, State Soil Geographic (STATSGO) database soil hydrologic group CD, and the depth to water table (Table 3; Tables S4 and S9) [64]. The 4-variable TN MLR equation had relatively good fit with the test data and was able to predict streams with median TN above 0.69 mg/L (sensitivity) about 89% of the time. The same model, however, was less specific in predicting streams with median TN below 0.69 mg/L, having a specificity of 76%. Based on the MLR, median TN in streams were likely to be elevated above the EPA guideline in watersheds with greater urban development and numbers of major NPDES-permitted facilities. Additionally, lower percentages of soil hydrologic group CD (sandy clay to clay loam with high runoff potential) and shallower depths to water table also predicted higher median TN. Stream TN was predominantly in the form of dissolved $\text{NO}_3 + \text{NO}_2$ at the SESQA urban sites. Hydrology is a recognized control on $\text{NO}_3 + \text{NO}_2$ export in headwater agricultural streams [59, 65] and landscape hydrogeologic characteristics are recognized drivers of nitrogen removal by denitrification [66, 67, 68].

Additional insight into in-stream nitrogen controls was sought from NO_3 stable isotope results (Fig. 4) and concentration-flow correlations (Fig. 5). Samples from the 3rd (April) and 9th (June) sampling weeks were analyzed for δO^{18} and δN^{15} to evaluate potential sources of stream NO_3 [69, 70, 71] (Fig. 4). NO_3 stable isotope data were consistent with subsurface transport of TN in the soil prior to release to the stream as $\text{NO}_3 + \text{NO}_2$. With the exception of the single fertilizer-attributable June sample, the majority of samples (including reference) plotted in the region ascribed to soil nitrogen rather than to atmospheric or fertilizer sources [69, 70, 71]. Also consistent with soil processing and subsurface delivery, $\text{NO}_3 + \text{NO}_2$ concentrations across all reference and urban stream sites in the SESQA study area were generally higher during low-flow conditions, even for sites without permitted discharges (Fig. 5A–F). Notably, significant negative correlations between unit-area

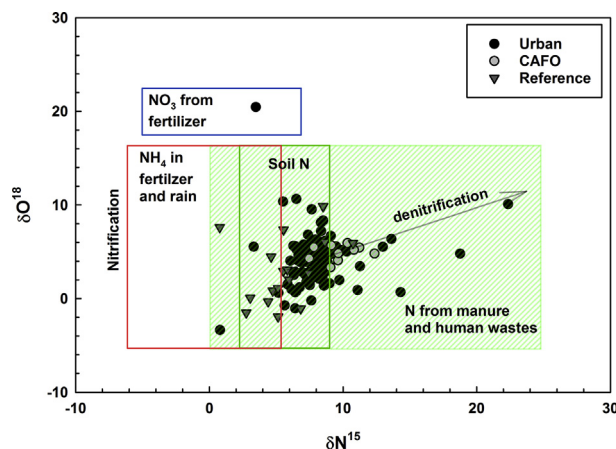


Fig. 4. Ranges of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of nitrate overlain with isotopic values in stream-water samples from urban, confined animal feeding operation (CAFO), and reference sites in the SESQA study area in April and June 2014 [70, 71].

