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Key Points:

- Stream nitrate during autumn varied with inputs of atmospheric nitrate
- Autumn leaf fall and stormflow events affected stream nitrate and DON dynamics
- Stream nitrate and DON during autumn storms originated from riparian areas not hillslopes

Supporting Information:

- Supporting tables, figures, and method descriptions, S1–S6
- Data release, S6 and S7
- Figure S1, S2

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Coupled hydrological and biogeochemical processes controlling variability of nitrogen species in streamflow during autumn in an upland forest

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Abstract Autumn is a season of dynamic change in forest streams of the northeastern United States due to effects of leaf fall on both hydrology and biogeochemistry. Few studies have explored how interactions of biogeochemical transformations, various nitrogen sources, and catchment flow paths affect stream nitrogen variation during autumn. To provide more information on this critical period, we studied (1) the timing, duration, and magnitude of changes to stream nitrate, dissolved organic nitrogen (DON), and ammonium concentrations; (2) changes in nitrate sources and cycling; and (3) source areas of the landscape that most influence stream nitrogen. We collected samples at higher temporal resolution for a longer duration than typical studies of stream nitrogen during autumn. This sampling scheme encompassed the patterns and extremes that occurred during base flow and stormflow events of autumn. Base flow nitrate concentrations decreased by an order of magnitude from 5.4 to 0.7 $\mu\text{mol L}^{-1}$ during the week when most leaves fell from deciduous trees. Changes to rates of biogeochemical transformations during autumn base flow explained the low nitrate concentrations; in-stream transformations retained up to 72% of the nitrate that entered a stream reach. A decrease of in-stream nitrification coupled with heterotrophic nitrate cycling were primary factors in the seasonal nitrate decline. The period of low nitrate concentrations ended with a storm event in which stream nitrate concentrations increased by 25-fold. In the ensuing weeks, peak stormflow nitrate concentrations progressively decreased over closely spaced, yet similarly sized events. Most stormflow nitrate originated from nitrification in near-stream areas with occasional, large inputs of unprocessed atmospheric nitrate, which has rarely been reported for nonsnowmelt events. A maximum input of 33% unprocessed atmospheric nitrate to the stream occurred during one event. Large inputs of unprocessed atmospheric nitrate show direct and rapid effects on forest streams that may be widespread, although undocumented, throughout nitrogen-polluted temperate forests. In contrast to a week-long nitrate decline during peak autumn litterfall, base flow DON concentrations increased after leaf fall and remained high for 2 months. Dissolved organic nitrogen was hydrologically flushed to the stream from riparian soils during stormflow. In contrast to distinct seasonal changes in base flow nitrate and DON concentrations, ammonium concentrations were typically at or below the detection limit, similar to the rest of the year. Our findings reveal couplings among catchment flow paths, nutrient sources, and transformations that control seasonal extremes of stream nitrogen in forested landscapes.

1. Introduction

Autumn is a season of dynamic change in temperate forest streams. Base flow concentrations of stream nitrate (NO_3^-) decrease and concentrations of dissolved organic matter (DOM) increase when fallen deciduous leaves decompose in streams [Mulholland and Hill, 1997; Meyer et al., 1998]. Additionally, catchment hydrology changes after leaf fall. Corresponding to decreases in evapotranspiration when deciduous leaves fall, catchment wetness, and streamflow are more responsive to rainfall events after leaf fall than during the vegetative growing season [Zon, 1927; Hornbeck, 1973]. Beyond these generalities and an expansive literature on biological uptake of nutrients during in-stream leaf decomposition [Kaushik and Hynes, 1968, 1971; Webster and Benfield, 1986; Webster et al., 1999], little has been reported on how interactions of biogeochemical transformations, nitrogen (N) source variation, and catchment flow paths have affected the dynamic variation of stream nitrogen during autumn.

An experiment in which enriched-abundance ^{15}N tracer was added to a stream has revealed changes to in-stream nitrogen cycling during autumn base flow [Tank *et al.*, 2000]. In addition, many publications from long-term studies have included figures that show that nitrate concentrations in stream waters of the eastern United States have typically decreased for a week or two during autumn [Murdoch and Stoddard, 1993; Likens and Bormann, 1995; Mulholland and Hill, 1997; Swank and Vose, 1997; Lovett *et al.*, 2000; Buffam *et al.*, 2001; Mulholland, 2004]. While indicative of general changes to stream nitrate processing during autumn over many years, the weekly resolution of those data has precluded much inference about: (1) particular biogeochemical or hydrological processes; or (2) the timescales over which the speciation and concentrations of dissolved nitrogen return to preleaf fall levels. In particular, little has been reported on stream dissolved organic nitrogen (DON) concentration responses to leaf fall or how storm events affect stream nitrogen speciation during autumn.

Transformations are less likely to affect stream nitrogen concentrations during stormflow when in-stream biological processing has decreased [Mulholland, 2004; Roberts and Mulholland, 2007; Roberts *et al.*, 2007] and solutes have been transported from riparian and upland source areas to streams [Mulholland, 1993; Inamdar *et al.*, 2000; Sebestyen *et al.*, 2009a]. Since few studies of nitrogen dynamics during autumn have included more than weekly samples or intrastorm samples from rainfall-runoff events, little is known about the magnitude of changes in nitrogen concentrations or the time scales of recovery to preleaf fall concentrations.

Stream nitrate and DON concentrations in upland temperate forests may increase several fold during stormflow events [Inamdar *et al.*, 2004; Mitchell *et al.*, 2006; Sebestyen *et al.*, 2009a]. For example, stream nitrate concentrations at the Sleepers River Research Watershed in Vermont, USA have always increased during stormflow. Nonetheless, peak concentrations were highly variable among stormflow events and nitrate concentration-discharge relationships have widely differed among events [Shanley, 2000; Sebestyen *et al.*, 2008, 2009a, 2009b]. The reasons for these differences among stormflow events have not always been clear, but have been attributed to the routing of water along different hydrological flow paths through catchment soils and occasions when atmospheric nitrate was directly transported to streams during stormflow events [Ohte *et al.*, 2004; Sebestyen *et al.*, 2008, 2009b].

The condition of nitrogen saturation is the hypothesized extreme effect of nitrogen pollution on forests [Ågren and Bosatta, 1988; Aber *et al.*, 1989]. As forest nitrogen pollution increases, nitrogen inputs have been expected to exceed biological uptake capacity, with the excess nitrate being routed through soils and groundwater to streams [Aber *et al.*, 1989; Stoddard, 1994]. Although most temperate forests have not reached latter stages of nitrogen saturation even if atmospheric nitrate deposition has been elevated above preindustrial levels [Stoddard, 1994; Boyer *et al.*, 2002; Aber *et al.*, 2003; Campbell *et al.*, 2004], the effects of chronic atmospheric deposition in the northeastern United States have become apparent as increases in nitrate concentrations relative to other N species and elevated yields of stream nitrate during stormflow events [Stoddard, 1994; Perakis and Hedin, 2002].

Since the 1980s and 1990s, when metrics of nitrogen saturation effects on forests and streams were introduced [Ågren and Bosatta, 1988; Aber *et al.*, 1989; Stoddard, 1994], studies have shown that most nitrate from atmospheric deposition cycles through vegetation and the soil organic pool [Gundersen *et al.*, 2006]. Consequently, most stream nitrate has originated from organic nitrogen that has been mineralized and nitrified. Nonetheless, there have been exceptions. Increasing evidence from isotopic tracer studies in moderately to highly nitrogen-polluted, temperate forests has shown that some nitrate in rain or snowmelt may flow to streams during some stormflow events without having been biologically processed in upland or riparian soils [Kendall *et al.*, 1995; Spoelstra *et al.*, 2001; Williard *et al.*, 2001; Burns and Kendall, 2002; Schiff *et al.*, 2002; Pardo *et al.*, 2004; Buda and DeWalle, 2009; Burns *et al.*, 2009; Sebestyen *et al.*, 2009b; Pellerin *et al.*, 2012]. Information on the timing and magnitude of these inputs of unprocessed atmospheric nitrate is critical to determining how upland, temperate forests respond to nitrogen pollution. Nonetheless, other than several spring or snowmelt events [Williard *et al.*, 2001; Sebestyen *et al.*, 2008; Pellerin *et al.*, 2012], these inputs have rarely been well constrained.

This study addresses how interactions of biogeochemical transformations, nitrogen source variation, and catchment flow paths affect stream nitrogen dynamics during autumn, which we compare to conditions during other seasons. Various nitrogen transformations including increased biological uptake [Goodale

et al., 2009; Webster *et al.*, 2009; Cheever *et al.*, 2012], decreased nitrification [Tank *et al.*, 2000], or increased denitrification [Mulholland and Hill, 1997] may contribute to lower stream nitrate concentrations during autumn base flow. Using isotopic tracers of nitrogen sources and transformations, we explored the variation of these processes during autumn. We also explored the duration and magnitude of change in stream DON concentrations, which we hypothesized should increase in response to DOM that is leached from decomposing leaves. We further hypothesized that the timing of storm events would be a control on recovery from the nitrate concentration minimum during leaf fall and that inputs of unprocessed atmospheric nitrate may explain reasons why nitrate concentration-discharge relationships have tremendously varied among storm events. To address these hypotheses, we collected samples at higher temporal resolution for a longer duration than typical studies of stream nitrogen dynamics during autumn. This sampling was intended to encompass the patterns and extremes in stream nitrogen dynamics that occur during base flow (before, during, and after leaf fall) and stormflow. We measured streamflow, water table elevations, solute concentrations, water isotopes, and nitrate isotopes, which allowed us to assess: (1) the timing, duration, and magnitude of changes to stream nitrate, DON, and ammonium concentrations; (2) changes in nitrate sources and cycling during autumn; (3) factors that affected the timing of recovery of stream nitrogen concentrations from the nitrate concentration minimum; (4) source areas of the landscape that most influence stream nitrogen dynamics in an upland forest; and (5) the intraevent and interevent timings of unprocessed atmospheric nitrate input to streams. In addition, we provide data presented in this study as supplemental information (2013WR013670-sup-0006-ds01 and 2013WR013670-sup-0006-ds02) in both Microsoft Excel Spreadsheet and PDF formats.

2. Site Description

We studied Watershed 9 (W-9) of the Sleepers River Research Watershed in northeastern Vermont, USA. This site is part of the Water Energy and Biogeochemical Budgets Program of the U.S. Geological Survey [Shanley, 2000; Glynn *et al.*, 2009]. The 40.5 ha catchment on the eastern slopes of the Kittredge Hills has a southerly aspect and the elevation ranges from 519 to 686 m above mean sea level. Pope Brook drains W-9 and forms the headwaters of Sleepers River, which flows to the Connecticut River. Upstream of the W-9 weir, three tributary streams from the A (16.9 ha), B (12.9 ha), and C (8.1 ha) subbasins merge to form Pope Brook (Figure 1). The section of the stream between the tributary confluences and the W-9 stream gauge on Pope Brook is low-gradient, and base flow sometimes was completely subsurface through several meters of sandy sediments. Further upstream, the tributaries cascade down steep hillslopes in gravel and boulder channels.

The bedrock is granulite (a calcareous silicate rock) with interbedded quartz-mica schists and micaceous quartzite [Hall, 1959]. Moderately to excessively well-drained Inceptisols and Spodosols overlay dense glacial tills. Poorly drained Histosols occur in riparian zones and wetlands, which comprise about 5% of the land area [Shanley *et al.*, 2003]. Past studies show that groundwater levels in W-9 vary up to 3 m in upslope soils depending upon wetness conditions [Shanley *et al.*, 2003] and that hydraulic conductivities on hillslopes increase exponentially toward the soil surface [Kendall *et al.*, 1999].

Northeastern Vermont has a humid continental climate and precipitation is evenly distributed throughout the year. From 1992 to 2003, the mean annual temperature was 4.6°C, the mean annual precipitation was 1334 mm, and the mean annual stream water yield from W-9 was 735 mm. Autumn leaf fall typically lasts several days to a week or more and occurs between mid-September and mid-October.

The site is typical of many upland landscapes of the northeastern United States that have chronic, elevated deposition of atmospheric reactive nitrogen. The average wet deposition of total inorganic nitrogen from 1992 to 1998 was 13.2 kg ha⁻¹ yr⁻¹ [Campbell *et al.*, 2004]. The mixed hardwood forest cover is sugar maple (*Acer saccharum*) with some yellow birch (*Betula alleghaniensis*), white ash (*Fraxinus americana*), red spruce (*Picea rubens*), and balsam fir (*Abies balsamea*) [Thorne *et al.*, 1988]. The stream is oligotrophic with low concentrations of all nitrogen species and no detectable dissolved inorganic phosphorus. Stream nitrate patterns are consistent with a stage-one response of nitrogen saturation [Stoddard, 1994] in which concentrations are seasonally low in summer except during stormflow events when stream nitrate concentrations increase by several fold [Shanley, 2000; Sebestyen *et al.*, 2009a]. At W-9, base flow nitrate concentration has typically been between 5 and 20 μmol L⁻¹ [Ohte *et al.*, 2004; Sebestyen *et al.*, 2008, 2009a], base flow DON concentration has ranged from 1 to 10 μmol L⁻¹, and base flow ammonium concentration has typically been less than a detection limit of 2.2 μmol L⁻¹.

