



The importance of in-stream uptake for regulating stream concentrations and outputs of N and P from a forested watershed: evidence from long-term chemistry records for Walker Branch Watershed*

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Abstract. Long-term, weekly measurements of streamwater nitrogen and phosphorus concentrations in the West Fork of Walker Branch, a 1st order forested stream in eastern Tennessee, were used to assess the importance of in-stream processes for controlling stream concentrations and watershed exports. Over the period from 1991 to 2002, there was a slight declining trend in watershed export of dissolved inorganic N via streamflow, despite relatively high and constant wet N deposition rates (5 kg/ha/y). The watershed retains >90% of N deposition inputs. Concentrations of NO_3^- and soluble reactive phosphorus (SRP) showed distinct seasonal patterns with autumn and early spring minima and summer maxima. An end-member mixing analysis indicated that these seasonal concentration patterns were largely a result of seasonal variations in in-stream uptake processes, with net uptake of NO_3^- and SRP having the greatest impact on streamwater concentrations in November (reductions of 29 $\mu\text{g N/l}$ and 2.5 $\mu\text{g P/l}$, respectively). This was likely a result of high rates of uptake by microbes colonizing new inputs of leaf detritus. For NO_3^- there was a secondary peak in net uptake in March and April (about 9 $\mu\text{g N/l}$) resulting from increased uptake by stream algae and bryophytes. Summer was a period of net release of NO_3^- to streamwater (peaking at 9 $\mu\text{g N/l}$ in July) and minimal net effects on SRP concentrations. On average, in-stream processes resulted in removal of about 20% of the NO_3^- and 30% of the SRP entering the stream from the catchment annually. This study, as well as other recent work, suggests that in-stream processes are important buffers on stream nutrient concentrations and exports reducing the effects of changes in inputs and retention in terrestrial portions of watersheds.

Introduction

Stream chemistry reflects the cumulative effects of hydrological and biogeochemical processes occurring throughout the catchment. Understanding the processes that control stream chemistry is important for the management of

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streams and their catchments. Many studies have demonstrated the importance of terrestrial processes in regulating streamwater nutrient concentrations, and historically these concentrations have been used to infer the status of nutrient cycling and retention in the terrestrial ecosystems drained by streams (Likens et al. 1977). In studies of forest nitrogen dynamics, stream NO_3^- concentrations have been used to assess effects of atmospheric nitrogen deposition and nitrogen saturation status (Aber et al. 1991, 2003; Stoddard 1994; Swank and Vose 1997; Lovett et al. 2000), effects of natural perturbations such as climate extremes (Mitchell et al. 1996; Aber et al. 2002) and insect defoliation (Swank 1988; Eshleman et al. 1998), and effects of historical land use (Goodale et al. 2000). However, often implicit in these studies is the assumption that processes in or near the stream minimally influence stream chemistry. This assumption is being challenged by recent reports of substantial in-stream retention of N (Alexander et al. 2000; Peterson et al. 2001; Seitzinger et al. 2002)

Investigations that have focused on the role of processes near and within streams have shown that these processes can have substantial effects on the concentrations and flux of nutrients in stream water leaving catchments. Nitrogen uptake and denitrification in the riparian zones of forests can reduce NO_3^- concentrations in drainage water entering streams (Groffman et al. 1996; Hill 1996). In-stream processes also can result in considerable reduction of nutrient concentrations in stream water. Mass balance studies of headwater streams or stream reaches have demonstrated prolonged periods of net retention of nutrients (Meyer and Likens 1979; Grimm 1987; Mulholland 1992; Burns 1998). Experimental nutrient tracer additions to streams have demonstrated high rates of net uptake during some seasons, particularly in early spring when light levels and autotrophic demand are high and in autumn after leaf input when heterotrophic demand is high (Mulholland et al. 1985, 2000; Tank et al. 2000). In a model of stream nitrogen dynamics based on N cycling rates determined from a series of ^{15}N -tracer additions to headwater streams across North America, Peterson et al. (2001) showed that in-stream processes could reduce the concentrations and flux of inorganic N by 50–75%. A simulation study of nitrogen fluxes in the Mississippi River basin also indicated considerable in-stream retention of N, with increasing retention rates with decreasing stream depth (Alexander et al. 2000).

