

RESEARCH ARTICLE

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Key Points:

- Groundwater was the dominant source of nitrified N to streams in low-gradient watersheds
- However, despite flat topography, precipitation was a measurable source of stream nitrate
- N delivery flow paths varied among adjacent watersheds with similar characteristics

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Dual nitrate isotopes clarify the role of biological processing and hydrologic flow paths on nitrogen cycling in subtropical low-gradient watersheds

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Abstract Nitrogen (N) is an important nutrient as it often limits productivity but in excess can impair water quality. Most studies on watershed N cycling have occurred in upland forested catchments where snowmelt dominates N export; fewer studies have focused on low-relief watersheds that lack snow. We examined watershed N cycling in three adjacent, low-relief watersheds in the Upper Coastal Plain of the southeastern United States to better understand the role of hydrological flow paths and biological transformations of N at the watershed scale. Groundwater was the dominant source of nitrified N to stream water in two of the three watersheds, while atmospheric deposition comprised 28% of stream water nitrate in one watershed. The greater atmospheric contribution may have been due to the larger stream channel area relative to total watershed area or the dominance of shallow subsurface flow paths contributing to stream flow in this watershed. There was a positive relationship between temperature and stream water ammonium concentrations and a negative relationship between temperature and stream water nitrate concentrations in each watershed suggesting that N cycling processes (i.e., nitrification and denitrification) varied seasonally. However, there were no clear patterns in the importance of denitrification in different water pools possibly because a variety of factors (i.e., assimilatory uptake, dissimilatory uptake, and mixing) affected nitrate concentrations. Together, these results highlight the hydrological and biological controls on N cycling in low-gradient watersheds and variability in N delivery flow paths among adjacent watersheds with similar physical characteristics.

1. Introduction

Nitrogen (N) is a limiting nutrient in many temperate forests [Vitousek and Howarth, 1991] and N cycling within a forested watershed can be controlled by several factors, including hydrology [e.g., Cirimo and McDonnell, 1997; Creed and Band, 1998] and biological processes [e.g., Groffman and Tiedje, 1989; Lovett et al., 2002]. Stream water nitrate is an integrative measure of N cycling at the watershed scale. Stream water nitrate concentrations can vary seasonally and on an event basis (i.e., snowmelt) [Mitchell et al., 1996; Kurian et al., 2013] and can be affected by anthropogenic activities such as timber harvest (without Best Management Practices) [Likens et al., 1970; McBroom et al., 2008], land use history [Goodale and Aber, 2001], or elevated N deposition [Aber et al., 1989; Stoddard, 1994]. However, in-stream N cycling can also be an important control on nitrate concentrations [Mulholland and Hill, 1997; Mulholland, 2004].

Many studies on N dynamics have occurred in upland temperate watersheds [e.g., Bormann et al., 1977; Henderson et al., 1978; Brookshire et al., 2011] and in watersheds with a significant snowpack [e.g., Groffman et al., 1999; Sebestyen et al., 2008]. In these watersheds, nitrate delivery to streams occurs primarily during snowmelt or when uptake by vegetation is low [Ohte et al., 2004; Barnes et al., 2008; Buda and DeWalle, 2009]. Less is known on N cycling in low-relief, groundwater-dominated watersheds with little snowfall and long growing seasons despite the prevalence of these watersheds across the globe (e.g., southeastern United States and coastal South America). Quantifying the dynamics and fate of N in subtropical, low-gradient watersheds is needed to understand the productivity of these ecosystems and the effects of exported N on downstream water bodies, especially coastal eutrophication [Vitousek et al., 1997; Rabalais et al., 2002].

The stable isotopes of nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$) can provide valuable information on nitrate sources and cycling within a watershed. In undisturbed forested watersheds, the two primary sources of nitrate are atmospheric deposition and nitrification. These sources of nitrate have distinct values of $\delta^{18}\text{O}_{\text{NO}_3}$ when determined from the denitrifier method [Casciotti *et al.*, 2002], with $\delta^{18}\text{O}_{\text{NO}_3}$ of atmospheric nitrate enriched ($> +60\text{‰}$) relative to $\delta^{18}\text{O}_{\text{NO}_3}$ from nitrification (-10 to $+10\text{‰}$) [Kendall *et al.*, 2007]. Measurements of $\delta^{18}\text{O}_{\text{NO}_3}$ in forested streams show that nitrification is the primary source of stream water nitrate [Burns and Kendall, 2002; Campbell *et al.*, 2002; Hales *et al.*, 2007]. However, the source of $\delta^{18}\text{O}_{\text{NO}_3}$ in stream water can vary seasonally and during precipitation and resulting channel stormflow events [Ohte *et al.*, 2004; Barnes *et al.*, 2008; Buda and DeWalle, 2009; Wexler *et al.*, 2014]. For instance, atmospheric (i.e., unprocessed) nitrate can comprise a large percentage of exported nitrate during snowmelt [Sebestyen *et al.*, 2008]. In subtropical, low-relief watersheds that do not receive significant snowfall, it is unclear if atmospheric nitrate is a measurable source of stream water nitrate.

Dual stable isotopes of nitrate can also be used to assess the occurrence and importance of denitrification in streams, soils, and groundwater [Aravena and Robertson, 1998; Sebiló *et al.*, 2003; Chen *et al.*, 2009; Lohse *et al.*, 2013]. Denitrification is an anaerobic respiration pathway that involves the conversion of nitrate to N_2 under reducing conditions and the presence of organic carbon. It is an important component of the N cycle and can affect water quality if nitrate is removed from the watershed before being transported downstream [Hill, 1996]. Denitrifiers preferentially use the light isotopes of nitrate in denitrification, resulting in increasing values of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ in the residual nitrate [Kendall *et al.*, 2007] in a 2:1 to 1:1 ratio [Böttcher *et al.*, 1990; Aravena and Robertson, 1998; Sigman *et al.*, 2005; Granger *et al.*, 2008]. In subtropical, low-relief watersheds with organic-rich riparian zones and warm summer temperatures, denitrification can be an important N removal pathway [Peterjohn and Correll, 1984; Lowrance *et al.*, 2000; Schaefer and Alber, 2007] and this process may be evident in dual nitrate isotope analysis.

Climate and land use change are projected to affect watersheds in the southeastern U.S. [Mulholland *et al.*, 1997; Sun *et al.*, 2008; Kunkel *et al.*, 2013]. For instance, changes in precipitation and stream flow may alter the delivery of N, and warmer temperatures may accelerate biological processes such as denitrification. Quantifying the sources, cycling, and fate of N in low-relief, subtropical watersheds is important in order to understand the hydrological and biological controls on N cycling, and how these controls may change under future climate and land management scenarios and affect downstream water quality.

Here we examined dissolved inorganic N dynamics in three low-relief, groundwater-dominated, forested watersheds with intermittent streams in the Upper Coastal Plain of South Carolina, USA to address the following questions: (1) what are the sources and delivery pathways of stream water nitrate? and (2) is denitrification an important N cycling process in these watersheds? We measured nitrate concentrations and stable isotopes of nitrate in various pools of water (stream water, groundwater, interflow, and throughfall) over a 2 year period. Patterns in ammonium concentrations were also investigated as a source of nitrate via nitrification. We hypothesized that nitrification would be a primary source of stream water nitrate instead of unprocessed nitrate from atmospheric deposition because of the lack of a winter snowpack. We further hypothesized that the dominant pathway of nitrate entry to streams would be via groundwater instead of through shallower, faster flow paths (i.e., overland or shallow subsurface flow) based on previous hydrological studies in these low-relief watersheds [Jackson *et al.*, 2014; Klaus *et al.*, 2015; (E. Du *et al.*, Interflow dynamics on a low relief forested hillslope: Lots of fill, little spill, submitted to *Journal of Hydrology*, 2016)]. Lastly, we hypothesized that denitrification would be a dominant N cycling process due to the warm temperatures and organic-rich soils in the riparian zones.