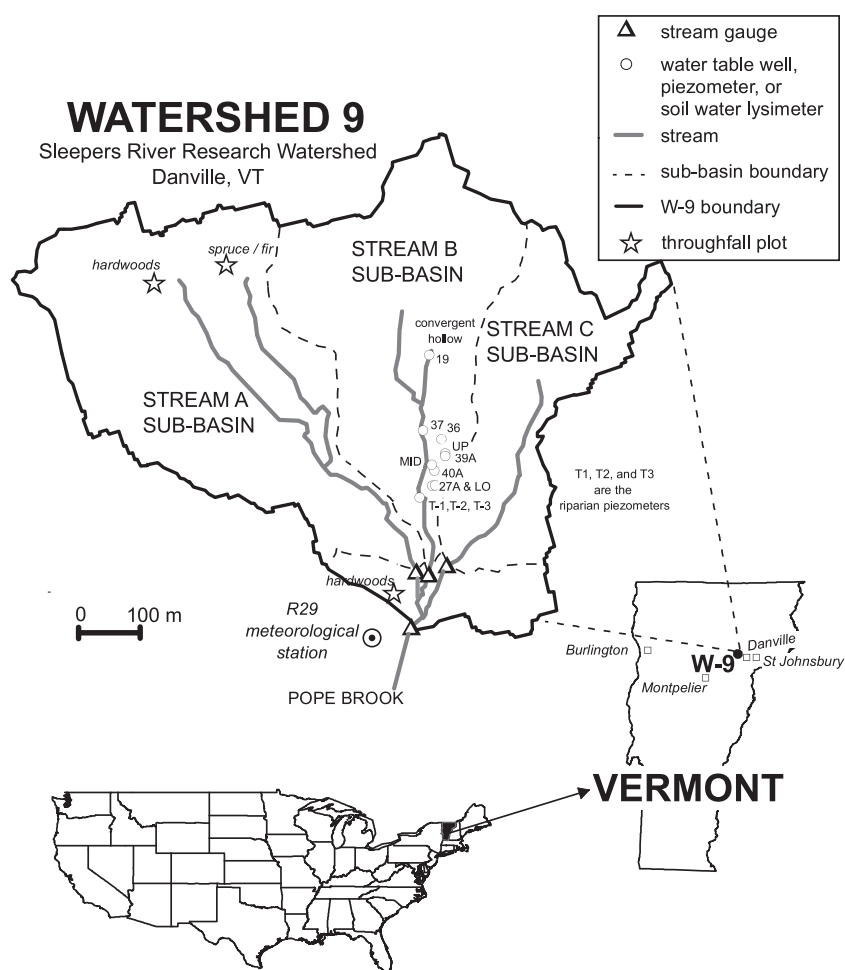


Figure 1. Map of sampling locations and landscape features. The numbers identify particular wells and piezometers.

3. Methods

Stream stage at W-9 was measured in a stilling well at the pool upstream of the 120° V-notch weir. At the A and B subbasins, stream stages were measured at 90° V-notch weirs. Stream gauging of stream C with a 60° trapezoidal flume began during our study on 7 October 2003. Specific discharges were calculated from stage-discharge relationships [Rantz, 1982].

During 2003, weekly grab samples of stream water were collected at each stream gauge. Additional grab samples were collected on other days, especially during October when stream waters were sampled several times a week. An ISCO automatic sampler at each stream gauge collected water when triggered by threshold changes in stream stage during stormflow events. Sampling intervals ranged from minutes to hours during events and samples were retrieved from automatic samplers every 1–3 days during October, which coincided with and allowed for the visual assessment of the timing of autumn leaf fall. Data from the intensive sampling during 2003 were compared to long-term data from weekly stream samples that were collected at W-9 from 1991 to 2007 and analyzed at the Durham, New Hampshire laboratory of the USDA Forest Service Northern Research Station (supporting information S2). We distinguished between base flow and stormflow samples using a digital base flow separation technique [Nathan and McMahon, 1990]. We have already described the application of this approach to W-9 streamflow data [Sebestyen et al., 2009a] and provide further information in supporting information S1.

Direct rainfall was measured with a weighing bucket collector and recorded every 5 min at a meteorological station near the W-9 stream gauge (Figure 1). Rainfall samples for chemical and isotopic analyses were

collected weekly and sometimes immediately after events (i.e., 15, 21, and 28 October 2003) from a polyethylene bucket in a wet-only precipitation collector.

In the 1990s, zero-tension soil lysimeters, recording wells, sampling wells, and piezometers were installed throughout W-9 (Figure 1) [Shanley *et al.*, 1995; Kendall *et al.*, 1999]. Water levels on upland hillslopes were recorded every 30 min by a Campbell CR10X datalogger for wells that had float and counterweight-driven potentiometers. A midhillslope recording well (site MID) was 50 m from and 8 m higher than the stream. An upslope recording well (site UP) was 70 m from and 13 m higher than the stream. To monitor the hydrological connectivity of near-surface saturation in riparian areas, groundwater levels were measured at piezometers T-1 (screened at 1.8 m) and T-3 (screened at 0.4 m), and well BW-19 with TruTrack water level recorders (www.TruTrack.com). Groundwater levels were recorded every 30 min and were manually measured at least monthly to verify the logged data.

Groundwater samples for chemistry were collected on four dates in autumn 2003 from two sites: BW-39A, a sampling well next to the UP recording well; and site LO DP where groundwater drains into a zero-tension lysimeter that is next to the LO recording well (~15 m from and 5 m higher than the stream). Samples were collected on 12 November 2003 at other locations to evaluate the spatial variation of groundwater chemistry (wells: BW-27A, BW-36, BW-37, and BW-40A; piezometers T-1, T-2, and T-3; and LO DP; Figure 1). We generally refer to groundwaters by geomorphic position relative to the stream: upper hillslope groundwater for wells BW-36, BW-37, BW-39A, and BW-40A; lower hillslope for the well BW-27A and lysimeter LO DP which are at the transition from hillslope to riparian soils; and riparian groundwater from piezometers T-1, T-2, and T-3. Wells and piezometers were purged prior to sampling to avoid collection of stagnant groundwater.

After rainfall, surficial soil water samples from the vadose zone were collected from the O horizon with zero-tension lysimeters that were next to the MID and UP wells and about 0.1 m below the soil surface. Four samples were collected at MID and two samples at UP. We refer to samples from these shallow lysimeters as surficial soil water. Samples from soil lysimeters that were deeper in the profile (0.4 and 1.0 m at MID and 1.1 m at UP) were collected for the 29 October event, the only date on which these samplers yielded water during our study.

3.1. Sample Processing and Analysis

Samples for nitrate, total dissolved nitrogen (TDN), nitrate isotope, and cation analyses were filtered through 0.45 μm membrane filters. Nitrate and nitrite concentrations were measured using suppressed conductivity detection and a Dionex IonPac AS14 column on a Dionex DX-500 ion chromatograph after auto-sampler injection with filtration through 20 μm filter caps. For a subset of the stream water samples, 10 mL aliquots were prepared for TDN analysis using the alkaline persulfate oxidation technique [Solorzano and Sharp, 1980] and measured by colorimetry on a Lachat AutoAnalyzer (QuikChem FIA⁺ 8000). Limited sample volumes precluded measurement of TDN concentration on some samples. Calcium concentrations were measured by atomic emission spectrometry on a Perkin-Elmer Elan 6000 quadrupole inductively coupled plasma mass spectrometer. The limit of detection was 0.2 $\mu\text{mol L}^{-1}$ for nitrate, 0.5 $\mu\text{mol L}^{-1}$ for TDN, and 0.5 $\mu\text{mol L}^{-1}$ for calcium.

Dissolved organic nitrogen concentrations were calculated by subtracting nitrate from TDN concentrations since nitrite was not detected. Ammonium was measured using automated continuous flow colorimetry on a Technicon AutoAnalyzer. During 2003, all the weekly samples had ammonium concentrations that were below the limit of detection (2.2 $\mu\text{mol L}^{-1}$). Because ammonium is a small component of TDN in stream waters at W-9, we assume that ignoring ammonium had a minimal effect on calculated DON concentrations of stream waters when unmeasured. Concentrations of DON were not calculated for soil waters, which likely had ammonium concentrations (not measured on any soil waters) that occasionally exceeded nitrate concentrations.

Concentrations of DOC were measured for most stream water, soil water, and groundwater samples, but not rain, by catalyzed persulfate wet oxidation on total organic carbon analyzers. Samples were filtered through 0.7 μm binder-free glass fiber filters into amber glass bottles. Limited sample volumes precluded measurement of DOC concentration for all samples.

Additional details of sample collection, preparation, and chemical analysis are provided in supporting information S3.

A subset of the water samples were analyzed for natural abundance nitrogen and oxygen isotopes in nitrate. Samples for nitrate isotope analysis were chosen to include samples before and after leaf fall with an emphasis on the peak leaf fall period and stormflow samples when stream nitrate concentrations were highest. Isotopic composition is reported in standard delta (δ) notation as the relative abundance on a permil (‰) basis

$$\delta = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1000 \quad (1)$$

where R_{sample} is the sample isotope ratio and R_{standard} is the isotope ratio of a recognized standard, Vienna Standard Mean Ocean Water for oxygen isotope values ($\delta^{18}\text{O}$) and atmospheric nitrogen gas (N_2) for $\delta^{15}\text{N}$ [Coplen, 1996]. Ten percent of samples were analyzed in duplicate.

The nitrate isotopic approach can be used to quantify amounts of atmospheric and microbial nitrate in catchment waters because nitrate in rain and snow has $\delta^{18}\text{O}$ (usually ranging from +60‰ to +100‰) that is distinguishable from that in nitrate derived from mineralization and nitrification [Durka et al., 1994; Kendall et al., 2007]. The oxygen in freshwater ($\delta^{18}\text{O}\text{-H}_2\text{O}$) typically ranges from −30‰ to +5‰ and the oxygen in air ($\delta^{18}\text{O}\text{-O}_2$) is +23.5‰ [Kendall, 1998]. The oxygen from water and air is assimilated into nitrate such that $\delta^{18}\text{O}$ -nitrate produced by nitrification typically ranges from −10‰ to +15‰ [Aleem et al., 1965; Hollocher et al., 1981; Andersson and Hooper, 1983; Hollocher, 1984; Mayer et al., 2001; Kendall et al., 2007; Spoelstra et al., 2007; Snider et al., 2009].

Nitrate isotope samples were prepared using the denitrifier method [Sigman et al., 2001; Casciotti et al., 2002] and analyzed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ on a Micromass IsoPrime mass spectrometer at the USGS Menlo Park Stable Isotopes Laboratory (MPSIL) in California. Only samples with concentrations $>1.0 \mu\text{mol NO}_3^- \text{L}^{-1}$ were analyzed, which precluded analysis of stream samples from 13 to 14 October 2003, some soil water, and most groundwater samples. All nitrate isotope samples were corrected and adjusted for exchange and fractionation against blanks and international nitrate isotopic standards USGS 34 and USGS 35 [Böhlke et al., 2003]. Analytical precision (1σ) for samples analyzed in duplicate was 0.2‰ for $\delta^{15}\text{N}$ and 0.7‰ for $\delta^{18}\text{O}$ of nitrate.

3.2. Nitrate Source Apportionment, Nitrate Transformation Modeling, and End-Member Mixing Analysis

Inputs of nitrate from nitrification (NIT) and atmospheric (ATM) sources to stream and soil water samples were calculated using a two-component mixing model

$$f_{\text{ATM}} + f_{\text{NIT}} = 1 \quad (2)$$

$$\delta^{18}\text{O}_{\text{stream}} = f_{\text{ATM}} * \delta^{18}\text{O}_{\text{ATM}} + f_{\text{NIT}} * \delta^{18}\text{O}_{\text{NIT}} \quad (3)$$

The fraction (f) from each source is identified by the ATM or NIT subscript. The value of $\delta^{18}\text{O}_{\text{ATM}}$ was set to the measured $\delta^{18}\text{O}$ of nitrate in rain water for each storm event. The value of $\delta^{18}\text{O}_{\text{NIT}}$ was assigned as the measured $\delta^{18}\text{O}$ of nitrate from nitrification in soil water or groundwater near the respective sampling date; +1.4‰ from 26 August to 27 September, −2.3‰ from 28 September to 9 November, and −4.2‰ from 10 to 29 November.

The simple isotopic mixing model (equations (2) and (3)) would only have been valid if denitrification had a negligible effect on the $\delta^{18}\text{O}$ of soil-derived nitrate. We evaluated this possibility and explored our data to make inferences about biogeochemical transformations that may have affected the concentration and isotopic composition of stream nitrate [Kendall et al., 2007].