Reports of the strong influence of in-stream processes on the chemistry of stream water in forested catchments are not surprising given the hydrological and chemical characteristics of many of these streams. Forest streams receive large inputs of carbon-rich but nutrient poor organic materials that support high rates of fungal and bacterial productivity and a large demand for N and P (Triska and Buckley 1978; Mulholland et al. 1984). High rates of primary production during periods of high light availability can also exert large demands for N and P (Hill et al. 2001). In headwater streams where water depths are shallow, high surface: volume ratios enhance the influence of biotic and abiotic processes on water. Accumulation of alluvial sediments can produce relatively large subsurface zones (hyporheic zones) with low water

velocities and chemical conditions favoring denitrification and other nutrient transformations not important in other environments. A number of studies have documented the importance of hydrologic exchange between surface and hyporheic zones for nutrient retention in streams (Triska et al. 1989; Valett et al. 1996; Mulholland et al. 1997).

In this paper, I focus on the effects of in-stream processes as controls on the concentration and flux of nutrients in stream water draining Walker Branch Watershed, a forested catchment in eastern Tennessee. In a previous report, Mulholland and Hill (1997) used an end-member mixing approach to evaluate the importance of in-stream biogeochemical processes versus hydrological and biogeochemical processes in terrestrial portions of the catchment for controlling nutrient concentrations in Walker Branch. The earlier report used data from 1991 to 1995 and found that streamwater concentrations of NO_3^- and soluble reactive phosphorus (SRP) were substantially lower during some seasons and somewhat higher during other seasons than predicted concentrations based on variations in catchment flowpaths and differences in nutrient concentrations among those flowpaths. In the study reported here we extend the earlier analysis using streamwater data for a 12-year period (1991–2002) and a more detailed analysis of seasonal variations in the effect of in-stream processes on N and P concentrations and fluxes.

Study site

The study was conducted in the West Fork of Walker Branch, a forested watershed drained by a 1st order stream on the US Department of Energy's Oak Ridge National Environmental Research Park (latitude $35^{\circ}58'N$, longitude $84^{\circ}17'W$). The climate is typical of the humid southern Appalachian region, with mean annual temperature of $14.5^{\circ}C$ and mean annual precipitation of 1400 mm distributed relatively evenly throughout the year. The West Fork of Walker Branch (hereafter referred to as Walker Branch) drains a 38.4 ha catchment underlain by siliceous dolomite that has weathered to form deep soils with abundant chert (McMaster 1963). Soils are acidic (pH 4.2–5.0) and low in exchangeable bases, nitrogen, and phosphorus (Johnson 1985). Surface infiltration rates are high resulting in little overland flow even during the most intense storms (Luxmoore 1983). Although surface soils have high hydraulic conductivity due to high macroporosity, hydraulic conductivity declines sharply in the B horizon and zones of perched saturation develop in the surface soil layers producing rapid lateral subsurface flow during larger storms (Mulholland et al. 1990; Wilson et al. 1991). The forest vegetation is of mixed age and consists primarily of oaks, red maple, yellow poplar, and pine (Johnson 1985).

The perennial stream is 1st order and is fed by several springs with relatively constant discharge and chemical composition (Mulholland 1992). Discharge is monitored at a site approximately 300 m downstream from the headwaters at a 120° v-notch weir with 15-min stage recordings. Precipitation in the catchment

is recorded hourly at two locations using weighing bucket collectors (prior to 1999) and electronic tipping bucket collectors thereafter.