2. Methods

2.1. Site Description

The three study watersheds (watersheds R, B, and C) are located in the National Environmental Research Park on the Department of Energy's Savannah River Site (SRS), near New Ellenton, South Carolina, USA and are within the Upper Atlantic Coastal Plain physiographic region. The SRS has a humid subtropical climate, a mean annual precipitation of 1225 mm, and a mean annual temperature of 18°C [Kilgo and Blake, 2005]. The three study watersheds are part of the larger Fourmile Branch, which drains to the Savannah River and then to the Atlantic Ocean. Prior to 1951, the area was rural and used for row crop agriculture with largely intact riparian corridors [Kilgo and Blake, 2005]. After 1951, the area was reforested with pine trees by

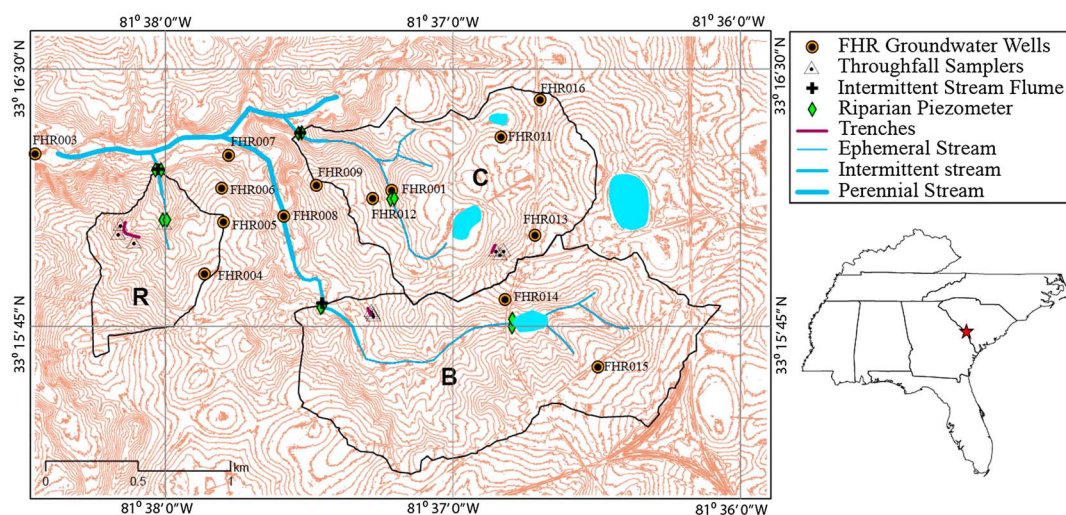


Figure 1. Sampling locations and equipment in the three study watersheds R (45 ha), B (169 ha), and C (117 ha) at the Savannah River Site in South Carolina, USA. Sampling locations in each watershed included an intermittent stream at the watershed outlet, two riparian piezometers near the outlet and two near an upstream site, two to five deep groundwater wells (forest hydrology research wells), three to four throughfall samplers, and an interflow interception trench.

the U.S. Forest Service and has been minimally managed since. Watershed uplands are characterized by gently rolling hills (~2–3% slope), well-drained sandy soils with a loamy to clayey subsoil, and vegetation dominated by pines, including longleaf pine (*Pinus palustris*), loblolly pine (*P. taeda*), and slash pine (*P. elliotii*). The riparian areas are characterized by flat, hummocky, floodplain valleys, organic-rich hydric soils, and mixed hardwood vegetation (primarily sweet gum; *Liquidambar styraciflua*). The first-order streams that drain these watersheds flow intermittently and have indistinct channels due to the flat topography. Stream water has high dissolved organic matter concentrations (i.e., blackwater streams), which is typical of streams in the southeastern U.S. [Meyer, 1990]. The characteristics (i.e., topography, geology, and vegetation) of the three adjacent watersheds are fairly similar, but the watersheds vary in size: watershed R is 45 ha, watershed B is 169 ha, and watershed C is 117 ha. Watersheds B and C have Carolina Bays, which are poorly drained and seasonally wet shallow depressions that are common to the Upper Coastal Plain (Figure 1).

2.2. Field Sampling

To examine N dynamics in these low-relief watersheds, we collected stream water, riparian groundwater (near streams; groundwater was 1.7–2.0 m below the soil surface), groundwater (groundwater was sampled 2.7–43.6 m below the soil surface), interflow water (i.e., shallow subsurface water flowing down the upland hillslopes during storms), and throughfall. The sampling locations in each watershed are shown in Figure 1. Sample collection for chemistry took place from January 2010 to February 2012, and sample collection for stable isotopes of nitrate began in February 2011. Sampling of stream water ended in April/May 2011 when flow ceased and all three streams dried up due to drought conditions at the site. Stream discharge also ceased in watershed B from June 2010 to February 2011 and in watershed C during July 2010.

Stream water samples were collected approximately weekly from the outlet of each watershed. Riparian groundwater was collected approximately monthly from two wells that were located near the outlet of each watershed and two wells that were located near an upstream sampling site (Figure 1). Groundwater (referred to as “deep groundwater” for the remainder of the paper) was sampled approximately monthly from 14 wells that were located across the three watersheds; two wells were within watershed R (FHR004 (groundwater was sampled 13.1 m below the soil surface) and 005 (13.0 m)), two wells were within watershed B (FHR014 (25.6 m) and 015 (37.6 m)), five wells were within watershed C (FHR001 (3.9 m), 011 (25.6 m), 012 (10.6 m), 013 (43.6 m), and 016 (28.6 m)), and five wells were located outside of the three watersheds and downstream of watershed B near Fourmile Branch (FHR003 (2.7 m), 006 (10.5 m), 007 (5.4 m), 008 (6.8 m), and 009 (12.4 m)) (Figure 1). Interflow was collected from trenches constructed on contour within a hillslope in each watershed (trenches were 120 m long in watershed R, 40 m long in watershed B, and 40 m long in watershed C) (Figure 1). During storms, water was collected by multiple drains (11 in watershed R, 5 in watershed B, and

5 in watershed C) which flowed into V notch weir boxes and then into a central collection zone where a composite sample was collected for chemistry. There was one storm in late March 2011 that generated enough interflow in watersheds R and C to collect samples for the duration of the event (~5 days).

Throughfall collectors were installed under the canopy; four throughfall collectors were located in watershed R, and three each were located in watersheds B and C (Figure 1). A 200 cm² funnel collected throughfall into a 3.8 L amber polypropylene bottle, and the water collected in all throughfall collectors per watershed was composited prior to sampling for chemistry. The throughfall collectors were sampled approximately weekly.

All water samples were brought back to the laboratory on ice, filtered (0.7 μm nominal pore size) into polyethylene bottles, and frozen at −20°C until analysis.

2.3. Chemical Analyses

Nitrate concentrations were measured using the cadmium reduction method [American Public Health Association (APHA), 2005] and ammonium concentrations were measured using the phenol hypochlorite method [APHA, 2005] on a SEAL Analytical AA3 autoanalyzer. Stable isotopes of nitrate ($\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$) were measured on all water samples that had nitrate concentrations greater than 14 μg N/L. Some sites do not have any nitrate stable isotope values reported because all samples were below the minimum concentration needed for analysis (i.e., groundwater well FHR015, most riparian well samples from watershed B, and most stream samples from watershed B). Isotope samples were analyzed at the UC Davis Stable Isotope Facility using the denitrifier method with *Pseudomonas aureofaciens* bacteria [Sigman *et al.*, 2001; Casciotti *et al.*, 2002]. A Thermo Finnigan Gas Bench and PreCon trace gas concentration system with a ThermoScientific Delta V Plus isotope-ratio mass spectrometer was used to measure the ratios of ¹⁵N/¹⁴N and ¹⁸O/¹⁶O in N₂O. $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ were determined against three nitrate standards (U.S. Geological Survey (USGS) 32, USGS 34, and USGS 35), and $\delta^{15}\text{N}_{\text{NO}_3}$ was reported relative to N₂ in air, and $\delta^{18}\text{O}_{\text{NO}_3}$ was reported relative to Vienna Standard Mean Ocean Water. The analytical precision was 0.4‰ for $\delta^{15}\text{N}_{\text{NO}_3}$ and 0.8‰ for $\delta^{18}\text{O}_{\text{NO}_3}$.

2.4. Stream Discharge and Precipitation

A two-foot H flume was installed at the outlet of each watershed, and water level was monitored every 15 min using a pressure transducer and an automated water sampler (ISCO 6712, Teledyne ISCO, Lincoln, NE). Stream discharge (in L/s) was calculated based on an established relationship between water level and discharge in the H flume.

To estimate interflow (in L/s), water level was measured every 10 min using a logging capacitance probe (Odyssey, Dataflow System, New Zealand) installed in each V notch weir box. The outflow from all V notch weir boxes was summed to determine total flow contributed by the trenched portion of the hillslope. Because the trenches were in different locations in each watershed (Figure 1) and drained different areas of hillslope, the amount of interflow was not compared among watersheds but rather interflow dynamics were examined within a watershed to examine chemistry responses to storm flow.

Precipitation (in mm) was measured at a central meteorological tower on the Savannah River Site, located approximately 3 km away from the study watersheds.

2.5. Calculations

The contribution of unprocessed nitrate from atmospheric deposition to interflow nitrate and stream water nitrate was determined using a general mixing model [Barnes *et al.*, 2008] and a Bayesian mixing model [Moore and Semmens, 2008; Stock and Semmens, 2015], respectively. Estimates of the atmospheric contribution of nitrate to stream and interflow nitrate are conservative because only direct (i.e., unprocessed) nitrate inputs were considered.