Denitrification trends were calculated as a Rayleigh fractionation using the equations

$$\delta^{18}\text{O}_f = \epsilon * \ln \left(\frac{C_f}{C_i} \right) + \delta^{18}\text{O}_i \quad (4)$$

$$\delta^{15}\text{N}_f = \epsilon * \ln \left(\frac{C_f}{C_i} \right) + \delta^{15}\text{N}_i \quad (5)$$

where C_i was the original nitrate concentration of a stream water, soil water, or groundwater sample; and $\delta^{18}\text{O}_i$ and $\delta^{15}\text{N}_i$ of nitrate were the original isotopic values in a stream water, soil water, or riparian

groundwater sample. Values of c_r , the concentration of nitrate remaining after 0% up to 100% had been denitrified, were substituted in the equation to model the isotopic composition of the remaining $\delta^{18}\text{O}_f$ or $\delta^{15}\text{N}_f$ of nitrate. Enrichment factors (ϵ) were assumed to be -5‰ to -10‰ with slopes between 0.5 and 1 to establish bounds for $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of residual nitrate that was not denitrified [McMahon and Böhlke, 1996; Sigman et al., 2005; Kendall et al., 2007; Granger et al., 2010]. Enrichment factors of -5‰ to -10‰ with denitrification have been measured in intermittently saturated soils [McMahon and Böhlke, 2006], similar to conditions that may have occurred in the W-9 stream reach especially when base flow mostly passed through the hyporheos. If certain criteria were met, then base flow nitrate concentrations may have been lowered by denitrification, as would be reflected in the dual isotope data. In the interpretation of dual isotope data, in-stream denitrification would be detected only if there was variation of the denitrification rate over time in response to changing stream biogeochemistry during leaf fall that had resulted in

1. nitrate concentrations in base flow that progressively decreased over successive days after inputs of leaves to the stream,
2. values of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate that were within the range of values calculated for plausible end-members using equations (4) and (5), and
3. a relationship between $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of base flow samples that was linear with a slope between 0.5 and 1.0.

The dual isotope data may also provide evidence of a source area of base flow nitrate. If base flow nitrate concentration was (1) less than that of a soil water or groundwater end-member that was hydrologically connected to the stream, (2) values of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate were within the range of values calculated for a soil water or groundwater using equations (4) and (5), and (3) the source area was hydrologically connected to the stream at the time the sample was collected as evidenced by end-member mixing analysis and water levels in riparian and hillslope areas, then that nitrate may have been affected by denitrification and transported from the source area of that end-member.

Recent research shows that the $\delta^{18}\text{O}$ of nitrate produced by nitrification is controlled by complex kinetic fractionation processes as well as $\delta^{18}\text{O}$ values of the two main sources of oxygen in nitrate, ambient water ($\delta^{18}\text{O}\text{-H}_2\text{O}$), and molecular oxygen ($\delta^{18}\text{O}\text{-O}_2$) [Kool et al., 2009; Snider et al., 2009; Buchwald and Casciotti, 2010; Snider et al., 2012]. Several studies report that the $\delta^{18}\text{O}$ -nitrate produced from nitrification in forest soils may be approximated from an empirically derived formula [Kendall et al., 2007; Spoelstra et al., 2007; Snider et al., 2009], where

$$\delta^{18}\text{O}_{\text{NO}_3^-} = \frac{2}{3} \delta^{18}\text{O}_{\text{H}_2\text{O}} + \frac{1}{3} \delta^{18}\text{O}_{\text{O}_2} \quad (6)$$

Samples that were analyzed for $\delta^{18}\text{O}$ -nitrate were also analyzed to measure the oxygen isotopic composition of water ($\delta^{18}\text{O}\text{-H}_2\text{O}$). Samples for measurement of $\delta^{18}\text{O}\text{-H}_2\text{O}$ were prepared by the carbon dioxide equilibration method [Epstein and Mayeda, 1953] and analyzed on a Finnigan MAT 251 mass spectrometer; or analyzed by laser absorption spectrometry using a Los Gatos Research 908 Liquid-Water Isotope Analyzer after cross-calibration of the two techniques. Analytical precision (1σ) for $\delta^{18}\text{O}\text{-H}_2\text{O}$ on samples analyzed in duplicate was 0.1‰ using the CO_2 equilibration method and 0.2‰ using the Water Isotope Analyzer.

Since most observations of stream $\delta^{18}\text{O}$ -nitrate values deviate by some degree from the empirical relationship of equation (6) [Snider et al., 2009], we compared $\delta^{18}\text{O}$ of nitrate in stream water, groundwater, and soil water to consider the relative $\delta^{18}\text{O}$ ranges in stream water that may have originated from nitrification in-stream (equation (7)), in the subsurface with water from groundwater (equation (8)), or in surficial soils with water from the vadose zone (equation (9))

$$\delta^{18}\text{O}_{\text{NO}_3^- \text{ stream}} = \frac{2}{3} \delta^{18}\text{O}_{\text{H}_2\text{O stream}} + \frac{1}{3} \delta^{18}\text{O}_{\text{O}_2} \quad (7)$$

$$\delta^{18}\text{O}_{\text{NO}_3^- \text{ stream}} = \frac{2}{3} \delta^{18}\text{O}_{\text{H}_2\text{O groundwater}} + \frac{1}{3} \delta^{18}\text{O}_{\text{O}_2} \quad (8)$$

$$\delta^{18}\text{O}_{\text{NO}_3^- \text{ stream}} = \frac{2}{3} \delta^{18}\text{O}_{\text{H}_2\text{O soil water}} + \frac{1}{3} \delta^{18}\text{O}_{\text{O}_2} \quad (9)$$

We compared observed $\delta^{18}\text{O}$ values of stream nitrate to theoretical values to evaluate if major shifts over time indicative of changing biogeochemical transformations occurred in response to autumn leaf fall.

We used end-member mixing analysis to examine sources and source areas that affected stream nitrate concentrations [Christophersen *et al.*, 1990]. We used calcium concentrations and $\delta^{18}\text{O}$ of water as conservative tracers because the values in groundwater and soil waters of W-9 do not overlap and these two solutes are minimally affected by biological cycling on the time scales of hydrological mixing of waters from distinct flow paths during stormflow [Kendall *et al.*, 1999; McGlynn *et al.*, 1999; Doctor *et al.*, 2008]. Mixing lines between groundwater and soil water were calculated using two-component mixing models

$$f_{\text{SW}} + f_{\text{GW}} = 1 \quad (10)$$

$$C_{\text{mixed}} = f_{\text{S}} * C_{\text{S}} + f_{\text{R}} * C_{\text{R}} \quad (11)$$

The fraction (f) from each source is identified by the S subscript for the surficial soil water end-member or the R subscript for riparian groundwater. The value of C_{S} was set to the mean concentration or isotopic value of two soil water samples from the MID lysimeter that were similar in composition between two sampling dates. The value of C_{R} was assigned to and solved for both a shallow and a deep riparian groundwater sample because these two samples had considerably different compositions. We assessed the resulting diagrams to determine if mixing of water and solutes from different source areas (shallow versus deep, riparian versus hillslope) and sources (soil water versus groundwater) were plausible explanations of stream nitrate variation under base flow.

3.3. Stream Water and Solute Mass Balances

A concentration was multiplied by the stream runoff for the interval bracketing a sample to calculate period-weighted solute yields. The interval started at the midpoint between a sample and the preceding sample and ended at the midpoint between that sample and the succeeding sample. These period-weighted solute yields were prorated to estimate daily stream solute yields.

Input/output mass balances were calculated to quantify solute losses and gains in the stream reach between the upstream (A, B, and C) and W-9 gauges. Daily mass balances were calculated from 8 October 2003 when the discharge measurements resumed at stream C to 30 November 2003. Solute mass balances are reported in two ways: as yields from the stream reach per day (normalized to the area of W-9, in units of $\text{mg m}^{-2} \text{d}^{-1}$),

$$\Delta \text{yield}_{\text{reach}} = \text{output}_{\text{W-9}} - \sum \text{tributary inputs} \quad (12)$$

and as the outflow relative to the tributary inputs

$$\text{net balance (\%)} = \left(\frac{\text{output}_{\text{W-9}}}{\sum \text{tributary inputs}} - 1 \right) * 100 \quad (13)$$

The net balance normalizes data and is a relative mass flux value that shows net losses (negative values) or gains (positive values) in the stream reach during base flow that would otherwise be obscured by the exponentially larger yields that occurred during stormflow.

4. Results

4.1. Stream Chemistry During Base Flow

In 2003, leaf fall started in late September and most leaves were shed from 9 to 15 October. During that week, stream nitrate concentrations progressively decreased from 5.4 to 0.7 $\mu\text{mol L}^{-1}$ during base flow recession (Figure 2). Nitrate concentrations $<4.0 \mu\text{mol L}^{-1}$ were not observed at any other time during 2003. For the long-term data from 1991 to 2003, such low stream nitrate concentrations only occurred in September or October corresponding to the expected timing of autumn leaf fall (Figure 3). Like the W-9 stream, nitrate concentrations at the A, B, and C tributary streams decreased during peak leaf fall during 2003 (supporting information S4). Inputs of nitrate from the A, B, and C tributaries exceeded the nitrate yield from W-9 between 8 and 14 October 2003 during base flow (Figure 4). Nitrate concentrations

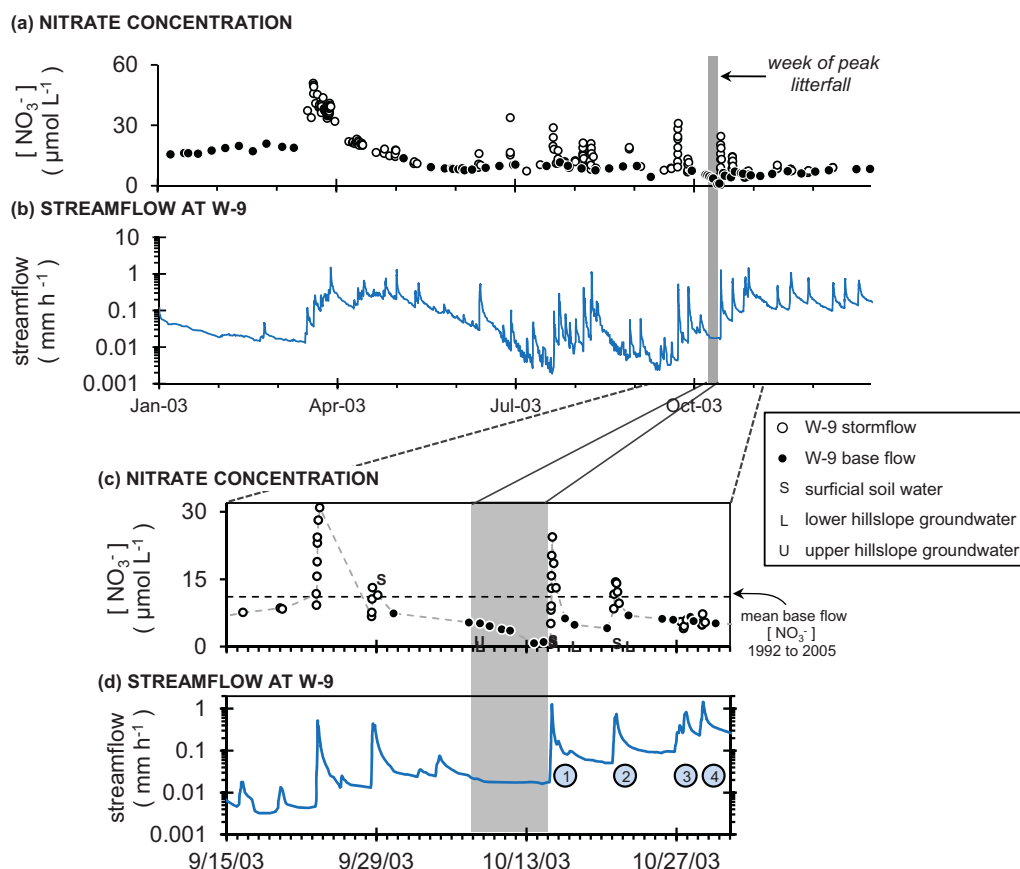


Figure 2. (a) Nitrate concentration and (b) streamflow at W-9 from January to December 2003. Nitrate concentrations were fairly stable during base flow relative to stormflow when concentrations increased during every event. Nitrate concentrations were lowest during the week of peak leaf fall and rebounded during the following stormflow event. Details of base flow and stormflow during autumn are shown in (c) and (d), with the circled numbers indicating the four closely spaced stormflow events during October.

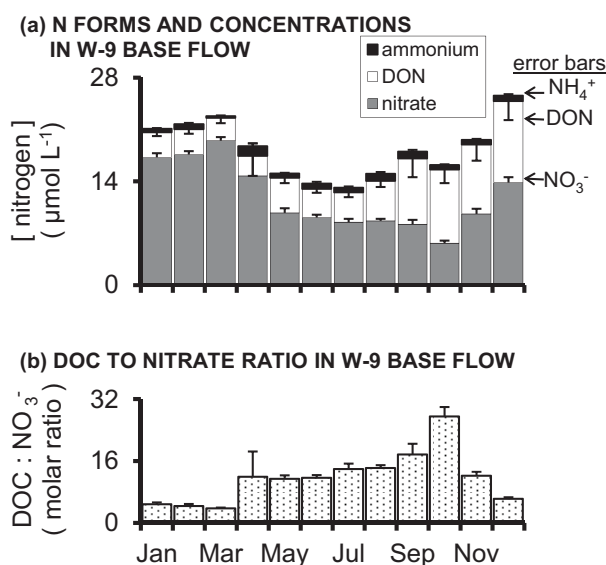


Figure 3. (a) From 1992 to 2005, the lowest nitrate concentrations and highest DON concentrations in base flow occurred in September or October when (b) organic carbon was more abundant relative to nitrate. The error bars show the standard errors of the mean monthly concentrations.

progressively decreased and net losses of nitrate (i.e., retention in the stream reach) increased each day from 21% on 8 October to 72% on 14 October (Figure 4c). In contrast, the stream reach was a net source of DON and DOC; base flow yields of DOC were 20–38% more and DON yields were 29–69% more at the W-9 stream gauge than inputs from the tributary streams (Figure 4).