Methods

Streamwater samples were collected weekly about 60 m upstream from the weir on Tuesdays between 0900 and 1200 h. Measurements of water temperature and specific conductance (Orion model 122) were made *in situ*. Samples for chemical analysis were collected in well-rinsed polyethylene bottles, immediately returned to the laboratory, and filtered (Nuclepore polycarbonate filters, 0.4- μm pore size) within 3 h of collection. Samples for major cations were acidified after filtration (0.5% HNO_3) and concentrations of Ca^{2+} and Mg^{2+} were determined by inductively coupled plasma emission spectrometry. Samples for Cl^- and SO_4^{2-} were refrigerated after filtration and analyzed by ion chromatography. Samples for N and P were frozen after filtration until analysis could be performed. Concentrations of NH_4^+ were determined by phenate colorimetry (American Public Health Association, APHA 1992) and $\text{NO}_2^- + \text{NO}_3^-$ by Cu–Cd reduction followed by azo dye colorimetry (APHA 1992), both using a Bran Lubbe autoanalyzer (TRAACS Model 800 or AA3). Because stream water was always relatively high in dissolved oxygen concentration (>6 mg/l) and because spot checks revealed minimal NO_2^- concentrations (<2 $\mu\text{g N/l}$), hereafter we refer to measurements of $\text{NO}_2^- + \text{NO}_3^-$ as NO_3^- . Concentrations of total dissolved nitrogen (TDN) were determined by UV/persulfate oxidation followed by NO_3^- analysis as described above prior to 1999 and by high temperature combustion (Shimadzu TNM-1), thereafter. Concentrations of dissolved organic nitrogen (DON) were determined as the difference between TDN and total inorganic N ($\text{NH}_4^+ + \text{NO}_3^-$) concentrations. Concentrations of SRP were determined by the ascorbic acid–molybdenum blue method (APHA 1992) using a 10-cm spectrophotometer cell to achieve low detection limits. Concentrations of total dissolved phosphorus (TDP) were determined by persulfate digestion followed by SRP analysis. Concentrations of dissolved organic phosphorus (DOP) were determined as the difference between TDP and SRP.

Annual outputs of N and P from the watershed via streamflow were determined by computing the discharge-weighted annual average concentration and multiplying by total annual discharge. Annual inputs of N to the watershed were determined from weekly collections of precipitation (National Atmospheric Deposition Program) at one location within the watershed.

End-member mixing analysis

A previous study of Walker Branch Watershed showed that Ca^{2+} and SO_4^{2-} concentrations could be used as conservative water tracers in an end-member

mixing analysis to distinguish among the dominant drainage water flow paths through the catchment (Mulholland 1993). This analysis indicated that stream discharge in Walker Branch is primarily a mixture of three subsurface water sources or flow paths (Figure 1): (1) bedrock zone water – water within bedrock fissures and cavities emerging as perennial springs with high Ca^{2+} (32 mg/l) and low SO_4^{2-} (1.6 mg/l) concentrations, (2) saturated zone water – water within the permanently saturated zone immediately above the bedrock and having low Ca^{2+} (4.3 mg/l) and low SO_4^{2-} (3.1 mg/l) concentrations, and (3) vadose zone water – water within transient saturated zones in the soil perched well above the permanent groundwater table with low Ca^{2+} (2.4 mg/l) and high SO_4^{2-} (8.5 mg/l) concentrations. The high vadose zone water SO_4^{2-} concentrations are the result of relatively high rates of atmospheric S deposition (mean wet S deposition rate of 8.8 kg/ha/y over the period from 1991 to 2002).

The end-member Ca^{2+} and SO_4^{2-} concentrations were means of samples collected over 6–12 month periods from two relatively constant flow springs for bedrock zone water, from a well within the permanently saturated zone near the stream headwaters for saturated zone water, and from a subsurface flume that intercepted lateral flow in the upper 3 m of soil on the upper hillslope for vadose zone water (Mulholland 1993). There was no evidence of seasonality in the end-member Ca^{2+} and SO_4^{2-} concentrations and coefficients of variation were 4 and 2% for bedrock zone water, 21 and 14% for saturated

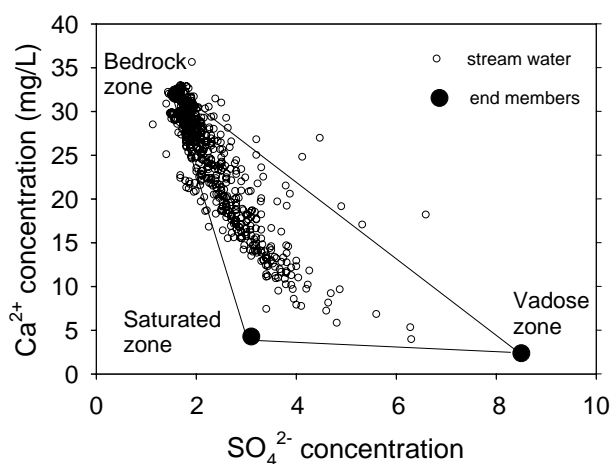


Figure 1. End-member mixing diagram for Walker Branch showing concentrations of Ca^{2+} and SO_4^{2-} in stream water and in the three dominant flow paths (water sources) contributing to stream discharge. The end-member Ca^{2+} and SO_4^{2-} concentrations were means of samples collected from two relatively constant flow springs for bedrock zone water, from riparian groundwater wells for saturated zone water, and from a subsurface flume that intercepted lateral flow in the upper 3 m of soil on the upper hillslope for vadose zone water (see Mulholland 1993).

zone water, and 7 and 22% for vadose zone water for Ca^{2+} and SO_4^{2-} , respectively.