To examine how the atmospheric contribution to interflow nitrate varied over a storm event, the contribution was calculated for each sample collected during the late March 2011 storm in each watershed ($n=9$ for watershed R and $n=6$ for watershed C) following Barnes *et al.* [2008]:

$$\text{Atmospheric nitrate contribution (\%)} = \frac{\delta^{18}\text{O}_{\text{interflow}} - \delta^{18}\text{O}_{\text{nitrification}}}{\delta^{18}\text{O}_{\text{atmospheric}} - \delta^{18}\text{O}_{\text{nitrification}}} \times 100, \quad (1)$$

where $\delta^{18}\text{O}_{\text{interflow}}$ was the $\delta^{18}\text{O}_{\text{NO}_3}$ value for a given interflow sample, and $\delta^{18}\text{O}_{\text{atmospheric}}$ was the mean $\delta^{18}\text{O}_{\text{NO}_3}$ value for all throughfall samples collected during the study period (+68.0‰). The estimated value

for $\delta^{18}\text{O}_{\text{nitrification}}$ assumed that nitrifying microorganisms use two atoms of oxygen from H_2O and one atom of oxygen from O_2 in the conversion of ammonium to nitrate [Andersson and Hooper, 1983; Kumar et al., 1983; Hollocher, 1984; Kendall et al., 2007]. The $\delta^{18}\text{O}_{\text{O}_2}$ was assumed to be +23.5‰ [Kendall et al., 2007] and the $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ for precipitation was the mean value (−2.8‰) determined from samples that were simultaneously collected from a precipitation collector in watershed R [Klaus et al., 2015]. However, the calculated mean $\delta^{18}\text{O}_{\text{nitrification}}$ was higher than $\delta^{18}\text{O}_{\text{interflow}}$ for one sample in watershed C, resulting in a negative % atmospheric nitrate contribution for that sample. Because the estimated value for $\delta^{18}\text{O}_{\text{nitrification}}$ was higher than $\delta^{18}\text{O}_{\text{interflow}}$, this suggests that the assumption of the 2:1 $\text{H}_2\text{O}:\text{O}_2$ ratio may be incorrect for these watersheds [Mayer et al., 2001; Casciotti et al., 2002; Barnes et al., 2008; Snider et al., 2010]. Instead, the lowest value measured for $\delta^{18}\text{O}_{\text{interflow}}$ (+4.5‰) was used to represent $\delta^{18}\text{O}_{\text{nitrification}}$ in the mixing model calculation (as in Barnes et al. [2008]).

We used a Bayesian mixing model (MixSIAR) [Moore and Semmens, 2008; Stock and Semmens, 2015] to determine whether the atmospheric contribution to stream water nitrate varied among watersheds. A Bayesian mixing model was not used for the interflow nitrate data because of the lack of variation in $\delta^{18}\text{O}_{\text{interflow}}$ (i.e., only one $\delta^{18}\text{O}_{\text{interflow}}$ value per time point). In the Bayesian mixing model for stream water nitrate, all $\delta^{18}\text{O}_{\text{NO}_3}$ values for stream water in each watershed were included as the “mixture.” The “sources” were $\delta^{18}\text{O}_{\text{nitrification}}$ and $\delta^{18}\text{O}_{\text{atmospheric}}$. $\delta^{18}\text{O}_{\text{nitrification}}$ was calculated as described above, except that $\delta^{18}\text{O}_{\text{nitrification}}$ was calculated from each $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ precipitation value measured during the study ($n = 14$) [Klaus et al., 2015]. Because there was no spatial variation in the estimate of $\delta^{18}\text{O}_{\text{nitrification}}$ (i.e., only one measurement location), but there was spatial variation in $\delta^{18}\text{O}_{\text{atmospheric}}$ (i.e., one throughfall measurement per watershed), the values for $\delta^{18}\text{O}_{\text{atmospheric}}$ were calculated as the mean $\delta^{18}\text{O}_{\text{NO}_3}$ of throughfall across watersheds on each sampling date ($n = 18$). In the Bayesian mixing model, watershed was a fixed effect and generalist priors were used [Stock and Semmens, 2015]. The data are presented as median and 95% Bayesian credible intervals (BCI) for each watershed. The Bayesian mixing model was run using the MixSIAR graphical user interface [Moore and Semmens, 2008] in R v. 3.2.2.

To examine whether among watershed variability in the contribution of atmospheric nitrate to stream water nitrate was due to differences in watershed characteristics, we calculated two watershed metrics: (1) the ratio of the stream (surface) area to watershed area (as in Wexler et al. [2014]) and (2) the C index [Langhoff et al., 2006]. The C index is the ratio of the wet riparian zone width to the effective stream width, with the effective stream width a product of stream sinuosity (unitless) and stream width. The wet riparian width is the portion of the riparian zone where the groundwater comes in contact with the soil surface. This wet portion of the riparian zone was determined from the depth-to-groundwater map (see Figure 7). The depth-to-groundwater was calculated from the difference in surface elevation (from a digital elevation model) and the water table surface, which was generated from 22 monitoring wells and piezometers across the study site combined with outcropped water surface levels (streams and wetlands). A small C index value suggests that groundwater enters the stream via seepage through the streambed, while a large C index value suggests that groundwater enters the stream via shallow subsurface flow or overland flow from the riparian zone [Langhoff et al., 2006].

We examined relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ and nitrate concentration [Kendall et al., 2007; Burns et al., 2009] to determine whether denitrification or mixing was an important control on nitrate concentrations in stream water, groundwater (deep and riparian), and interflow in watersheds R and C. We did not examine these relationships in watershed B due to small sample sizes (few samples collected in watershed B were above the detection limit needed for $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ analysis). Denitrification or mixing may be important if there are linear or curvilinear relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ and nitrate concentration [Kendall et al., 2007]. Mixing can be distinguished from denitrification as mixing will result in a linear relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and the inverse of nitrate concentration while fractionation due to denitrification will result in a negative, linear relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ and the natural log of nitrate [Kendall et al., 2007]. We also calculated apparent fractionation ($^{15}\epsilon$) as the slope of the relationship of $\delta^{15}\text{N}_{\text{NO}_3}$ and the natural log of nitrate concentration. $^{15}\epsilon$ ranges from −40 to −1.5‰ [Mariotti et al., 1981, 1988; Sebilo et al., 2003]. A low apparent fractionation (−1.5 to −3.6‰) [Sebilo et al., 2003] suggests that denitrification is diffusion limited (i.e., benthic-dominated denitrification), meaning that the rate of denitrification is dependent on the diffusion of nitrate to anoxic sites where denitrification occurs [Sebilo et al., 2003; Lohse et al., 2013]. A high apparent

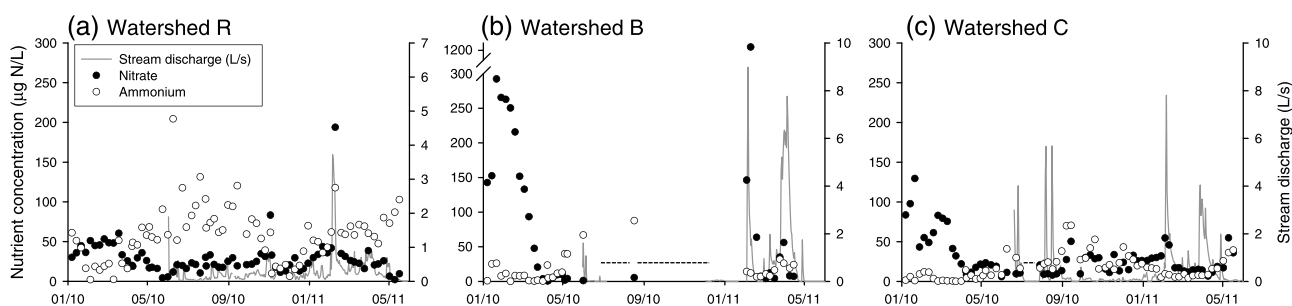


Figure 2. Nitrate (black circles) and ammonium (white circles) concentrations in stream water and stream discharge (grey line) in (a) watershed R, (b) watershed B, and (c) watershed C, from January 2010 to June 2011 when flow ceased in all streams. Streamflow measurements did not begin until June 2010 in all three watersheds. In watershed B, the stream was dry in June 2010 through early February 2011 (black dotted line), but there was one date in August 2010 when there was briefly water in the stream and a sample was collected. In watershed C, the stream was dry in July 2010 (black dotted line).

fractionation (-18‰) [Sebilo *et al.*, 2003] suggests that denitrification is not diffusion limited (i.e., riparian-dominated denitrification) [Sebilo *et al.*, 2003; Chen *et al.*, 2009]. The slope between $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ was also calculated, with a slope of 0.5 to 1 suggesting that $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ are affected by fractionation due to denitrification, and thus, denitrification is an important control on nitrate dynamics [Böttcher *et al.*, 1990; Aravena and Robertson, 1998; Sigman *et al.*, 2005; Kendall *et al.*, 2007; Granger *et al.*, 2008].