Dissolved organic nitrogen was a larger component of stream TDN during and after autumn leaf fall than during the rest of the year (Figure 5). In other years, too, DON and DOC concentrations as well as the DOC to nitrate ratio (DOC:nitrate) during base flow were consistently higher after

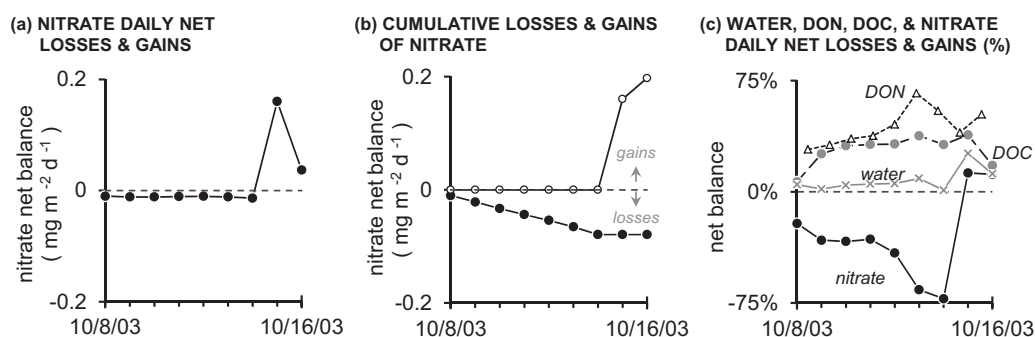


Figure 4. (a) Daily net balances of nitrate for the stream reach between the A/B/C tributaries and the W-9 stream gauge, from equation (12). (b) Cumulative losses and gains are shown for nitrate, from equation (12). (c) The daily net balance of water, nitrate, DON, and DOC are expressed as percent losses and gains in the stream reach, from equation (13).

autumn leaf fall (Figure 3). During the week of peak leaf fall, base flow DOC concentrations of $133\text{--}150 \mu\text{mol L}^{-1}$ (Figure 5) were 20–36% higher than the long-term mean of $111 \mu\text{mol L}^{-1}$.

4.2. Stream Chemistry and Catchment Hydrology During Stormflow

There were many stormflow events from September to November. Four closely spaced events during October were fairly similar in durations and peak magnitudes. These October events offer particular insight on stream nitrogen dynamics because the first event immediately followed peak leaf fall and stream N dynamics were markedly different among those four events.

Stream nitrate, DON, and DOC concentrations increased during storm events (Figure 6). The week of low nitrate concentrations at the time of peak litterfall ended when nitrate concentrations increased with stormflow on 15 October (Figure 2). For the four closely spaced October events, responses attenuated over successive events as peak concentrations were progressively lower at equal or higher streamflow (Figures 2 and 5, see supporting information S4 for nitrate details).

Riparian water table fluctuations were synchronous with streamflow (Figure 7). Water levels were higher in the deep riparian piezometer (T-1) than in the shallow piezometer (T-3), with a mean hydraulic head difference of $0.19 \pm 0.08 \text{ m}$ (ranging from 0.02 to 0.46 m). This persistent upward hydraulic gradient showed that groundwater always discharged along this portion of the stream. The highest water levels occurred during storm events when water levels in riparian piezometers and wells rapidly rose to within several centimeters of the soil surface from depths of 0.3 to 0.6 m before the events.

At the midslope well, water levels began to rise from the till and bedrock interface during late September (Figure 7). Water levels were even more damped and lagged relative to streamflow at the upslope well where water level fluctuations did not synchronize with streamflow until mid-November. Water levels were always deeper than 0.9 m below the surface at the midslope well and deeper than 0.5 m at the upslope well.

4.3. End-Member Chemistry and Isotopic Apportionment of Nitrate Sources

At the repeatedly sampled hillslope sites (BW-39A and LO), groundwater nitrate varied little from September to November relative to stream and soil waters. On 12 November 2003, when more locations were sampled than other times, groundwater nitrate concentration ranged from <0.2 to $4.7 \mu\text{mol L}^{-1}$ for hillslope wells and $4.7 \mu\text{mol L}^{-1}$ to $10.3 \mu\text{mol L}^{-1}$ in riparian piezometers (Figure 7). Nitrate and DOC concentrations and DOC:nitrate were higher in surficial soils than deeper soils (Figure 8).

Mixing diagrams show that base flow calcium and $\delta^{18}\text{O}$ -water were similar to riparian groundwater (Figure 9). In contrast, concentrations and $\delta^{18}\text{O}$ of nitrate were dissimilar between base flow and riparian groundwater for most samples. Concentrations and $\delta^{18}\text{O}$ of nitrate were dissimilar between base flow and soil waters, and base flow and upper hillslope groundwater for all samples. Concentration and $\delta^{18}\text{O}$ of base flow nitrate were not bounded by the measured end-members.

While base flow nitrate concentrations decreased and base flow DOC:nitrate increased during leaf fall, $\delta^{18}\text{O}$ of base flow nitrate increased by several permil relative to that expected if nitrate was nitrified with oxygen

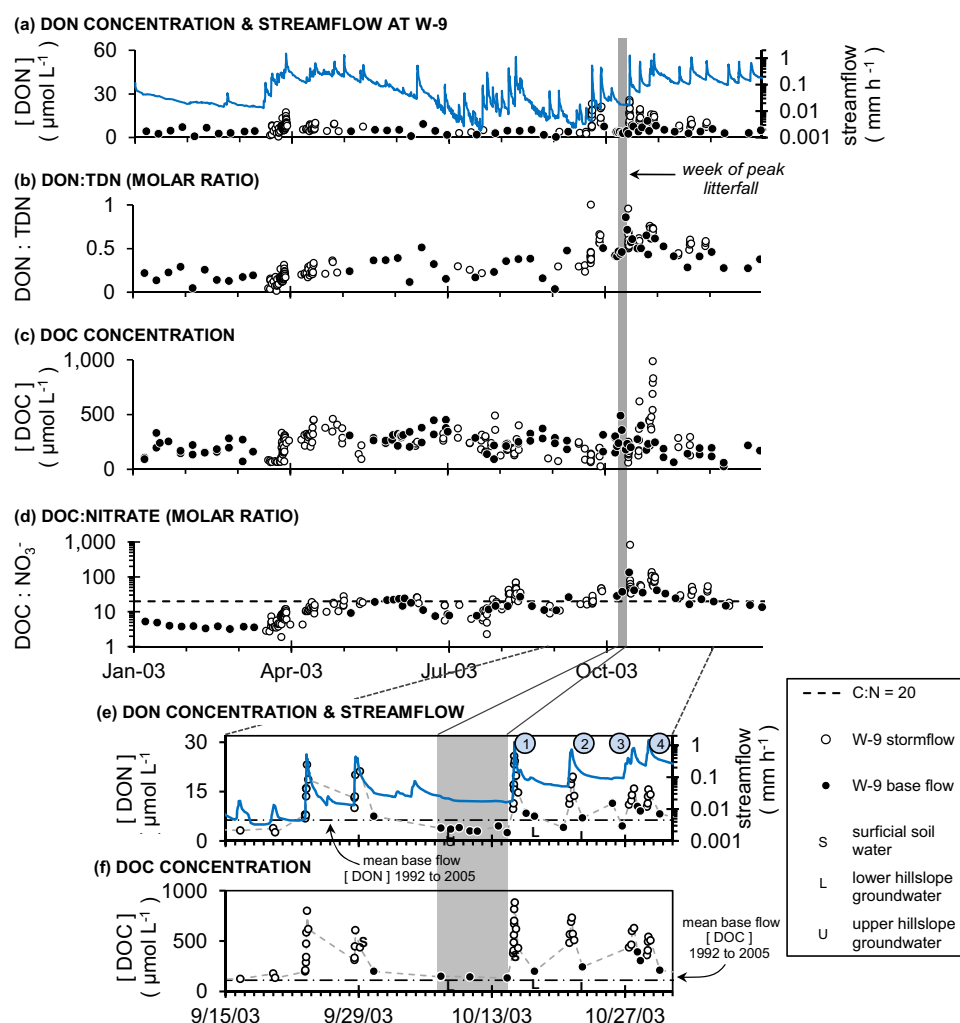


Figure 5. (a) Concentrations of DON and streamflow at W-9 from January to December 2003. (b) DON was a larger fraction of TDN during base flow and stormflow of autumn. Concentrations of DON and (c) DOC were fairly stable during base flow relative to stormflow when concentrations increased. Note that few stormflow samples from summer were analyzed for DON. (d) During and after leaf fall the DON to nitrate ratio was consistently above 20 during base flow and stormflow. Details of base flow and stormflow during autumn are shown in (e) and (f). The circled numbers show the four closely spaced stormflow events during October.

from stream water and O_2 or groundwater and O_2 (Figure 10). Throughout the study, $\delta^{15}N$ values ($1.8\text{‰} \pm 0.5\text{‰}$) of base flow nitrate were nearly invariant, except for one value of 0.7‰ on 9 October 2013 during the week of peak leaf fall (supporting information S5). The $\delta^{15}N$ -nitrate values of base flow, stormflow, groundwater, and rainfall overlapped among the water types (Figure 12; supporting information S5). Values of soil water $\delta^{15}N$ were at least 4‰ less than the values in base flow, groundwater, and rainfall.

The $\delta^{18}O$ -nitrate values of precipitation ($+70.0\text{‰}$ to $+101.3\text{‰}$) were distinct from groundwater (-4.2‰ to $+2.0\text{‰}$) and base flow (-0.5‰ to $+5.8\text{‰}$; supporting information S5). During the 21 October event, the rain nitrate concentration ($36.2 \mu\text{mol L}^{-1}$) was higher than any other rainfall event during our study (Figure 11) and stream $\delta^{18}O$ -nitrate values peaked at $+32.1\text{‰}$ (supporting information S4). As calculated from a two-component mixing model (equations (2) and (3)), a maximum of 33% of stream nitrate was unprocessed from an atmospheric source (Figure 11c). Over the entire 21 October event, 16% of the total stream nitrate yield retained the isotopic signature of unprocessed atmospheric nitrate that originated from rainfall. As stormflow receded before the next rainfall event, stream nitrate concentration and inputs of unprocessed atmospheric nitrate decreased to preevent levels (Figure 11).

While the 21 October event had the largest input of unprocessed atmospheric nitrate (33%), the 23 September, 15 October, 27 October, and 13 November events had maximum amounts of 10–13% unprocessed

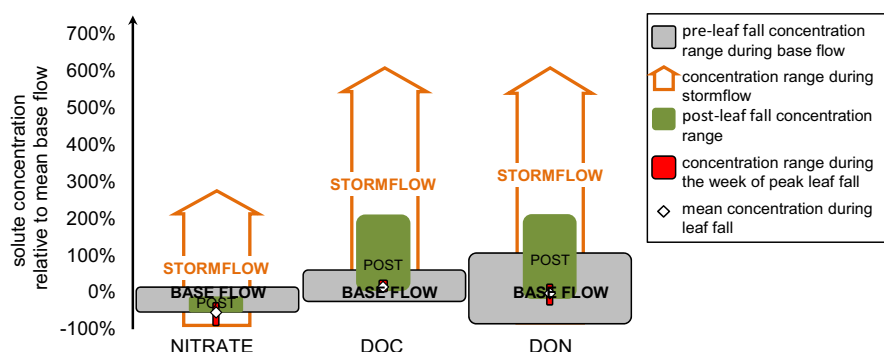


Figure 6. Variation of stream nitrate, DOC, and DON during stormflow (STORMFLOW), summer base flow (BASE FLOW), base flow during the week of peak leaf fall (LEAF FALL), and base flow after leaf fall (POST) relative to mean base flow concentrations. The words STORMFLOW, BASE FLOW, and POST as well as the diamond symbols for leaf fall are centered on the means of the relative variations while the vertical dimension of each shape marks the relative range of variation. Shape widths are scaled to the durations of base flow during summer, leaf fall, and post leaf fall as well as stormflow.

atmospheric nitrate (Figure 11c). For all other stormflow events, $\delta^{18}\text{O}$ values of stream nitrate were between -0.1‰ and $+5.6\text{‰}$, which was consistent with $>90\%$ of stream nitrate originating from nitrification in the catchment. Cumulative inputs of nitrate with wet deposition and from the apportioned sources of stream nitrate are shown in Figure 10f.