The fractional contributions (f_1 , f_2 , and f_3) of each of these end-member water sources to stream discharge were determined on all dates that stream water chemistry was measured by solving the following set of equations:

$$[\text{Ca}^{2+}]_1 f_1 + [\text{Ca}^{2+}]_2 f_2 + [\text{Ca}^{2+}]_3 f_3 = [\text{Ca}]_S \quad (1)$$

$$[\text{SO}_4^{2-}]_1 f_1 + [\text{SO}_4^{2-}]_2 f_2 + [\text{SO}_4^{2-}]_3 f_3 = [\text{SO}_4^{2-}]_S \quad (2)$$

$$f_1 + f_2 + f_3 = 1 \quad (3)$$

where subscripts 1, 2, and 3 refer to the three different end-member water sources, the subscript S refers to stream water, and $[\text{Ca}^{2+}]$ and $[\text{SO}_4^{2-}]$ are the concentrations of these ions in water. While both the bedrock zone and saturated zone water sources contribute to stream discharge under all flow conditions, the importance of the former is greatly reduced and the latter greatly increased at higher discharge. The existence of three flow paths in Walker Branch was also supported by analysis of stream discharge recession curves following storm events (Mulholland 1993).

The concentrations of NO_3^- and SRP in water from the different flow paths vary considerably, based on samples collected at the same locations as the end-member Ca^{2+} and SO_4^{2-} samples and presented by Mulholland and Hill (1997). Concentrations of NO_3^- (mean = 5 $\mu\text{g N/l}$, SD = 7 $\mu\text{g N/l}$) and SRP (mean = 0.9 $\mu\text{g P/l}$, SD = 0.7 $\mu\text{g P/l}$) were lowest in the vadose zone, based on volume-weighted mean concentrations for four events generating flow through the subsurface flume. Concentrations were slightly higher in the saturated zone, as determined from well samples collected on four dates (NO_3^- : mean = 7 $\mu\text{g N/l}$, SD = 3 $\mu\text{g N/l}$; SRP: mean = 1.7 $\mu\text{g P/l}$, SD = 0.7 $\mu\text{g P/l}$). Concentrations were highest in the bedrock zone, as determined from 54 samples collected from a spring over an 18-month period (NO_3^- : mean = 46 $\mu\text{g N/l}$, SD = 3 $\mu\text{g N/l}$; SRP: mean = 5.0 $\mu\text{g P/l}$, SD = 0.7 $\mu\text{g P/l}$).

Differences in flowpath NO_3^- and SRP concentrations appear to be the result of biological processes in soil and geochemical processes at bedrock surfaces. The low vadose and saturated zone NO_3^- and SRP concentrations are likely the result of high rates of microbial and plant uptake in the highly nutrient deficient forest soils throughout the year (Mulholland et al. 1990). High bedrock zone P concentrations are likely due to sources in the dolomite bedrock, as indicated by high correlation between SRP and Ca^{2+} concentrations in water discharged at springs and measurements of P in samples of dolomite collected from outcrops (Mulholland 1992). Nitrate has been reported in carbonates in other areas (Chalk and Keeney 1971) and the high

bedrock zone NO_3^- concentrations may also be due to bedrock sources in Walker Branch.