2.6. Statistics

We examined the factors influencing nitrate and ammonium concentrations using simple and multiple regression and examined whether nitrate and ammonium concentrations were correlated using Pearson's correlation. To examine whether nutrient concentrations varied among the different water pools (stream, riparian groundwater, deep groundwater, interflow, and throughfall), one-way analysis of variance (ANOVA) was carried out for each watershed. A corrected p value of 0.017 was used for significance due to the multiple comparisons ($n = 3$ watersheds) for this ANOVA. A Tukey's post hoc significant difference test was carried out if the main effect was significant. All data were transformed using natural log if necessary to meet parametric assumptions and statistical tests were done in SYSTAT v. 13.

3. Results

3.1. Stream Water Nitrogen Concentrations

Stream water nitrate and ammonium concentrations were variable across watersheds and through time (Figure 2). On average, nitrate concentrations were higher and more variable in watershed B (mean \pm standard deviation = $111.5 \pm 227.3 \mu\text{g N/L}$, $n = 33$) than in watersheds R ($29.3 \pm 23.9 \mu\text{g N/L}$, $n = 73$) and C ($29.6 \pm 24.3 \mu\text{g N/L}$, $n = 67$). In contrast, mean ammonium concentrations were higher in watershed R ($58.6 \pm 34.2 \mu\text{g N/L}$) than in watersheds B ($17.2 \pm 19.1 \mu\text{g N/L}$) and C ($17.2 \pm 15.4 \mu\text{g N/L}$). Ammonium concentrations were generally higher than nitrate concentrations in watershed R (Figure 2a), and nitrate concentrations were generally higher than ammonium concentrations in watersheds B (Figure 2b) and C (Figure 2c).

Across all three watersheds, stream water temperature was a significant predictor of both ammonium and nitrate concentrations (range of $r^2 = 0.17$ to 0.63 , all $p \leq 0.0004$), with ammonium concentrations increasing (Figure 3a) and nitrate concentrations decreasing (Figure 3b) with increasing temperature. Despite these patterns, nitrate and ammonium concentrations were not significantly correlated (watershed R: $R = -0.05$, $p = 0.70$, watershed B: $R = -0.15$, $p = 0.42$, and watershed C: $R = -0.20$, $p = 0.11$). Nitrate concentrations also increased during some high flow events (e.g., early February 2011 storm in watersheds R and B; Figures 2a and 2b); however, stream discharge was not a significant predictor of nitrate concentrations (all $p > 0.05$). Ammonium concentrations decreased with stream discharge in watershed C (multiple linear regression with temperature and stream discharge: $r^2 = 0.42$, $p < 0.0001$), and there were no significant relationships in watersheds R and B (all $p > 0.05$).

3.2. Watershed Nitrogen Concentrations

Within each watershed, nitrate and ammonium concentrations differed significantly among water pools (one-way ANOVAs, all $p < 0.0001$). There was a general pattern of higher ammonium and nitrate

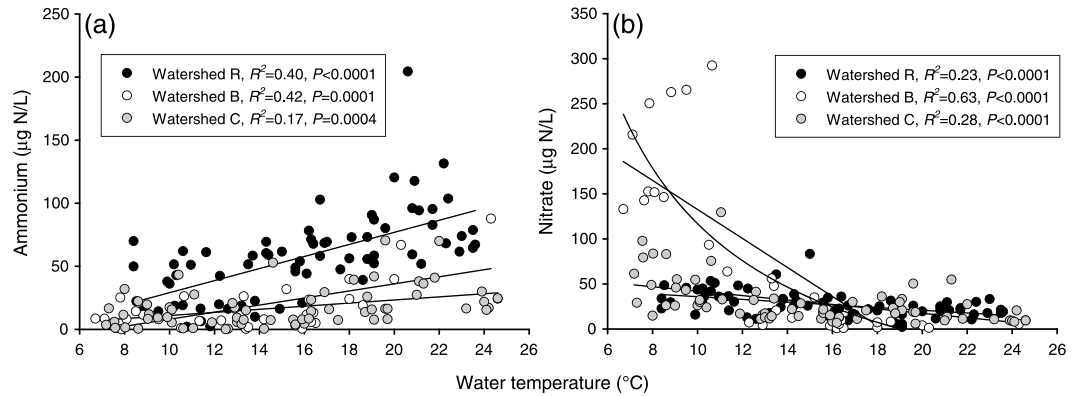


Figure 3. (a) Ammonium concentrations increased and (b) nitrate concentrations decreased with stream water temperature in watersheds R (black circles), B (white circles), and C (grey circles). The best fit for the relationship between stream water temperature and nitrate in watershed B was exponential, and the highest nitrate concentration measured during the study (1266 µg N/L) was removed from the regression as this high concentration appeared to be driven by the resumption of flow in February 2011.

concentrations in throughfall, intermediate concentrations in interflow and groundwater, and lower concentrations in stream water; however, these patterns varied by nutrient type (nitrate and ammonium) and watershed (Figure 4). Ammonium and nitrate concentrations in throughfall were significantly higher than in stream water in all watersheds (Tukey's post hoc tests, all $p < 0.05$) (Figure 4). Nitrate concentrations in interflow were significantly higher than in stream water in watersheds R and C (Figures 4a and 4c) (there were no interflow chemistry data for watershed B). These patterns were different for ammonium: in watershed R, ammonium concentrations were higher in stream water than in interflow (Figure 4d), and in watershed C, ammonium concentrations were lower in stream water than in interflow (Figure 4f). Nitrate concentrations

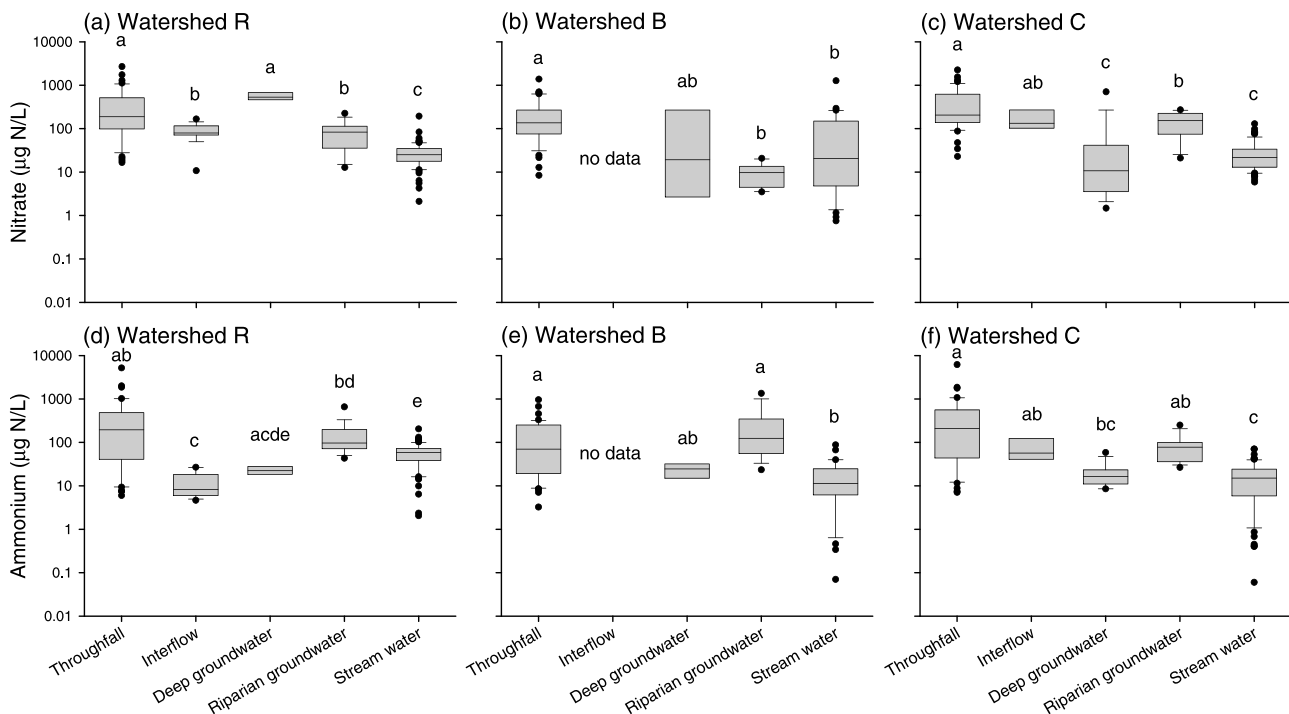


Figure 4. (a–c) Nitrate and (d–f) ammonium concentration in throughfall, interflow, deep groundwater, riparian groundwater, and stream water in (a, d) watershed R, (b, e) watershed B, and (c, f) watershed C, from January 2010 to February 2012 (note the y axes are on a log scale). Box plots represent weekly concentrations in stream water (when flowing) and throughfall, monthly concentrations in groundwater, and storm concentrations when interflow was present. Different letters represent significant differences in nitrate or ammonium concentration between water pools for a given watershed based on Tukey's post hoc significant difference test following a significant ($p < 0.017$; corrected for multiple comparisons) ANOVA result.