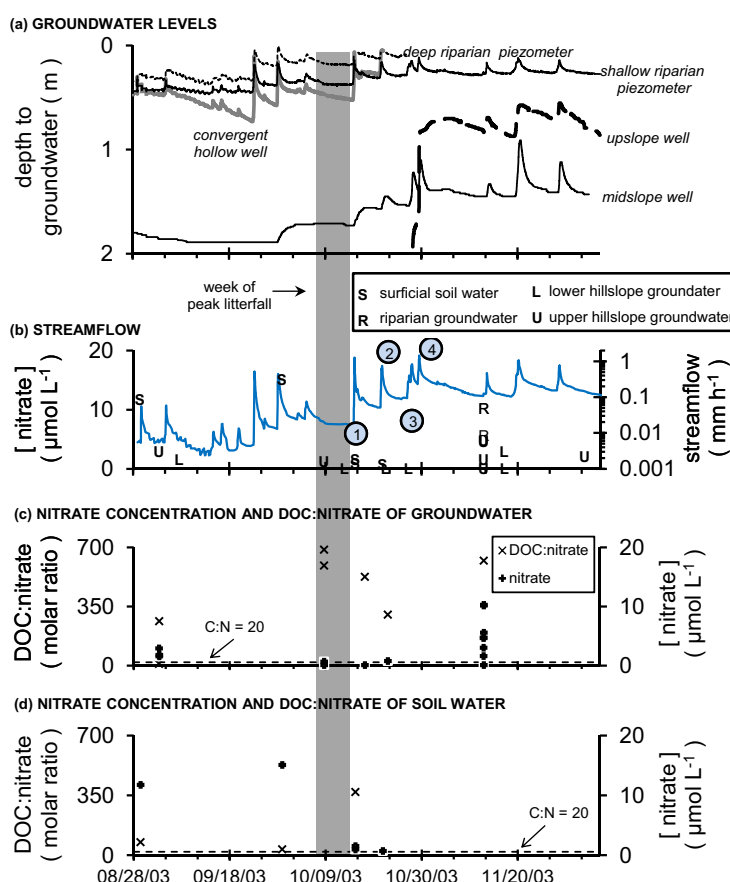


Figure 7. (a) Changes in groundwater levels at midslope and upslope sites were lagged and damped relative to near-stream areas (riparian piezometers and the convergent hollow well) where water levels synchronously fluctuated with (b) streamflow. Data gaps in the records for the deep riparian piezometer and convergent hollow reflect datalogger failures. The circled numbers show four closely spaced stormflow events during October. Patterns of nitrate concentration and DOC:nitrate varied inversely in (c) groundwater and (d) soil water. Groundwater was sampled from more sites on 12 November than the previous dates.

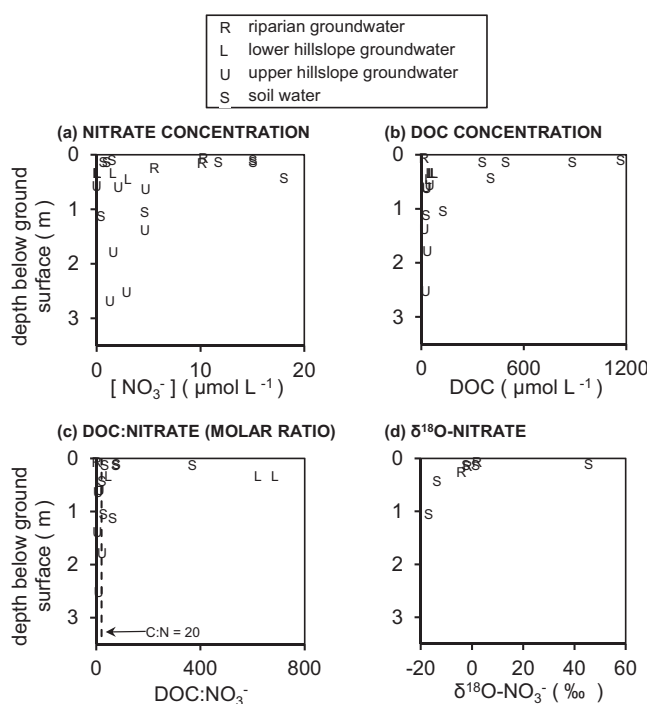


Figure 8. (a) Nitrate concentration, (b) DOC concentration, (c) DOC:nitrate, and (d) $\delta^{18}\text{O}$ of nitrate for soil water and groundwater versus depth below the soil surface.

A soil water sample was collected from each of two sites during the 30 August storm event. The $\delta^{18}\text{O}$ of nitrate in one sample was $+1.4\text{‰}$, within the range of nitrate that originated from nitrification (supporting information S5). The $\delta^{18}\text{O}$ of nitrate in the other sample was $+45.5\text{‰}$, which amounted to 38% unprocessed atmospheric nitrate. On 29 September, $\delta^{18}\text{O}$ -nitrate in surficial soil water was -2.3‰ , which amounted to 100% nitrate from nitrification. Concentrations of soil water nitrate collected after leaf fall were too low for measurement of nitrate isotopes.

5. Discussion

Base flow nitrate concentrations dramatically decreased during the week of peak litterfall, then rebounded to pre-leaf fall levels

after a storm event. In contrast, base flow DON concentrations increased after the week of peak litterfall and remained high for more than a month. Corresponding to the shifts in nitrogen speciation, base flow DON concentration increased relative to nitrate concentration and was a larger fraction of TDN compared to base flow during the rest of the year (Figure 5b). These changes reflected the effects of seasonal biogeochemical transformations and source variation on stream nitrogen chemistry.

5.1. Nitrogen Sources During Base Flow

During the peak of autumn litterfall, nitrate was retained in and DON was released from the W-9 stream network (Figure 4). Higher DON yields from the stream reach during leaf fall (Figure 4) and persistent increased base flow concentrations after leaf fall (Figure 6) from the stream reach indicated a shift in DON availability. The increased base flow concentrations and stream-reach yields of DON and DOC are easily explained by leaching of soluble organic matter from leaves that had fallen into the stream [Meyer *et al.*, 1998]. The increased DON concentrations and yields were consistent with past findings of DON having been released after microbes had colonized decomposing leaves in stream channels [Wetzel and Manny, 1972; Gosz *et al.*, 1973; Howarth and Fisher, 1976]. The measured DOC gains in the W-9 stream reach (e.g., Figure 4) show an increased DOM supply during and after autumn leaf fall, which is also consistent with past findings [Meyer *et al.*, 1998].

Biogeochemical transformations within landscapes have been important controls on nitrate and DOM supplies that have been available for transport to streams [McDowell and Likens, 1988; Aber *et al.*, 1989; Campbell *et al.*, 2004]. Surficial soils are particularly important. For example, nitrate, DON, and DOC concentrations in temperate forest soil waters have been found to be highest near the ground surface where organic matter is most abundant, inorganic nitrogen pools are largest, and mineralization rates are highest [Thurman, 1986; Garten, 1993; Nadelhoffer *et al.*, 1999; Michalzik *et al.*, 2001; Booth *et al.*, 2005; Ross *et al.*, 2012] (Figure 8). These vertical solute concentration patterns have also reflected biological processing in and solute absorption on deeper soils as water vertically flows through soils [Aiken and Cotsaris, 1995; Ohte *et al.*, 2004; Campbell *et al.*, 2006; Kothawala and Moore, 2009]. Nonetheless, surficial soils on W-9 hillslopes were unsaturated throughout autumn which precluded hydrological connectivity of these potential source areas of nitrate and DOM to streams (Figure 7). Therefore, progressive changes in stream chemistry during base flow

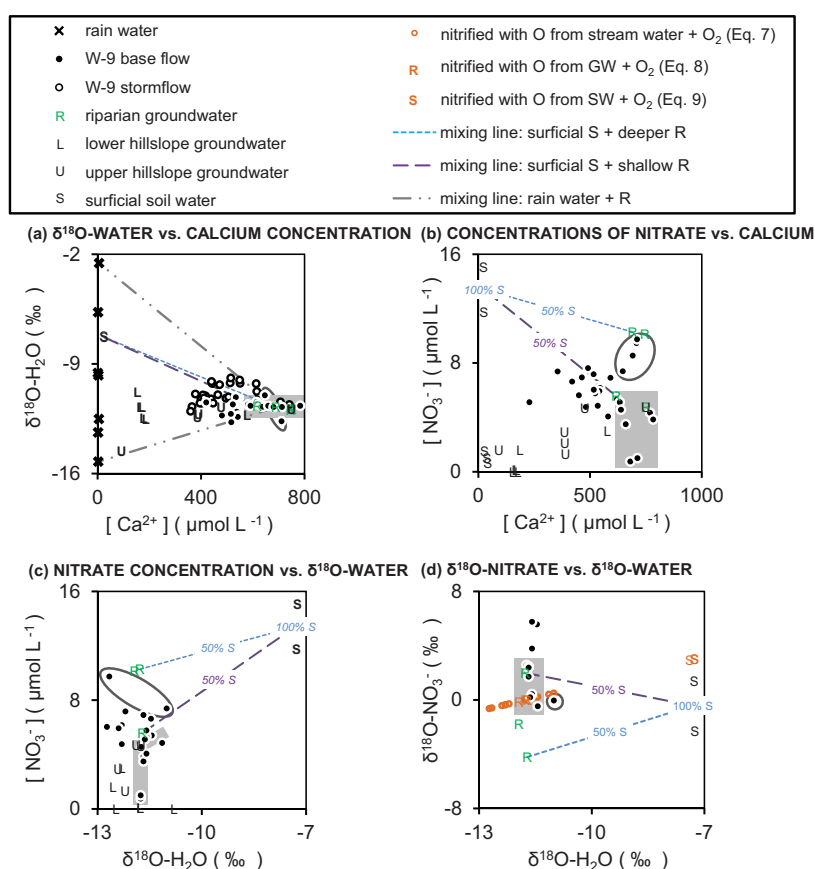


Figure 9. Mixing diagrams using (a) $\delta^{18}\text{O}$ of water and calcium concentrations, (b) nitrate and calcium concentrations, (c) nitrate concentration and $\delta^{18}\text{O}$ of water, and (d) $\delta^{18}\text{O}$ of nitrate and $\delta^{18}\text{O}$ of water. (a) The envelope of mixing lines between rain water and riparian end-members was wide because $\delta^{18}\text{O}$ of rain water was highly variable among rainfall events. More data are shown in (a) and (b), since calcium concentration was measured on most base flow samples relative to the more limited data in (c) and (d) from the subset of base flow samples that were analyzed for water and nitrate isotopes. The gray shading shows base flow samples that were collected during the week of peak leaf fall and points inside ovals were collected before peak leaf fall.

and solute losses or gains in the stream reach (Figure 4b) had to reflect changes in biogeochemical processes in the stream, hyporheic zone, or hydrologically connected riparian areas.

5.2. Nitrate Transformations During Base Flow

In-stream processes have been shown to be important seasonal regulators of stream nitrogen dynamics [Mulholland and Hill, 1997; Tank et al., 2000; Roberts and Mulholland, 2007; Valett et al., 2008; Goodale et al., 2009] and leaf fall has been found to be the time when in-stream nitrogen transformations have been highest in a temperate forested catchment [Mulholland, 2004].

Our data from W-9 show little variation of nitrogen forms and concentrations during base flow from late spring until leaf fall, and then pronounced changes during peak litterfall (Figures 2 and 5). Many inorganic nitrogen transformations were possible and transformations may have been coupled. During and after leaf fall, many factors such as increased photoautotrophy, increased heterotrophic cycling, decreased nitrification, and increased denitrification may have affected in-stream nitrogen processing during base flow. Perennially low ammonium concentrations in W-9 streams were consistent with the known rapid uptake or conversion of ammonium to nitrate [Richey et al., 1985; Mulholland et al., 2000; Tank et al., 2000; Peterson et al., 2001]. We expected minimal photoautotrophic uptake of inorganic nitrogen due to fallen leaves that shaded the W-9 streams, as observed. This expectation is consistent with findings of light limitation on in-stream gross primary production when deciduous leaf litter covered and shaded headwater streams from sunlight [Mulholland and Hill, 1997; Bernhardt and Likens, 2004; Roberts et al., 2007]. In the absence of inorganic nitrogen uptake by primary producers, leaf colonizing bacteria and fungi likely were primary sinks of inorganic nitrogen in the W-9

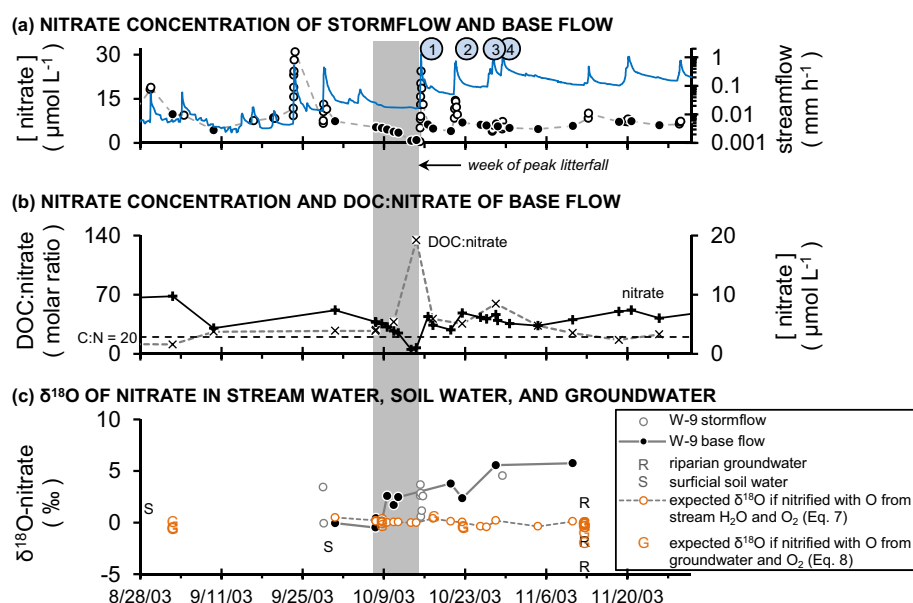


Figure 10. (a) Nitrate concentration variation in the stream. The circled numbers show the four closely spaced stormflow events during October. (b) Patterns of nitrate concentration and DOC:nitrate varied inversely in base flow. (c) $\delta^{18}\text{O}$ of base flow and stormflow nitrate including the expected $\delta^{18}\text{O}$ for stream nitrate that would have been nitrified with oxygen from H_2O and O_2 (equation (7)). Stormflow samples are shown for events with $>90\%$ nitrate that originated from nitrification.

stream reach under base flow [Kaushik and Hynes, 1971; Webster and Benfield, 1986; Sanzone et al., 2001; Gulis and Suberkropp, 2003]. Nonetheless, in-stream transformations in addition to heterotrophic cycling may explain some of the variation of stream nitrate concentrations during leaf fall.