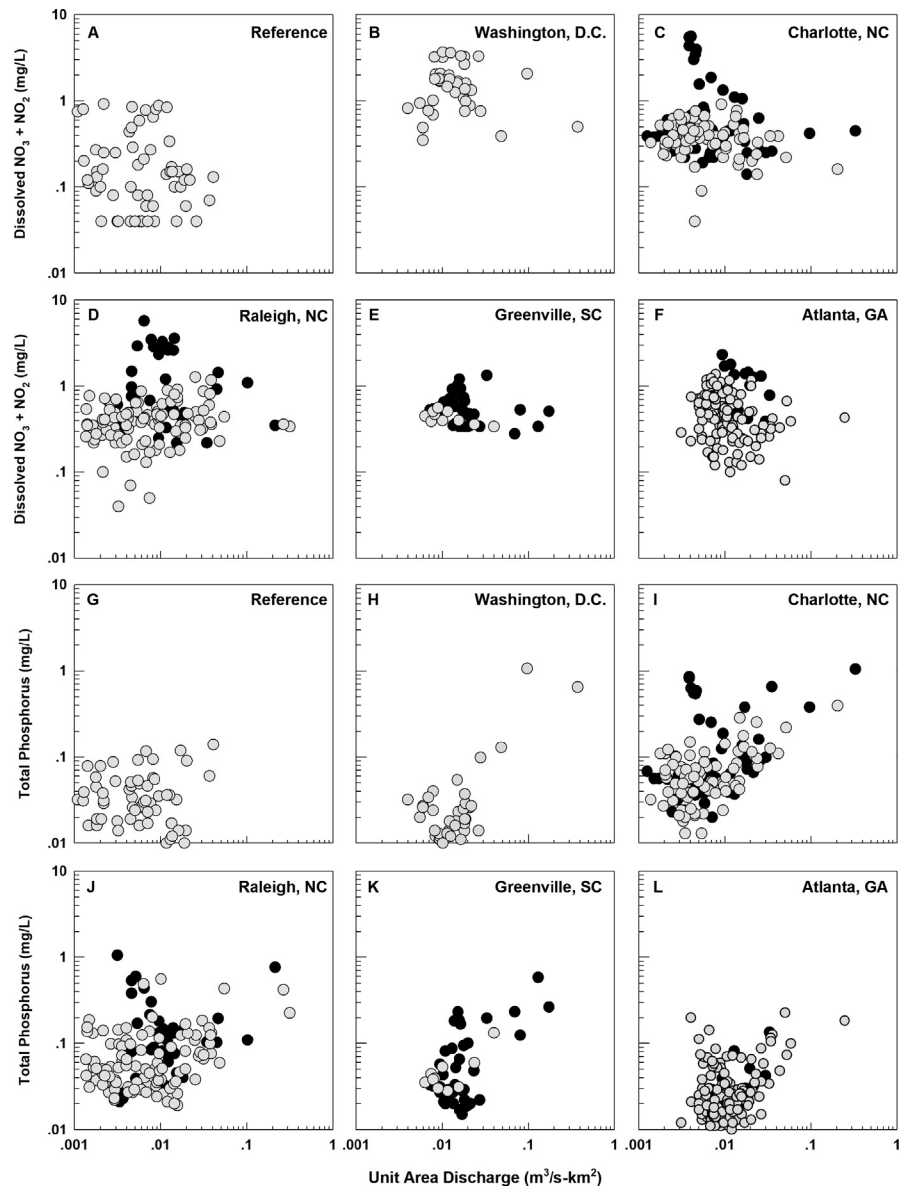


Fig. 5. Scatter plots of dissolved $\text{NO}_3 + \text{NO}_2$ sample concentrations at (A) reference, (B) Greenville, (C) Raleigh, (D) Charlotte, (E) Atlanta, and (F) Washington DC sites and total phosphorus sample concentrations at (G) reference, (H) Greenville, (I) Raleigh, (J) Charlotte, (K) Atlanta, and (L) Washington DC sites, with (black dots) and without (grey dots) National Pollutant Discharge Elimination System (NPDES) permitted wastewater discharges, versus unit area discharge ($\text{m}^3/\text{s}\cdot\text{km}^2$) from April–June 2014.

stream discharge and $\text{NO}_3 + \text{NO}_2$ concentrations were observed in streams in both Atlanta with no permitted discharges ($\rho = -0.332$, $p = 0.0001$) and in Greenville with permitted discharges ($\rho = -0.372$, $p = 0.018$) (Table S8). Based on concentration-flow correlations, stable isotopes, and MLR analyses, the increased $\text{NO}_3 + \text{NO}_2$ concentrations observed at reference streams during low-flow conditions is most readily attributed to shallow groundwater discharge, with the potential

for additional groundwater contributions to urban-stream $\text{NO}_3 + \text{NO}_2$ concentrations under low-flow conditions due to subsurface wastewater infrastructure failure.

A 3-variable median TP MLR equation also included NLCD 2011 developed land, number of 2002 NPDES-permitted major facilities, and a hydrologic metric (topographic wetness index) (Table 3; Table S9). The median TP MRL had a poorer fit than the median TN MLR, and was able to predict streams that had median TP above the 0.037 mg/L guideline about 78% of the time. The TP MLR model, however, was more specific in predicting streams with median TP below the guideline, having a specificity of 83%. The MLR analysis suggests that median TP concentrations in streams were likely to be above EPA guidelines in watersheds with greater urban development, more point-source discharges, and a higher topographic wetness index (the natural logarithm of the upslope contributing area per unit contour length (m) divided by the tangent of the local slope). A higher topographic wetness index indicates an increased potential for overland flow. Elevated median TP concentrations at SESQA stream sites were also correlated with greater soil clay content, available water capacity of soil, and Horton overland flow and with reduced permeability. These individual correlations suggest that the delivery of TP to streams during storm runoff may be important.

Streams in the Greenville, Raleigh, Charlotte, and Washington, DC, urban centers tended to exhibit greater variability in flow and TP than reference sites, while TP in the Atlanta streams were observed within the range of the reference samples under most flow conditions (Fig. 5G–L; Table S8). A bimodal pattern of higher TP concentrations versus discharge was typically exhibited in urban streams with permitted discharges, whereby higher TP concentrations were observed during low-flow conditions, indicating point-source influence, while higher TP concentrations were also observed during higher flow conditions, consistent with non-point source, storm-runoff influences (e.g., Raleigh, and Charlotte streams with permitted discharges; Fig. 5G–L). Significant positive correlations of unit-area discharge and TP concentrations were observed in streams in Washington, DC, Atlanta, and Charlotte, regardless of the presence or absence of permitted discharges (Table S8). Together, concentration-flow relations and MLR analyses are consistent with the importance of point-source drivers of TP concentrations under low-flow conditions and increasing landscape contributions under higher flow conditions.

4. Conclusions

Dissolved $\text{NO}_3 + \text{NO}_2$ concentrations dominated TN and generally exceeded EPA guidelines and reference conditions in streams draining poultry CAFO or urban centers. Urban land use, NPDES permitted major wastewater facilities, and soil infiltration metrics best predicted TN exceedances. TP exceedances of EPA guidelines and

reference-watershed conditions were common in CAFO-influenced streams and in urban watersheds without particulate-phosphorus controls (Greenville, Charlotte, Raleigh). Urban land use, NPDES permitted major wastewater facilities, and surface runoff metrics best predicted TP exceedances. Concentration-flow relations and regression results indicate the importance of point-source drivers of TP concentrations under low-flow conditions and increasing landscape contributions under higher flow conditions. The results illustrate the importance of increased regional monitoring of in-stream nutrient condition and the potential benefits of watershed nutrient controls in the highly-urbanized Piedmont Ecoregion.

Declarations

Author contribution statement

Celeste Journey, Peter Van Metre, Ian Waite, Jimmy Clark, Daniel Button, Naomi Nakagaki, Sharon Qi, Mark Munn, Paul Bradley: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

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The authors declare no conflict of interest.

Additional information

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