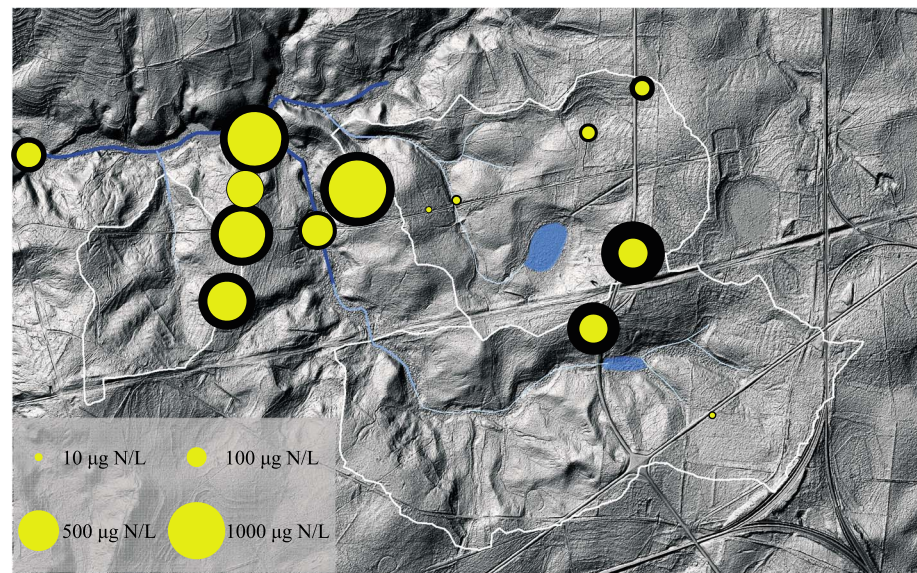


Figure 5. Nitrate concentration in 14 deep groundwater wells located across watersheds R, B, and C, and the larger Fourmile Branch watershed with concentration proportional to circle size. Yellow circles represent mean concentrations measured over the sampling period ($n = 3-5$ samples per well), and black circles represent one standard deviation. Note the LiDAR image shows terracing on the hillslopes from row crop farming that took place prior to the 1950s.

in stream water and deep groundwater were similar in watersheds B and C (Figures 4b and 4c) and higher in deep groundwater than stream water in watershed R (Figure 4a). Specifically, nitrate concentrations in deep groundwater were spatially variable, and there was an area of high nitrate in deep groundwater between watersheds R and C and downstream of watershed B, with mean concentrations near 1 mg N/L (Figure 5). Ammonium concentrations in stream water and deep groundwater were similar in all three watersheds (Figures 4d–4f). Nitrate and ammonium concentrations in riparian groundwater were significantly higher than in stream water in all watersheds except nitrate in watershed B (Tukey’s post hoc test, $p = 0.13$).

3.3. Dual Stable Isotopes: Sources of Stream Water Nitrate

The biplots of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ revealed different patterns of nitrate cycling among the three adjacent watersheds (Figure 6). In watershed R, there was overlap in $\delta^{15}\text{N}_{\text{NO}_3}$ versus $\delta^{18}\text{O}_{\text{NO}_3}$ biplot space between stream water and riparian groundwater in watershed R (Figure 6a), while there was little overlap in interflow and stream water $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ (Figure 6a). A Bayesian mixing model using measurements of $\delta^{18}\text{O}_{\text{NO}_3}$ in stream water and throughfall and estimates of $\delta^{18}\text{O}_{\text{nitrification}}$ suggested that the median contribution of precipitation to stream water nitrate in watershed R was 10% (95% BCI = 5–16%).

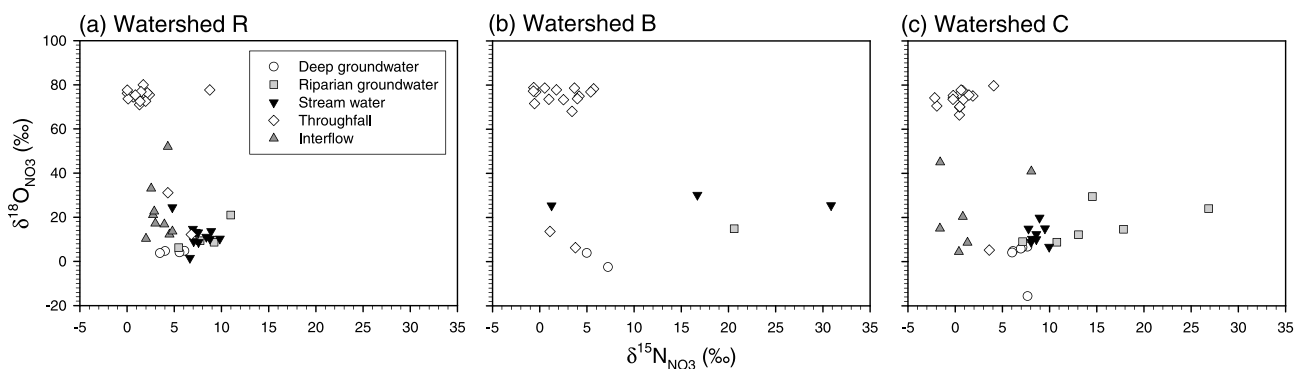


Figure 6. Biplots of $\delta^{18}\text{O}_{\text{NO}_3}$ versus $\delta^{15}\text{N}_{\text{NO}_3}$ for stream water (black triangles), riparian groundwater (light grey squares), deep groundwater (white circles), interflow (dark grey triangles), and throughfall (white diamonds) in (a) watershed R (adapted from [Klaus et al., 2015]), (b) watershed B, and (c) watershed C. Sampling for stable isotopes of nitrate occurred from February 2011 to February 2012. Sampling of stream water ended in April/May 2011 when flow ceased and all three streams dried up due to drought conditions at the site.

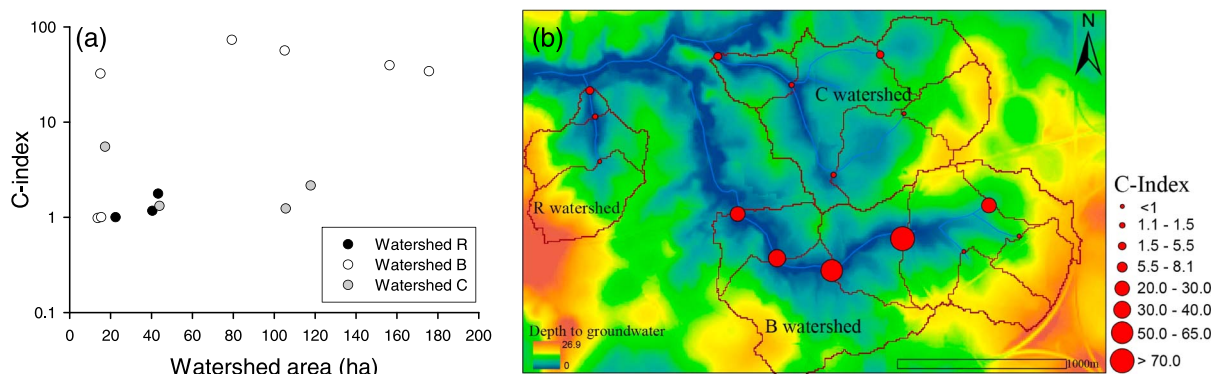


Figure 7. (a) The C index (on a log scale) versus watershed area for watersheds R (black circles), B (white circles), and C (grey circles). A small C index value suggests that groundwater enters the stream via seepage through the streambed, while a large C index value suggests that groundwater enters the stream via shallow subsurface flow or overland flow from the riparian zone [Langhoff *et al.*, 2006]. (b) Depth-to-groundwater map for the three study watersheds and the C index estimated at various locations within each of the watersheds.

In watershed C, the biplots of $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ revealed similar patterns to watershed R, but stream water $\delta^{15}\text{N}_{\text{NO}_3}$ and $\delta^{18}\text{O}_{\text{NO}_3}$ plotted between riparian and deep groundwater (Figure 6c). Interflow nitrate was most dissimilar to stream water nitrate, with low $\delta^{15}\text{N}_{\text{NO}_3}$ values and high $\delta^{18}\text{O}_{\text{NO}_3}$ values (Figure 6c). Similar to watershed R, a Bayesian mixing model suggested that the median contribution of precipitation to stream water nitrate in watershed C was 10% (4–16% (95% BCI)).