Nitrification may have decreased during leaf fall as the organic matter supply increased and streams became more nitrogen limited. Above a C:N of about 18–21 (molar ratio), heterotrophic bacteria outcompete nitrifiers for ammonium, which limits nitrification [Strauss and Lamberti, 2000; Bernhardt and Likens, 2002]. In W-9 streams, the carbon and nitrogen stoichiometry of base flow shifted with autumn leaf fall when base flow DOC:nitrate changed from 20 or less during spring and summer to values that were always >20 during and after autumn leaf fall through 18 November (Figure 5). Nitrification was likely to have decreased with this shift in relative nitrate abundance. Importantly, consumption by assimilative uptake may have reduced stream nitrate concentration and in-stream replenishment of nitrate may have been suppressed due to decreased nitrification. This possible feedback on low base flow nitrate concentrations at W-9 is consistent with low nitrification rates that have been measured in streams during autumn leaf fall [Tank et al., 2000], and in response to DOC additions to laboratory assays or streams [Strauss and Lamberti, 2000; Bernhardt and Likens, 2002; Strauss and Lamberti, 2002].

Isotopic data from W-9 provide evidence that reduced in-stream nitrification was part of the reason why base flow nitrate concentrations decreased during autumn leaf fall. Concentrations of conservative tracers in base flow were similar to those of riparian groundwater. However, base flow nitrate concentration was too low and $\delta^{18}\text{O}$ -nitrate values of base flow and riparian groundwater were too dissimilar during peak leaf fall to have resulted from simple mixing of end-member waters (Figure 9). Nitrate concentrations, $\delta^{18}\text{O}$ -nitrate, and $\delta^{15}\text{N}$ -nitrate during base flow would not differ from those values of the riparian end-member if nitrate in base flow had originated from groundwater without further in-stream transformations. $\delta^{18}\text{O}$ of base flow nitrate when measured during winter and spring has typically been several permil higher than that in groundwater [Ohte et al., 2004; Sebestyen et al., 2008]. Before peak leaf fall, the $\delta^{18}\text{O}$ of base flow nitrate was similar to the isotopic composition expected from nitrification with oxygen from dissolved oxygen and stream water or groundwater (Figure 10c). During peak litterfall, nitrate concentrations decreased and the observed $\delta^{18}\text{O}$ of base flow nitrate increased to values that were about 2‰ higher than the expected nitrification values (Figure 10). Decreasing nitrate concentrations in base flow and higher $\delta^{18}\text{O}$ of residual nitrate without a change in $\delta^{15}\text{N}$ due to preferential uptake of nitrate with low $\delta^{18}\text{O}$ values are

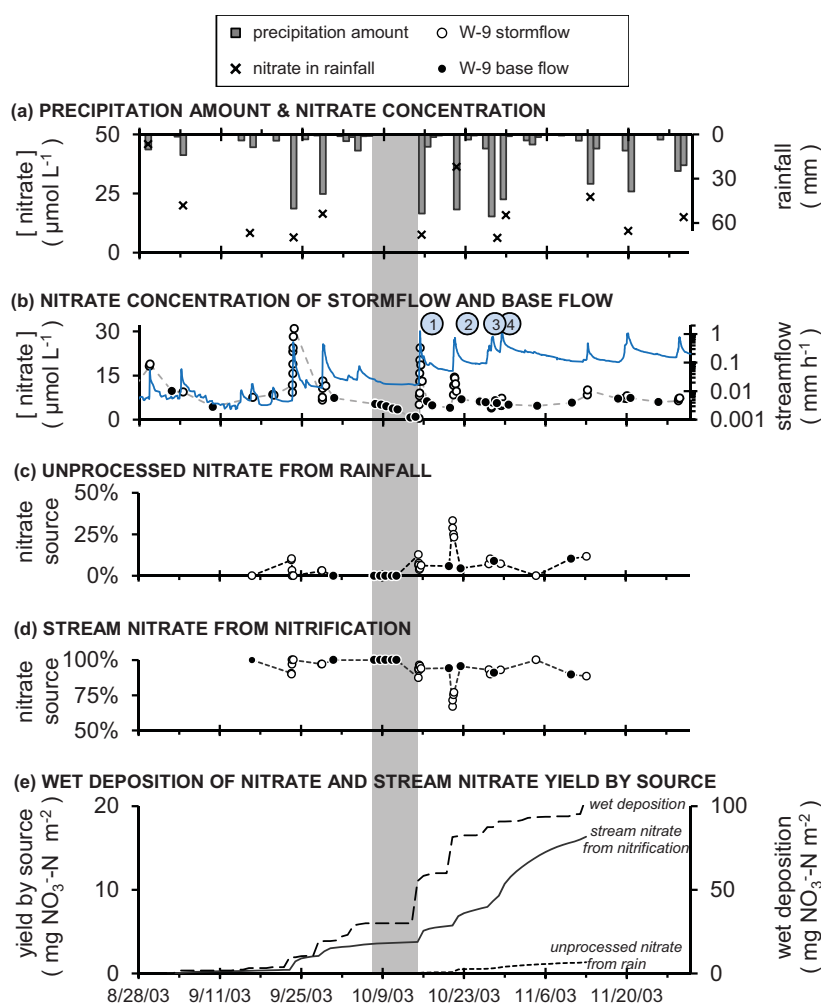


Figure 11. (a) Rainfall amount and rain nitrate concentration. (b) Nitrate concentration variation in the stream. The circled numbers show the four closely spaced stormflow events during October. Apportioned stream nitrate sources (equations (2) and (3)): (c) unprocessed nitrate from rainfall and (d) stream nitrate from nitrification. (e) Cumulative inputs with wet deposition and apportioned sources from 1 September to 12 November. The gray shading shows the week of peak litterfall.

conditions that are consistent with decreased nitrification during and after peak litterfall when base flow DOC:nitrate was well above 20 (Figure 10).

Increased denitrification along hyporheic flow paths during base flow has been speculated to occur with increased DOM availability during autumn leaf fall [Mulholland and Hill, 1997]. Addition of DOC along hyporheic flow paths has stimulated denitrification [Baker et al., 1999; Sobczak et al., 2003; Zarnetske et al., 2011]. Nonetheless, low concentrations of base flow nitrate may have limited the nitrate supply to denitrifiers [Verhagen and Laanbroek, 1991; Holmes et al., 1996; Bernhardt and Likens, 2002; Mulholland et al., 2009] and measured denitrification rates have been lowest when nitrate and ammonium concentrations have been lowest [Groffman et al., 2006; Mulholland et al., 2009]. Furthermore, when DOC:nitrate falls between 4 and 20, nitrifiers outcompete denitrifiers for nitrate, and heterotrophs outcompete both nitrifiers and denitrifiers when DOC:nitrate is >20 [Taylor and Townsend, 2010]. In the W-9 stream, nitrate was least abundant, relative to the rest of the year and relative to DOC (i.e., DOC:nitrate), during autumn leaf fall (Figure 5). Given the stoichiometric limitation that DOC:nitrate was >20 after autumn leaf fall, an increase of denitrification after autumn leaf fall was highly unlikely.

The nitrate isotope data provide further evidence that denitrification was less important as a control on stream nitrate concentrations during autumn than heterotrophic cycling and suppressed nitrification. If some nitrate remained in the stream and no other transformations had changed the nitrate isotopic composition, a linear relationship between $\delta^{18}\text{O}$ -nitrate and $\delta^{15}\text{N}$ having a slope between 0.5 and 1.0 would be

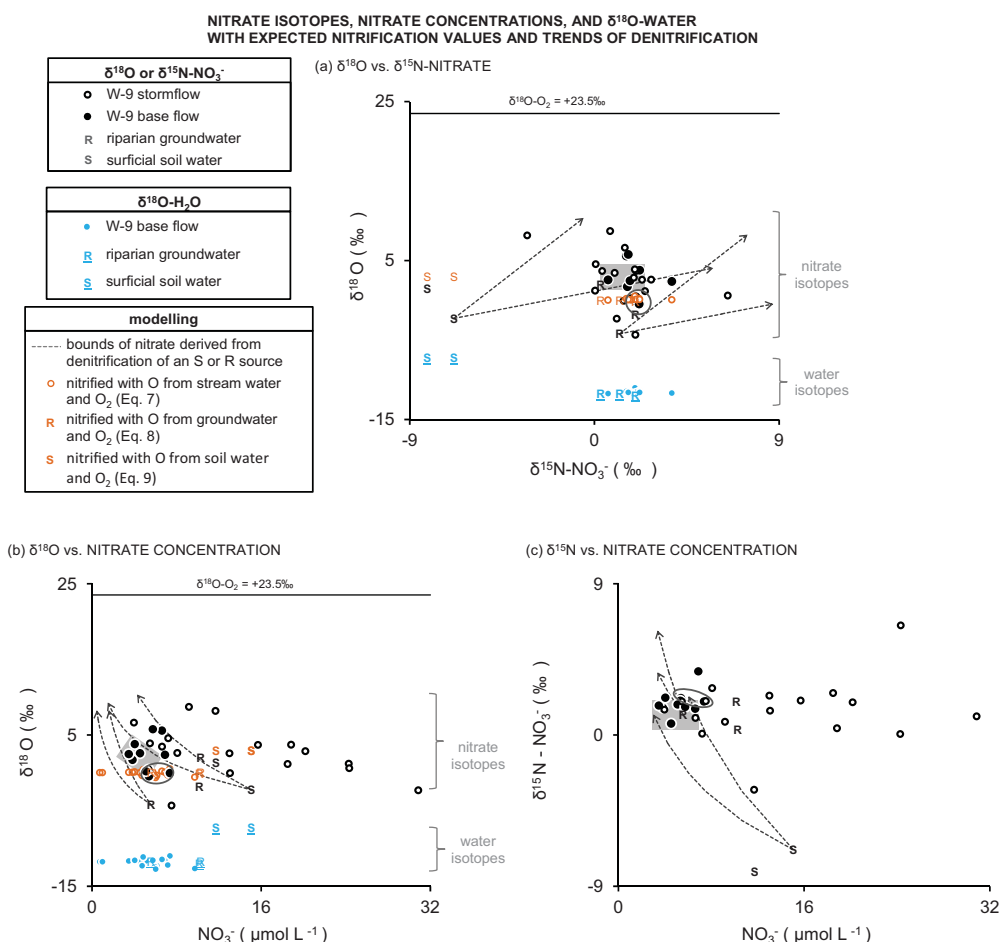


Figure 12. (a–c) Nitrate isotopes, nitrate concentrations, and $\delta^{18}\text{O}$ of water. Stormflow samples are shown for samples with $>90\%$ nitrate that originated from nitrification. The denitrification trend (from equations (4) and (5)) emerges from the shallow riparian groundwater which had a chemistry that was most similar to base flow chemistry. The other denitrification trend emerges from a soil water sample. However, soil waters were not nitrate sources during base flow when surficial soils on the hillslope were disconnected from streamflow. The gray shading shows base flow samples that were collected during the week of peak leaf fall and points inside ovals were collected before peak leaf fall.

diagnostic of rates of nitrate consuming reactions such as denitrification and uptake that had changed over the time [Böttcher *et al.*, 1990; Kendall *et al.*, 2007]. This possible diagnostic pattern did not emerge as stream nitrate concentrations progressively decreased during leaf fall (Figure 12). Furthermore, when observed in other studies, shifts in $\delta^{15}\text{N}$ of nitrate due to denitrification have been upward of $5\text{--}20\text{‰}$ [McMahon and Böhlke, 2006; Chen *et al.*, 2009; Osaka *et al.*, 2010; Cohen *et al.*, 2012; Lohse *et al.*, 2013], which greatly contrasts with the rather stable $\delta^{15}\text{N}$ values in base flow that we observed (supporting information S5). The absence of enrichment of $\delta^{15}\text{N}$ of base flow nitrate while $\delta^{18}\text{O}$ -nitrate values increased is additional evidence that rates of denitrification and uptake did not substantially increase during autumn. Since denitrification is a ubiquitous process in upland soils, groundwater, riparian areas, and stream sediments [Seitzinger *et al.*, 2006], denitrification must have occurred in W-9, especially in upland soils. Nonetheless, rates of in-stream denitrification at W-9 did not appear to have substantially increased during peak leaf fall. Thus, in-stream cycling by heterotrophs along with decreased nitrification during base flow were likely important contributors to the decrease in stream reach yield of nitrate, decrease in nitrate concentrations, and increase in $\delta^{18}\text{O}$ of base flow nitrate during peak leaf fall at W-9.