There does not appear to be much seasonal variation in flowpath NO_3^- and SRP concentrations in Walker Branch. Mean volume-weighted vadose zone concentrations for samples collected in June ($\text{NO}_3^- = 3 \mu\text{g N/l}$; $\text{SRP} = 1.7 \mu\text{g P/l}$) were similar to mean volume-weighted concentrations for samples collected in January ($\text{NO}_3^- = 1 \mu\text{g N/l}$; $\text{SRP} = 0.4 \mu\text{g P/l}$) and March ($\text{NO}_3^- = 8 \mu\text{g N/l}$; $\text{SRP} = 0.8 \mu\text{g P/l}$). There was little seasonal variation in bedrock zone NO_3^- and SRP concentrations as well, as is evident from the low coefficients of variation for samples collected from the springs (6 and 14% NO_3^- and SRP, respectively). Seasonal variation in saturated zone NO_3^- and SRP concentrations is unknown because all samples were collected during the dormant season.

The net effects of in-stream biogeochemical processes on stream NO_3^- or SRP concentrations were assessed by comparing the stream concentrations predicted from the flowpath analysis with those measured on each sampling date (either as observed/predicted ratios or differences). Predicted stream NO_3^- or SRP concentrations based on the flowpath analysis are considered to be the expected stream concentrations if only hydrological and terrestrial biogeochemical processes regulate stream chemistry. The predicted NO_3^- or SRP concentrations were determined by calculating the fraction of stream discharge contributed by each flow path (f_1, f_2, f_3) using the Ca^{2+} and SO_4^{2-} concentrations of each streamwater sample, and then multiplying the flowpath NO_3^- or SRP concentrations by the flowpath fractions and summing the products. Data for dates with stream discharges $> 50 \text{ l/s}$ (11 data points) were excluded from the analysis of seasonal trends because it is likely that in-stream processes would have minimal effect on nutrient concentrations due to short water travel times during large storms. A more thorough description of this analysis and flowpath chemistry details is given in Mulholland and Hill (1997).

The annual average net effect of in-stream processes on NO_3^- or SRP exports from the catchment was determined by calculating average monthly observed/predicted concentration ratios from the stream sample data, weighting the average monthly ratios by average monthly stream discharge over the period of record, and summing the monthly discharge-weighted ratios. To provide an estimate of uncertainty, observed/predicted concentration ratios were also calculated using uncertainty ranges in predicted stream NO_3^- and SRP concentrations determined using the approach of Genereux (1998). This involved calculating the uncertainty (70%) in the three flowpath fractions, based on the measured variation in the end-member Ca^{2+} and SO_4^{2-} concentrations as described by Genereux (1998), and in the end-member nutrient (NO_3^- or SRP) concentrations for each flowpath component (calculated as the t value \times SD for each nutrient in each flowpath). The uncertainty in the predicted stream NO_3^- or SRP concentrations ($W_{N_{\text{pred}}}$) was then calculated as follows using the general framework for propagating uncertainty described by Genereux (1998)

$$W_{N_{\text{pred}}} = \sqrt{(N_1 W_{f1})^2 + (f_1 W_{N1})^2 + (N_2 W_{f2})^2 + (f_2 W_{N2})^2 + (N_3 W_{f3})^2 + (f_3 W_{N3})^2} \quad (4)$$

where the f terms refer to the fraction of streamflow due to each flow path and the numbered subscripts to the three different flowpaths, the W terms refer to the uncertainty in the flowpath fractions (W_{f1} , W_{f2} , and W_{f3}) or the flowpath nutrient concentrations (W_{N1} , W_{N2} , and W_{N3}), and the N terms refer to the flowpath nutrient concentrations (either NO_3^- or SRP). Observed/predicted concentration ratios were then calculated using predicted concentrations $\pm W_{N_{\text{pred}}}$ for each nutrient N (NO_3^- or SRP) and each stream sampling date.

Results

Although precipitation is relatively uniform seasonally, daily discharge is strongly seasonal in Walker Branch, with higher baseflows and larger and more frequent stormflow events during the winter and spring months than during summer and autumn (Figure 2). The seasonality in discharge is the result of high rates of evapotranspiration by the deciduous forest producing large soil water deficits during the growing season.

Watershed input–output budgets for N indicate that <10% of the wet deposition inputs of N are exported as DIN via streamflow (Figure 3a). Although there was no significant trend in annual wet N deposition rate (mean of 5.1 kg/ha/y), there was a slight downward trend in annual DIN output in streamflow over the 12-year period (-0.013 kg/ha/y, $p = 0.037$). There are no measurements of P input, but P outputs varied between 0.015 and 0.08 kg/ha/y, and there were no significant trends with time (Figure 3b). Annual DIN and SRP outputs were positively related to annual runoff (r of 0.59 and 0.93, respectively).