The stream water nitrate stable isotope data revealed different patterns in watershed B, as the $\delta^{18}\text{O}_{\text{NO}_3}$ of stream water was high (mean = +27.0‰; Figure 6b), and the contribution of atmospheric nitrate to stream water was significantly higher (based on nonoverlapping 95% BCIs) than in watersheds R and C (median (95% BCI) = 28% (18–46%)). The highest $\delta^{18}\text{O}_{\text{NO}_3}$ value measured in stream water (+30.2‰) coincided with peak stream discharge after a late March 2011 storm; however, the small stable isotope data set for stream water in watershed B ($n=3$) precluded examination of more detailed patterns over time. Stream water $\delta^{15}\text{N}_{\text{NO}_3}$ in watershed B was more variable (range = +1.3 to +30.9‰) than in the other watersheds (Figure 6b). Low concentrations of nitrate in riparian groundwater and the lack of interflow during the study period restricted comparison of stream water nitrate isotopes to these pools; however, the one riparian groundwater sample that had sufficient nitrate concentrations for stable isotope analysis fell within the ranges observed in watersheds R and C.

Variation in the proportion of stream water nitrate from atmospheric deposition across watersheds may be due to differences in stream channel area relative to the total watershed area or a dominance of shallow subsurface flow paths contributing to stream flow (i.e., the C index). In watershed B, the stream channel area comprised a greater proportion of the watershed (0.33%) than in watershed C (0.22%) and watershed R (0.10%). The C index calculated at the outlet of watershed B was also larger (34.2) than in watershed C (2.2) and watershed R (1.8) (Figure 7a), and this pattern was consistent when the C index was calculated at various locations in each watershed (Figure 7b).

3.4. Dual Stable Isotopes: Sources of Interflow Nitrate

Over a period of 6 days from 26 March 2011 to 31 March 2011, 72.8 mm of rain fell, with the majority falling on 27 March 2011 (32.1 mm), during which time the trenches in watersheds R and C started flowing (Figure 8) (no flow was measured in the watershed B trench). During the storm, nitrate peaked at 167 $\mu\text{g N/L}$ in watershed R interflow, and the nitrate peak occurred slightly after the peak in $\delta^{18}\text{O}_{\text{NO}_3}$ (+52.0‰) (Figure 8a). In watershed C interflow, nitrate peaked at 425 $\mu\text{g N/L}$, and this corresponded to the peak in $\delta^{18}\text{O}_{\text{NO}_3}$ of +45.0‰ (Figure 8b). During the storm, atmospheric contributions to interflow nitrate varied, with the highest contributions of 75% in watershed R and 64% in watershed C (Table 1).

3.5. Dual Stable Isotopes: The Importance of Denitrification and Mixing

Both denitrification and mixing affected nitrate concentrations in the study watersheds but their relative importance was difficult to distinguish. In deep groundwater in watershed R, the importance of

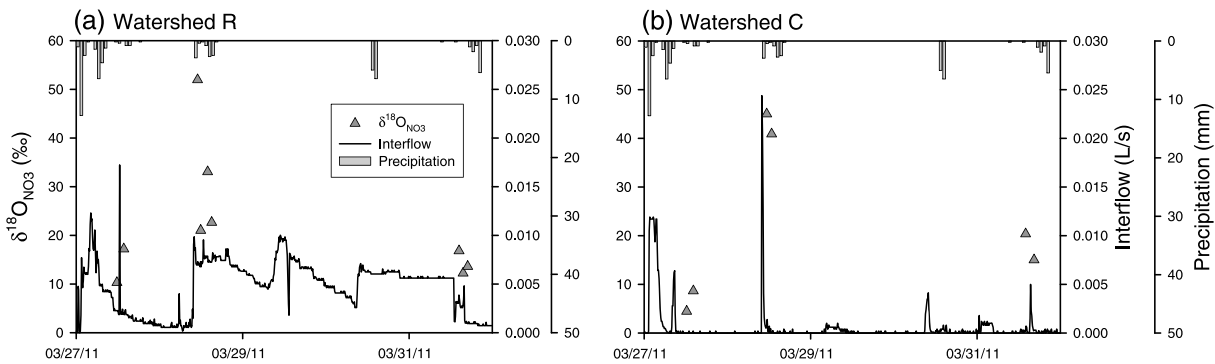


Figure 8. Response of interflow to a precipitation event in late March 2011 in (a) watershed R and (b) watershed C. $\delta^{18}\text{O}_{\text{NO}_3}$ (dark grey triangles) and interflow (in L/s; black line) were measured at the interflow interception trench and precipitation (in mm; grey bars) was measured at the nearby rain gage. There was no flow at the trench in watershed B during this event.

denitrification and mixing was indistinguishable because the relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration ($r^2 = 0.95, p = 0.03$) (Figure 9a) and between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the inverse of nitrate concentration ($r^2 = 0.92, p = 0.04$) (Figure 9c) were both significant. In riparian groundwater in watershed R, there was a significant relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration ($r^2 = 0.91, p = 0.046$) (Figure 9a) but not with the inverse of nitrate ($r^2 = 0.68, p = 0.18$) (Figure 9c). The apparent fractionation ($^{15}\epsilon$) values calculated from $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate relationships were -1.8‰ for riparian groundwater and -6.6‰ for deep groundwater. The slopes of the relationships between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ were 0.2 for deep groundwater and 2.4 for riparian groundwater; however, these relationships were not statistically significant (deep groundwater $r^2 = 0.22, p = 0.53$ and riparian groundwater $r^2 = 0.72, p = 0.15$). For stream water and interflow in watershed R, there were no significant relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration (Figure 9a) or between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the inverse of nitrate concentration (all $r^2 < 0.21, p > 0.15$) (Figure 9c).

In deep groundwater and riparian groundwater in watershed C, there were no significant relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration (Figure 9b) or between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the inverse of nitrate concentration (Figure 9d) (all $r^2 < 0.05, p > 0.67$); however, the relationship between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration for riparian groundwater in watershed C ($r^2 = 0.89, p = 0.02, ^{15}\epsilon = -2.6$) (Figure 9b), and the relationship with the inverse of nitrate concentration was not significant ($r^2 = 0.74, p = 0.06$) (Figure 9d).

Table 1. Estimated Percentage of Atmospheric Nitrate That Contributed to Interflow Nitrate in Watersheds R and C During a Storm in March 2011

Date and Time	Interflow Nitrate From Atmospheric Deposition (%)
<i>Watershed R</i>	
3/27/2011 11:45	9%
3/27/2011 13:45	20%
3/28/2011 11:00	75%
3/28/2011 11:55	26%
3/28/2011 13:55	45%
3/28/2011 15:05	29%
3/31/2011 14:25	19%
3/31/2011 15:35	12%
3/31/2011 16:55	14%
<i>Watershed C</i>	
3/27/2011 12:15	0%
3/27/2011 14:10	7%
3/28/2011 11:20	64%
3/28/2011 12:45	57%
3/31/2011 14:00	25%
3/31/2011 16:25	17%

There were significant relationships between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentration ($r^2 = 0.69, p = 0.01$) (Figure 9b) and between $\delta^{15}\text{N}_{\text{NO}_3}$ versus the inverse of nitrate concentration ($r^2 = 0.73, p = 0.007$) (Figure 9d) for stream water in watershed C; however, the directions of these relationships were opposite to that expected for denitrification (negative) and mixing (positive). There were no significant relationships between $\delta^{15}\text{N}_{\text{NO}_3}$