5.3. Catchment Hydrology and Stream N Transport During Stormflow

Stream nitrate concentrations rebounded after leaf fall during stormflow (Figure 2c), which shows that the timing of stormflow events will have important implications for the duration and persistence of the

seasonally low nitrate concentrations at the Sleepers River Research Watershed and other similarly nitrogen-polluted forests in temperate climates. Beyond creating a temporal limit to changes in base flow nitrogen transformations with leaf fall, stormflow events have initiated complex interactions of ecosystem processes because atmospheric deposition, nitrification in the landscape, mobilization of DOM, and hydrological transport have additively affected how various sources and source areas have been linked to stream solute dynamics [Hornberger *et al.*, 1994; Creed *et al.*, 1996; Sebestyen *et al.*, 2008]. In-stream biogeochemical transformations including net primary production [Roberts *et al.*, 2007], respiration [Roberts *et al.*, 2007], nitrification [Cooper, 1983], and nutrient uptake [Mulholland, 2004] all have typically decreased during stormflow. Thus, in-stream transformations during stormflow tend to have little or no effect on stormflow nitrogen dynamics. Rather, source variation and transport processes that link landscape areas to a stream dominate the variation of stream chemistry during stormflow.

Concentration attenuation across multiple stormflow events has typically been interpreted to indicate a limit on the amount of nitrate that was available to be transported via flushing from a forest soil to a stream. However, the stormflow nitrate concentration dynamics during the four closely spaced events in October provide evidence that interpretation of a flushing source limitation may also be confounded by source variation. Stream nitrate concentrations were higher during the 15 October event than during the next three events (Figure 2c) and flushing of nitrate that had originated from nitrification was the cause of high concentrations in the stream (Figure 11). High concentrations of nitrate, DON, and DOC must have originated from near-stream source areas during October because water levels on the hillslope were too low for flow along highly transmissive lateral flow paths in surficial soils (Figure 7) [Kendall *et al.*, 1999; McGlynn *et al.*, 1999; Shanley *et al.*, 2003]. In contrast, water levels in riparian areas rose into surficial soils during stormflow, establishing hydrological connectivity to areas where soil nitrification rates may have been higher relative to deeper soils. For example, surficial riparian soils in W-9 [Ross *et al.*, 2009] and other forested catchments [Garten, 1993] have had higher rates of nitrification than drier areas on hillslopes, implying that nitrification in surficial soils of riparian areas sometimes was a source of stormflow nitrate.

Most nitrate from atmospheric deposition has been cycled through the soil organic pool before some fraction of the input has been transported via flowing water to a stream [Durka *et al.*, 1994; Ohte *et al.*, 2004; Hales *et al.*, 2007; Sebestyen *et al.*, 2008; Curtis *et al.*, 2011; Lovett and Goodale, 2011; Mitchell, 2011] and high stormflow nitrate concentrations have mostly originated from nitrification of previously fixed nitrogen [Hales *et al.*, 2007; Barnes *et al.*, 2008; Lovett and Goodale, 2011; Mitchell, 2011]. Nonetheless, large fractions of nitrate in stormflow have occasionally originated from atmospheric nitrate in precipitation. At peak flow on 21 October, a maximum of 33% of the stream nitrate originated from an atmospheric source (Figure 11c). These inputs of unprocessed atmospheric nitrate to the W-9 stream must have occurred when rain fell to the stream channel or was rapidly transported along highly transmissive surficial flow paths in near-stream variable source areas [Sebestyen *et al.*, 2008]. Hydrological connectivity to near-stream source areas would also explain the remaining 67% of stormflow nitrate that originated from nitrification.

After 21 October, unprocessed atmospheric nitrate was about 10% or less of the stormflow nitrate and stormflow nitrate concentrations barely increased relative to the three previous storms. While atmospheric nitrate was a small component of the overall cumulative stream nitrate yield (Figure 11e), the occasional presence of atmospheric nitrate in the stream reflects conditions that we expect to be relevant to nitrogen-polluted catchments in similar hydrological settings throughout the northeastern United States: (1) an atmospheric pollutant had direct and immediate effects on stream nitrogen dynamics; (2) some nitrate bypassed biological processing in catchment soils; and (3) large inputs of unprocessed atmospheric nitrate to streams occurred during autumn despite being previously unreported and, apparently, sporadic in occurrence.

Stream nitrate that has sometimes originated from the rapid transport of atmospheric nitrate (Figure 11) [Sebestyen *et al.*, 2008; Goodale *et al.*, 2009] confounds a common conceptualization that nitrogen saturation can be assessed from elevated stream nitrate concentrations during stormflow relative to base flow and reference conditions [Murdoch and Stoddard, 1993; Stoddard, 1994]. Instead, elevated stream nitrate concentrations during stormflow sometimes reflect how water and solutes have been routed along preferential flow paths through catchments and that biological processing of atmospheric nitrate has been bypassed. As such, patterns of stream nitrate variation may not always be useful in the assessment of nitrogen saturation.

Although practical as a means to document ecosystem responses to nitrogen pollution and as evidence that atmospheric nitrate may be transported to streams when a forest is not nitrogen saturated [Sebestyen *et al.*, 2009b], inputs of unprocessed atmospheric nitrate have been poorly constrained for most events and streams for several reasons. First, high-frequency sampling with end-member analysis and natural abundance nitrate isotope tracers that is needed for accurate quantification [Ohte *et al.*, 2004] is rare. Consequently, the timing, duration, magnitude, and frequency of atmospheric inputs are mostly unknown, except for several snowmelt events. Second, although end-member mixing analysis is needed to apportion stream nitrate sources, the value of the nitrification end-member has often been unmeasured. Instead, the isotopic composition has sometimes been assumed from an empirical relationship that has often been shown to be inaccurate [Kool *et al.*, 2009; Snider *et al.*, 2009; Buchwald and Casciotti, 2010; Snider *et al.*, 2012]. Consequently, apportionments of nitrate sources may be incorrect or highly uncertain when the nitrification end-member value has been calculated rather than measured. Overall, more high-frequency, multitracer studies with end-member quantification are needed to improve scientific knowledge on the timing, locations, sources, source areas, and processes that control the variation and speciation of reactive nitrogen in forest streams.

In comparison to studies that have accurately quantified inputs of unprocessed atmospheric nitrate during snowmelt, the maximum 33% from an atmospheric source in our study was large and similar to the range (15–48%) measured during three separate snowmelt events at W-9 [Sebestyen *et al.*, 2008; Pellerin *et al.*, 2012] and maximum amounts of about 50% elsewhere in the eastern and northeastern United States [Williard *et al.*, 2001; Pardo *et al.*, 2004; Goodale *et al.*, 2009]. The finding of unprocessed nitrate during a time other than snowmelt is important documentation of the range of hydrological and ecosystem conditions during which unprocessed atmospheric nitrate may be transported to streams in catchments that are not in advanced stages of nitrogen saturation. For example, Ohte *et al.* [2004], Sebestyen *et al.* [2008], and Pellerin *et al.* [2012] presented high-frequency data that showed that the highest concentration and percentage of unprocessed atmospheric nitrate occurred days to weeks before peak streamflow during snowmelt events. The duration of autumn events was much shorter than snowmelt, yet the highest percentage of unprocessed nitrate also preceded the peak of stormflow during any particular late summer and autumn event. However, unlike snowmelt events when the highest percentage of nitrate from an atmospheric source occurred at the time of the peak nitrate concentration, the maximum percentage of stream nitrate from an atmospheric source preceded the peak nitrate concentration during late summer or autumn stormflow events.

6. Conclusions

Many studies have demonstrated seasonal and interannual patterns in stream nitrogen dynamics. However, few studies have documented chemical variation with enough temporal resolution to show stream nitrogen dynamics and how controls on the speciation and concentrations of stream nitrogen have varied under base flow conditions or during a series of rainfall-runoff events. Our findings from the Sleepers River Research Watershed show that coupling of nitrogen processing, source variation, and routing of solutes along various hydrological flow paths controls stream nitrogen dynamics over time during autumn. Furthermore, shifts in the forms and concentrations of nitrogen were rapid and extreme.

Dynamic changes of stream nitrogen chemistry show that autumn leaf fall has effects on whole-ecosystem processing of nitrogen [Mulholland and Hill, 1997; Mulholland, 2004; Roberts and Mulholland, 2007; Roberts *et al.*, 2007]. The duration of effects will be determined by organic matter supply during base flow, which changes stream stoichiometry, as well as the timing and magnitude of stormflow events. At W-9, stream nitrate concentrations rebounded when solutes were flushed along surficial flow paths in near-stream areas that transiently connected to streams. Surficial soils on hillslopes were not source areas from which high concentrations of stream nitrate or DON originated which may be common in similar temperate ecosystems until catchments rewet with autumn rains. Autumn was the only time of year when DON concentrations exceeded nitrate concentrations in the stream. This pattern is reflective of increased DON availability and higher DON inputs after leaf fall than during the rest of the year.

Quantifying nitrate sources will be critical to a general understanding of stream nitrate increases during stormflow. The nitrate stable isotope data showed that unprocessed nitrate from rainfall contributed to the

variation of nitrate concentration in soil and stream waters during certain stormflow events. Expanding upon previous studies [Spoelstra et al., 2001; Schiff et al., 2002; Ohte et al., 2004; Pardo et al., 2004; Campbell et al., 2006; Sebestyen et al., 2008; Goodale et al., 2009], the measured nitrate contributions from atmospheric sources allowed us to identify and quantify a direct link between anthropogenic nitrogen pollution and stream nitrate concentrations for a nitrogen-polluted catchment that is not in an advanced stage of nitrogen saturation. These inputs of unprocessed atmospheric nitrate controlled patterns of stream nitrate variation during some autumn storm events and the amounts were larger than commonly acknowledged for nonsnowmelt periods. The data also show subtle differences in the intrastorm timing of nitrate inputs to streams from atmospheric sources between late summer/autumn and snowmelt events.

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Supporting Information Submission for Paper 2013WR013670
Coupled hydrological and biogeochemical processes controlling variability of nitrogen species in streamflow during autumn in an upland forest.
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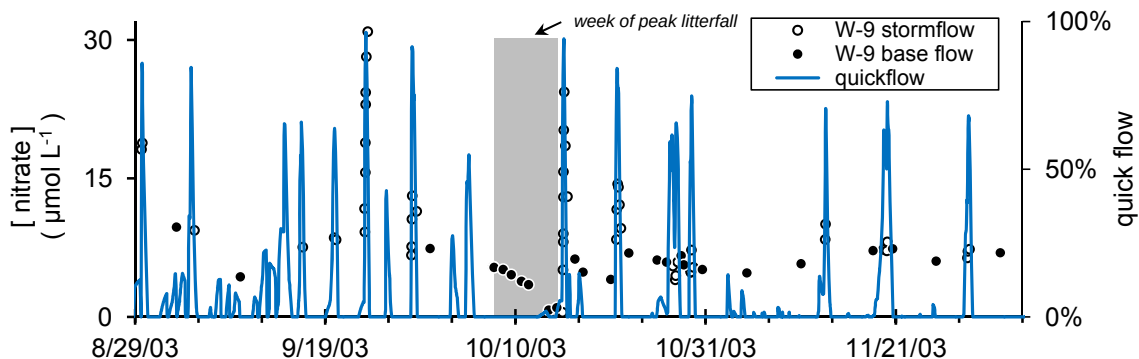
Introduction

The Supporting Information contains figures and additional information to supplement descriptions of the base flow/stormflow separation (S1); and water sample collection, preparation, and analysis (Supporting Informations S2-S3). Nitrate concentrations for the tributary streams are presented in Supporting Information S4. Nitrate and water isotope data variation over time are shown in Supporting Information S5. Solute concentration and isotopic composition data are provided in spreadsheets in Supporting Information S6. Streamflow, precipitation amount, and water level data are provided in spreadsheets in Supporting Information S7.

Supporting Information S1: Base flow and stormflow separation.

The *Nathan and McMahon* [1990] base flow recession technique was used to calculate quick flow ($q_{\text{quick flow}}$) and slow flow fractions from 30-minute streamflow data. Any sample collected when $q_{\text{quick flow}}$ was greater than 10% of streamflow was considered to have been sampled from stormflow. Mixing diagrams and review of the hydrograph showed that one sample (from 10/15/03 18:23 EST) was more appropriately considered as having been collected during stormflow.

Stream nitrate concentration shown relative to the fraction of quick flow.



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Nathan, R. J., and T. A. McMahon (1990), Evaluation of automated techniques for base flow and recession analyses, *Water Resour. Res.*, 26(7), 1465-1473.

Supporting Information S2: Long-term stream sampling and analytical methods.

For the long-term monitoring, concentrations of nitrate and ammonium were measured by automated colorimetry (continuous flow analysis on a Technicon AutoAnalyzer), TN by catalytic oxidation combustion (Antek 720C chemiluminescent nitrogen detector coupled with a Shimadzu TOC-5000A, no measurements before 1996), and dissolved organic carbon (DOC) concentrations by continuous flow analysis on a Technicon Autoanalyzer before 1996 and by combustion oxidation on a Shimadzu TOC-5000A thereafter [Bailey *et al.*, 1995].

Table of limits of detection.

Analyze	Method Detection Limits ($\mu\text{mol L}^{-1}$)
Ammonium	2.2
DOC before 1996	17
DOC after 1996	8
Nitrate	2.9
TN	1.4

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Bailey, S. W., C. T. Driscoll, and J. W. Hornbeck (1995), Acid-base chemistry and aluminum transport in an acidic watershed and pond in New Hampshire, *Biogeochemistry*, 28(2), 69-91, doi:10.1007/BF02180678.

Supporting Information S3: Additional sampling and analytical information for samples collected during this study.

Samples from ISCO samplers were usually retrieved weekly in 2003 and within twelve hours during October. Grab and ISCO samples were collected in new half-liter LDPE bottles that were first tripled rinsed with sample water, then filled, and stored refrigerated. In October, some stream nitrogen samples were syringe-filtered in the field. Filters were first flushed with sample. Bottles were then rinsed with filtered sample and then filled.

In the lab, unfiltered samples were syringe filtered into pre-cleaned sample bottles, HDPE bottles for nitrogen species and amber bottles with Teflon-lined caps for DOC analysis. All HDPE bottles were pre-rinsed and leached with deionized water (resistance exceeding $18.0 \Omega\text{ohms cm}^{-1}$).

Aliquots for nitrogen species concentrations were filtered and refrigerated. Nitrate isotope samples were filtered and then frozen until analyzed. Nitrate concentrations were measured at the EW Boyer Water Chemistry Lab. Paired unfiltered grab and field-filtered samples were collected to test for differences of filters and the effects of holding time on nitrate concentrations. Unfiltered grab and field-filtered samples were auto-injected into the ion chromatograph through $20 \mu\text{m}$ filter caps at the time of analysis. Analysis of sample pairs showed no detectable difference between field and laboratory filtering and no effect of storage time on nitrate concentrations for up to 5 years. Total dissolved nitrogen was measured in the WL Silver Ecosystem Laboratory at the University of California, Berkeley.

Whatman GF/F filters ($0.7\text{-}\mu\text{m}$ nominal pore size) used for lab filtering and glass bottles for DOC samples were pre-baked for six hours at 450° Celsius. For field filtered samples, PURA-DISC GF/F disposable in-line syringe filters were used

though these filters could not be baked. Because samples spanned the duration of several different studies of DOC, concentrations were measured at multiple laboratories: USGS MPSIL by the persulfate wet oxidation method on an OI Analytical 1010 TIC/TOC analyzer [Doctor *et al.*, 2008]; USGS, Boulder, CO by the persulfate wet oxidation method on an OI Analytical 700 TOC Analyzer [Aiken, 1992]; and the MJ Mitchell Biogeochemistry Lab, SUNY-ESF, Syracuse, NY by the UV-persulfate oxidation method on a Tekmar-Dohrmann Phoenix 8000 TOC analyzer.

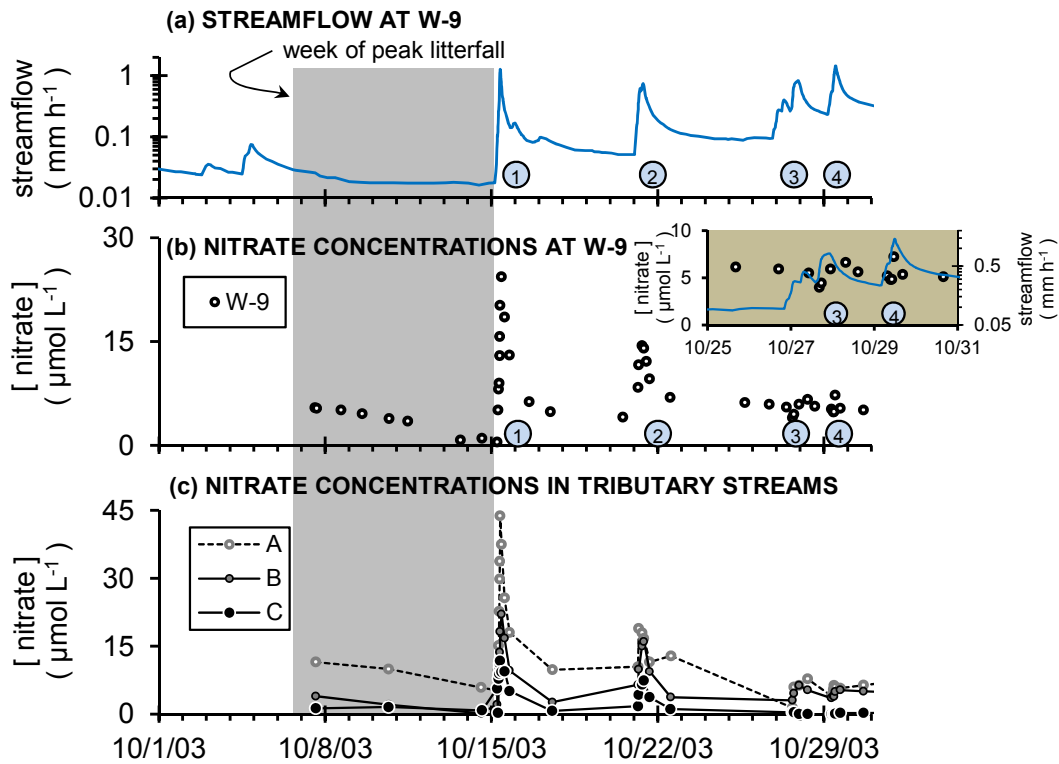
Deionized water blanks were analyzed every ten to twenty samples. No contamination of samples from collection, storage, or processing was detected from blanks.

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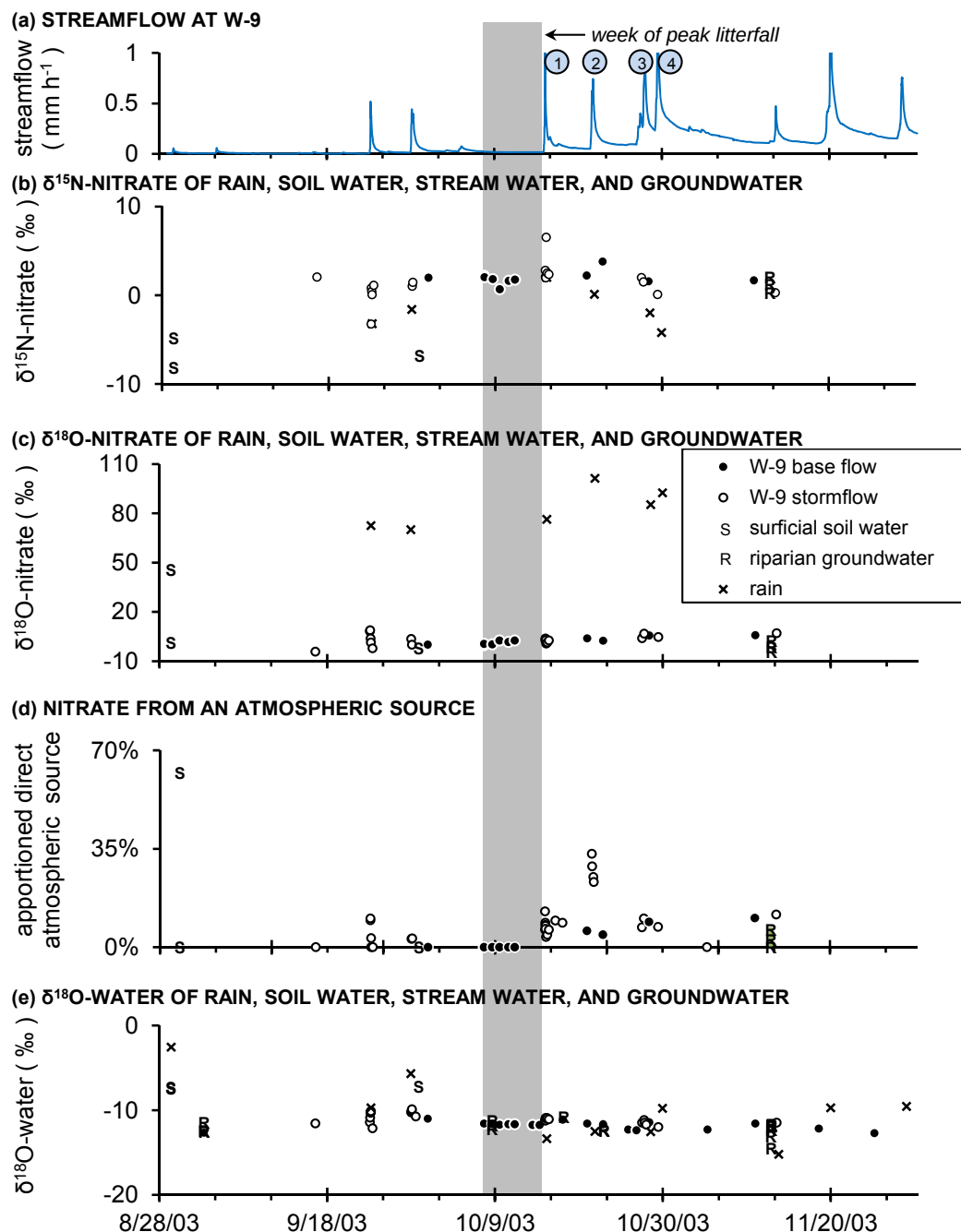
Supporting Information S4: Nitrate concentrations at the three tributary streams.

(a) Streamflow at the W-9 stream gage. At the (b) W-9 stream and (c) the three upstream tributaries, nitrate concentrations decreased from 7 to 14 October during the week of peak leaf fall. The circled numbers show the four closely-spaced stormflow events during October. The inset figure in (b) shows details of slight nitrate increases during the third and fourth storm events during October.



Supporting Information S5: Nitrate and water isotope in stream water, precipitation, soil water, and groundwater.

(a) Streamflow at the W-9 stream gage. (b) $\delta^{15}\text{N}$, and (c) $\delta^{18}\text{O}$ of nitrate in rain, soil water, stream water, and groundwater. (d) Percentages of nitrate in soil and stream waters that originated from an atmospheric source, calculated using Eq. 2 and 3. (e) $\delta^{18}\text{O}$ of water in rain, soil water, stream water, and groundwater samples.



Supporting Information S6: 2013WR013670-sup-0006-ds01.xlsx and 2013WR013670-sup-0006-ds01.pdf

Worksheet "DATA": Physical, chemical, and isotope data for each sample included in Figures 2-12 and Auxiliary Materials S4 and S5. The worksheet includes samples of stream, precipitation, soil, and ground water. Solute concentrations are reported in units of $\mu\text{mol L}^{-1}$, $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ are reported in units of permil (‰), and times are Eastern Standard Time (UTC/GMT-5 h). The method detection of limits are reported for each solute and analytical precisions are reported for isotopes.

Worksheet "MAP": Map of sampling locations and landscape features. The numbers identify particular wells and piezometers. Reproduction of Figure 1 from 2013WR013670.

Worksheet "METADATA": Metadata for chemical and isotope data. This text is reproduced from the methods section and Supporting Information S3 of 2013WR013670.

Supporting Information S7: 2013WR013670-sup-0006-ds02.xlsx and 2013WR013670-sup-0006-ds02.pdf

Worksheet "STREAMFLOW": Breakpoint streamflow data for the four stream gages: W-9, 2003 calendar year; W-9A, 9/1/2003 to 11/30/2003; W-9B, 9/1/2003 to 11/30/2003; and W-9C, 9/1/2003 to 11/30/2003. Specific discharge is reported in units of mm h^{-1} . These data are plotted in Figures 2, 5, 7, 10, and 11 as well as Supporting Information S5.

Worksheet "PRECIPITATION": Daily precipitation amounts from the weighing bucket rain gage at the R-29 meteorological station. Precipitation amounts are reported in units of mm d^{-1} . These data are plotted in 11.

Worksheet "WATER LEVELS": Breakpoint water level data for wells (BW-19, UP, and MID) and piezometers (T-1 and T-3). Water levels are m below the ground surface.

Worksheet "METADATA": Metadata for measurement of streamflow, precipitation, and water level elevations in wells and piezometers. This text is reproduced from the methods section and Supporting Information S3 of 2013WR013670.

Worksheet "MAP": Map of sampling locations and landscape features. The numbers identify particular wells and piezometers. Reproduction of Figure 1 from 2013WR013670.

All times are Eastern Standard Time (UTC/GMT-5 h)