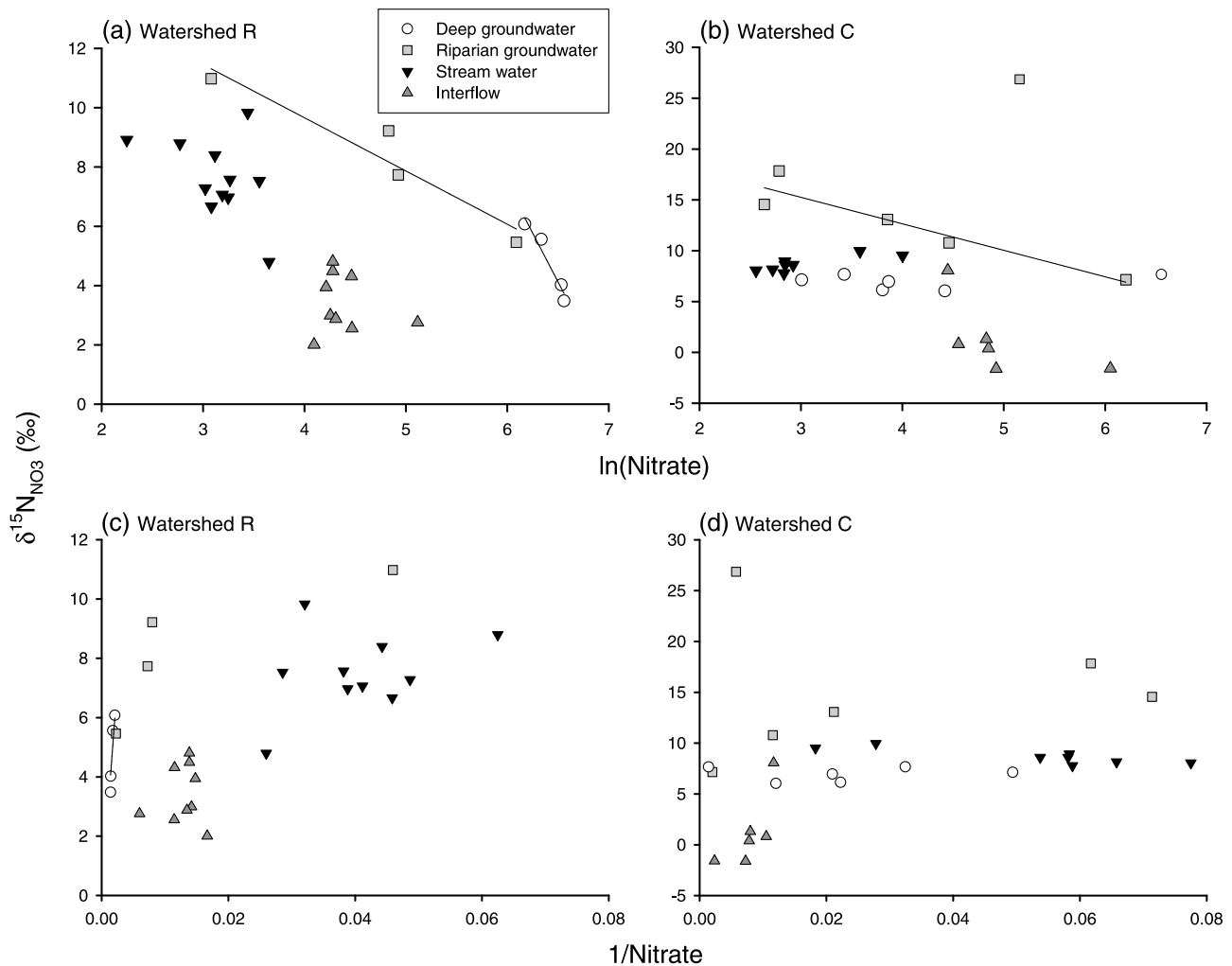


Figure 9. Relationships between (a, b) $\delta^{15}\text{N}_{\text{NO}_3}$ and the natural log of nitrate concentration and (c, d) $\delta^{15}\text{N}_{\text{NO}_3}$ and the inverse of nitrate concentration for deep groundwater (white circles), riparian groundwater (light grey squares), stream water (black triangles), and interflow water (dark grey triangles) in (a, c) watershed R and (b, d) watershed C. Significant, negative relationships between (a, b) $\delta^{15}\text{N}_{\text{NO}_3}$ and the natural log of nitrate suggest that denitrification affected nitrate concentrations in riparian groundwater in watersheds R and C. However, the relationship for riparian groundwater in watershed C was only significant when one data point with a high $\delta^{15}\text{N}_{\text{NO}_3}$ value (+26.9‰) was removed from the regression. A significant, negative relationship between (a) $\delta^{15}\text{N}_{\text{NO}_3}$ and the natural log of nitrate and a significant, positive relationship between (c) $\delta^{15}\text{N}_{\text{NO}_3}$ and the inverse of nitrate in watershed R suggests that the importance of denitrification and mixing was indistinguishable. There were no significant relationships for stream water or interflow water nitrate.

versus the natural log of nitrate concentration ($r^2 = 0.37, p = 0.20$) (Figure 9b) or versus the inverse of nitrate concentration for interflow ($r^2 = 0.54, p = 0.10$) (Figure 9d).

4. Discussion

4.1. Watershed Nitrogen Cycling and Sources of Stream Water Nitrate

A combination of N concentrations and stable isotopes of nitrate ($\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$) together suggest that the predominant flow path of N in these low-relief watersheds is from precipitation to lateral interflow, to groundwater, and then to stream water. This sequence is consistent with hydrological measurements in these watersheds [Klaus et al., 2015; E. Du et al., submitted to, 2016] and with analytical assessments of hill-slope flow processes [Jackson et al., 2014]. Nitrate concentrations were generally highest in throughfall, intermediate in interflow and riparian groundwater, and lowest in groundwater and stream water. The decrease in nitrate concentrations along this flow path sequence was likely due to biological uptake and N transformations. A synthesis by Sudduth et al. [2013] of nitrate cycling in 40 forested watersheds from around the world

found that stream water nitrate concentrations were roughly half of the concentrations in soil water likely due to uptake and transformations in soils before reaching the streams. Stream water nitrate and ammonium concentrations in the three study watersheds were similar to ranges reported for other forested watersheds in the southeastern United States [Chescheir *et al.*, 2003; Amatya *et al.*, 2007; Beltran *et al.*, 2010].

The overlapping $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ signatures of groundwater and stream water also suggest that groundwater was the predominant source of stream water nitrate. These findings are corroborated by detailed hydrological and water isotope measurements in our study watersheds that showed that groundwater discharging into the stream valley was the predominant source of stream water [Klaus *et al.*, 2015; E. Du *et al.*, submitted to, 2016]. While interflow in hillslope soils was observed during storms, the hillslopes were rarely directly hydrologically connected to the streams. A combination of high hydraulic conductivity of the soils and relatively flat topography resulted in a high water percolation rate through the underlying argillic (clay) layer and into the groundwater (E. Du *et al.*, submitted to, 2016). Interflow at the soil-argillic interface can contribute to stream water near the riparian zone; however, the contributing distance was at most 30 m [Jackson *et al.*, 2014]. The different $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ signatures of interflow and stream water also suggest that nitrate does not directly enter streams from interflow (and likely enters via a groundwater pathway) or it is transformed prior to entering the stream.

While the $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ values of stream water and groundwater in watersheds R and C overlapped, the $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ values of stream water in watershed B showed a greater contribution of nitrate from atmospheric deposition. A Bayesian mixing model suggested that 28% of stream water nitrate was from precipitation, and this was significantly higher than in watershed R (10%) and watershed C (10%). Several variables may explain the higher proportion of atmospherically derived nitrate in watershed B stream water. The stream draining watershed B was dry more often and had higher peak discharges than in watersheds R and C (Figure 2), and watershed B also had a higher C index (ratio of the wet riparian zone width to the effective stream width) [Langhoff *et al.*, 2006] than watersheds R and C. These hydrologic variables suggest that shallower, quick-delivery flow paths may be important in delivering nitrate to the stream in watershed B and thus may explain the greater contribution of atmospheric nitrate to stream water nitrate. The proportion of stream channel area to total watershed area in watershed B was also greater than in watersheds R and C, suggesting direct precipitation onto the interflow contributing areas, riparian zone, and stream channel together may provide enough flow relative to stream discharge to observe the atmospheric nitrate signal (as in Wexler *et al.* [2014]). The nitrate data set in watershed B was limited as several samples were below the nitrate concentration necessary for stable isotope analysis; longer-term data sets are needed to better elucidate the patterns of N cycling in this watershed.

That nitrate in precipitation contributed to stream water nitrate in all three watersheds was surprising for these low-relief, low runoff ratio, groundwater-dominated streams [Klaus *et al.*, 2015]. Most studies that have used stable isotopes of nitrate to show the importance of precipitation-derived (i.e., unprocessed) nitrate in stream water have been conducted in the northeastern United States where watersheds tend to be of steeper topography, and most nitrate delivery to streams from precipitation occurs during snowmelt or in the winter when vegetative uptake is low [Ohte *et al.*, 2004; Barnes *et al.*, 2008; Sebestyen *et al.*, 2008; Buda and DeWalle, 2009]. However, summer rain events can also be important in northeastern U.S. watersheds. At Watershed 3 (WS3) at the Hubbard Brook Experimental Forest, New Hampshire, USA, a summer rainfall contributed 29–34% of stream water nitrate during that event. This high percentage of rainfall-derived nitrate was due to the large area of stream channel relative to watershed area intercepting precipitation and directly exporting it to the stream [Wexler *et al.*, 2014]. While precipitation events can result in large atmospheric contributions of nitrate to stream water, annual contributions of atmospheric nitrate are lower. For example, annual contributions of atmospheric nitrate were 7% in a snowmelt-dominated watershed in Vermont, USA [Sebestyen *et al.*, 2008] and 11–12% in forested watersheds in Connecticut and Massachusetts, USA [Barnes *et al.*, 2008]. The annual contributions of atmospheric nitrate to stream water nitrate in these northeastern U.S. watersheds were similar to the median February through May contributions in the Coastal Plain watersheds (10–28%). However, these measurements took place for 4 months prior to stream discharge ceasing due to a regional drought, and it is not known whether atmospheric nitrate contributes to stream water nitrate during wetter periods. Overall, our results suggest that atmospheric contributions to stream water nitrate in low-relief, subtropical watersheds can be as high as in upland, snowmelt-dominated catchments in the northeastern U.S.

4.2. Nitrogen Cycling Processes

We predicted that denitrification would be an important component of watershed N cycling in these three study watersheds due to the organic-rich soils in the riparian zones and the warm temperatures. The seasonal patterns in stream water ammonium and nitrate concentrations and relationships with water temperature suggest that indeed temperature may be an important seasonal driver on N cycling processes (i.e., denitrification and nitrification) in these watersheds. Nitrate concentrations were lower and ammonium concentrations were higher in summer, with the opposite pattern occurring in winter. Warmer temperatures in summer may increase anoxic microsites for denitrification due to lower oxygen solubility and thus decrease nitrate concentration. At the same time, nitrification, which requires oxic conditions, may decrease and lead to an accumulation of ammonium.

Seasonal patterns in stream water nutrient concentrations differ across watersheds, suggesting that the drivers of N cycling are site or region specific. For instance, high nitrate concentrations have been observed in streams draining northeastern forested watersheds in winter, and these higher winter concentrations have been attributed to lower biological activity in the watershed [Sickman *et al.*, 2003; Judd *et al.*, 2007]. In forested watersheds that experience warmer winters, stream water nitrate concentrations are high in summer because decreased light availability and reduced organic matter standing stocks lower in-stream autotrophic and heterotrophic nutrient demand [Mulholland, 2004; Lutz *et al.*, 2012]. In contrast, stream water nutrient concentrations in the three study watersheds in the Upper Coastal Plain appear to be driven by seasonality in nitrification and denitrification rates.

Based on the temporal patterns in ammonium and nitrate concentrations, we expected to see a strong signature of denitrification in stable isotopes of nitrate. The high values of $\delta^{15}\text{N}_{\text{NO}_3}$ in stream and groundwater in watersheds B and C (compared to watershed R) in the absence of any current anthropogenic sources (e.g., manure and fertilizers) suggests that significant fractionation factors influence $\delta^{15}\text{N}_{\text{NO}_3}$ in these watersheds. It is possible that the higher $\delta^{15}\text{N}_{\text{NO}_3}$ values reflect legacy nitrogen from farming activities that occurred on the site prior to the 1950s [Kilgo and Blake, 2005]; however, the highest $\delta^{15}\text{N}_{\text{NO}_3}$ values were measured in watershed C, which tended to have lower nitrate concentrations in groundwater. Relationships between $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ and between $\delta^{15}\text{N}_{\text{NO}_3}$ and nitrate concentration can help elucidate the importance of denitrification; however, these relationships in the study watersheds were inconclusive and differed among watersheds. It should be noted that we could not apply these techniques to watershed B due to the low sample size. Denitrification may be important in riparian groundwater in watersheds R and C based on linear plots of $\delta^{15}\text{N}_{\text{NO}_3}$ versus the natural log of nitrate concentrations. However, both denitrification and mixing relationships were significant for deep groundwater in watershed R, suggesting that both processes affected nitrate concentrations in that water pool. The apparent fractionation calculated ($^{15}\epsilon$) for groundwater ranged from -1.8 to -6.6 , which is consistent with the range for groundwater-dominated (and thus diffusion limited) denitrification [Sebilo *et al.*, 2003]. However, the slopes of the relationships of $\delta^{18}\text{O}_{\text{NO}_3}$ and $\delta^{15}\text{N}_{\text{NO}_3}$ were both above (2.4 for riparian groundwater in watershed R) and below (0.2 for deep groundwater in watershed R) the predicted range of 0.5 to 1.0 [Böttcher *et al.*, 1990; Aravena and Robertson, 1998; Sigman *et al.*, 2005; Granger *et al.*, 2008]. These inconclusive patterns suggest that many factors (e.g., mixing, assimilatory uptake, and dissimilatory uptake) may together affect nitrate concentrations and stable isotope signatures in these watersheds.

Overall, the lack of a definitive measure of the importance of denitrification using nitrate stable isotopes in these watersheds suggests that additional measurements (i.e., denitrification potential and N_2/Ar gas measurements) are necessary to characterize denitrification. Also, our period of observation using stable isotopes was short (i.e., 4 months) as sampling for stable isotopes was not initiated until early 2011, and 4 months later, stream flow ceased due to a drought in the region. Therefore, we do not have any stream water stable isotope data from the summer when the stream water nitrate concentration data (via relationships with temperature) suggest that denitrification may be most important. Extending this data set over multiple years will be necessary to see how nitrate cycling processes vary at event, seasonal, and interannual time scales.

4.3. Variation Among Watersheds

Adjacent watersheds with similar characteristics can vary greatly in biogeochemical and hydrological properties [Lovett *et al.*, 2000; Vanni *et al.*, 2001; West *et al.*, 2001]. For instance, stream water nitrate concentrations vary widely across watersheds in the Catskills in New York State [Lovett *et al.*, 2000], although this variability does not appear to be driven by differences in groundwater nitrate concentrations [West *et al.*, 2001].

The opposite pattern was observed in our three study watersheds. Watersheds R, B, and C were located adjacent to one another and had similar topography, vegetation, and soils. However, groundwater nitrogen chemistry varied greatly among the watersheds while stream water nitrogen chemistry was more similar. Groundwater nitrate concentrations were consistently higher near watershed R and downstream of watershed B, which may reflect legacy effects [Harding *et al.*, 1998; Hamilton, 2012] from farming practices that occurred prior to 1950. Effects of human activities on groundwater quality can be lagged by up to several decades depending on water and solute transit times [Hamilton, 2012]; however, groundwater transit times have not been estimated for the R, B, and C watersheds. The depths to groundwater in the various sampling wells also varied (from 2.7 to 43.6 m below the soil surface), and thus, differences in our samples may have reflected differences in groundwater age. Understanding the effect of groundwater age on nitrogen cycling [e.g., Kennedy *et al.*, 2009] is an area for future research.

The estimated contribution of atmospheric nitrate via rapid transport to stream water nitrate also varied among watersheds and may be explained by differences in riparian zone area relative to total watershed area (as described previously). The variability among watersheds may be more pronounced because of the small size of the study watersheds. Stream water specific discharge and stream biogeochemistry tend to be less variable from subcatchment to subcatchment as watershed size increases to some representative elementary area [Wolock *et al.*, 1997]. Overall, these results highlight the importance of capturing variable biogeochemical processes in adjacent watersheds with fairly similar characteristics and the need to understand these processes at various spatial scales.

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5. Conclusions

Groundwater was the dominant source of streamflow in these watersheds [Jackson *et al.*, 2014; Klaus *et al.*, 2015; E. Du *et al.*, submitted to, 2016] and dual stable isotopes of nitrate also showed that this flow path was an important source of stream water nitrate. However, nitrate in atmospheric deposition was also a measurable source of stream water nitrate. We did not expect this because of the low runoff ratios in the study watersheds [Klaus *et al.*, 2015]. We also found considerable spatial variation in groundwater nitrate concentrations potentially due to legacy farming that occurred at the site prior to the 1950s. We expected to see a strong signal of denitrification due to the warm climate and organic-rich soils in the riparian zone and seasonality in stream water ammonium and nitrate concentrations, but there were no clear patterns from the stable isotopes of nitrate likely because a variety of interacting factors affected nitrate concentrations (i.e., assimilatory uptake, dissimilatory uptake, and mixing) at different time scales. Understanding these hydrologic and biological controls is important for predicting how N cycling may change in Upper Coastal Plain watersheds under climate change or land management scenarios.

Finally, two of these watersheds (watersheds B and C) are the sites of a watershed-scale experiment to examine the effect of short rotation loblolly pine production for bioenergy on water quality and hydrology, while watershed R will serve as the unmanipulated control watershed (all data presented in this manuscript are from the pretreatment period). As there was considerable variability in chemistry across the three study watersheds, the baseline data are crucial in characterizing these differences. From these baseline results showing a dominant groundwater flow path for water and nitrate, we hypothesize that fertilizers applied during pine silviculture that are not taken up by the vegetation may enter streams via groundwater, rather than a more rapid delivery via overland or interflow to the streams. The characteristics of watershed B suggest that superficial flow paths may be important in delivering atmospheric nitrate to stream water; however, this pathway was likely dominant only near the stream channel itself (i.e., within or near the riparian zone based on downslope travel distances calculated in Jackson *et al.* [2014]) and this area will remain intact as a stream-side management zone during pine production. Determining the travel time of excess fertilizers from application to delivery to the stream and the ultimate fate (downstream export versus denitrification) will be important in assessing the effects of short rotation pine for bioenergy on water quality.

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