The records of streamwater concentrations of N and P indicate no trends over time (Figure 4), although it appears that the annual minima in NO_3^- concentration may be declining slightly (Figure 4b). There was considerable and repeatable seasonal variation in the concentrations of NO_3^- and SRP (Figure 4c), however. The highest concentrations of NO_3^- and SRP occurred in summer and early autumn (NO_3^- : 50–60 $\mu\text{g N/l}$, SRP: 5–6 $\mu\text{g P/l}$). Lowest concentrations occurred in mid–late autumn, when concentrations were near the limits of detectability (1 $\mu\text{g N/l}$ and 0.5 $\mu\text{g P/l}$ for NO_3^- and SRP, respectively). A second concentration minimum usually occurred in early spring. There is some evidence of seasonality in NH_4^+ concentration (Figure 4a), with somewhat higher concentrations in summer than winter. NH_4^+ concentrations remained well below NO_3^- concentrations, however, except in late autumn when NO_3^- was near detection limits. DIN:SRP ratios (Figure 4d) were lowest in late autumn (usually <10) because the declines in NO_3^-

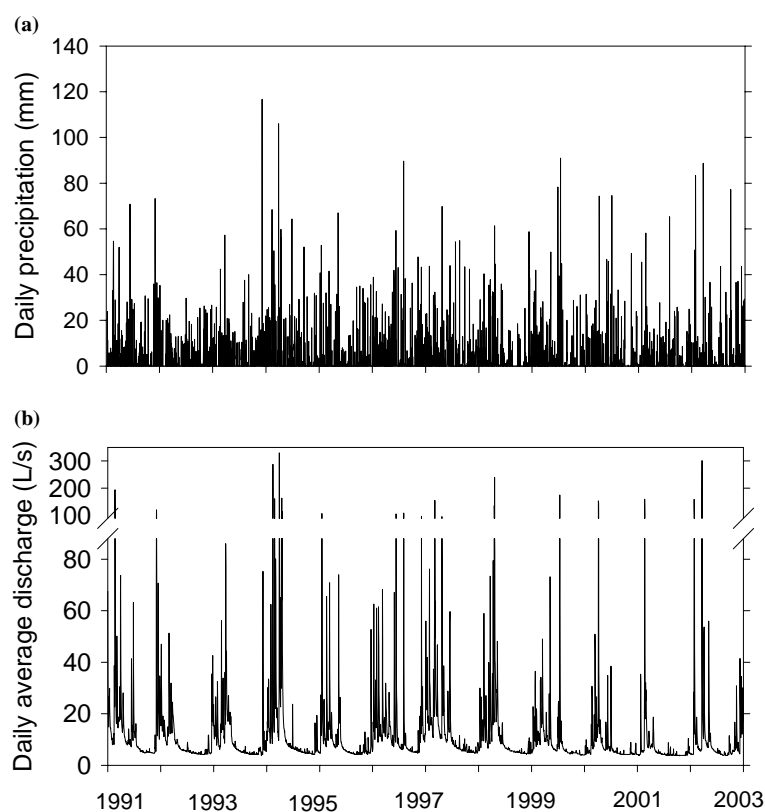


Figure 2. (a) Daily precipitation and (b) average daily discharge in the West Fork of Walker Branch.

concentration were proportionately much larger than the declines in SRP concentration at this time. For much of the year DIN:SRP ratios remained near 30 or greater.

There was little seasonality in the concentrations of organic forms of N and P (Figure 4e, f). DON concentrations were generally slightly lower than DIN concentrations, whereas DOP concentrations were often slightly higher than SRP concentrations, particularly in late summer and autumn.

The seasonality in discharge and in NO_3^- and SRP concentrations was more evident in a plot of average monthly values over the 12-year record (Figure 5). Discharge was greatest in late winter and early spring and lowest in late summer and autumn, generally opposite the trend in water temperature. The seasonal patterns in NO_3^- and SRP concentrations were not simply a function of stream discharge and temperature. Although peak NO_3^- and SRP concentrations occurred during the summer months when discharge was lowest and water temperatures were highest, the concentration minima in November also occurred during low discharge and moderate water temperatures.

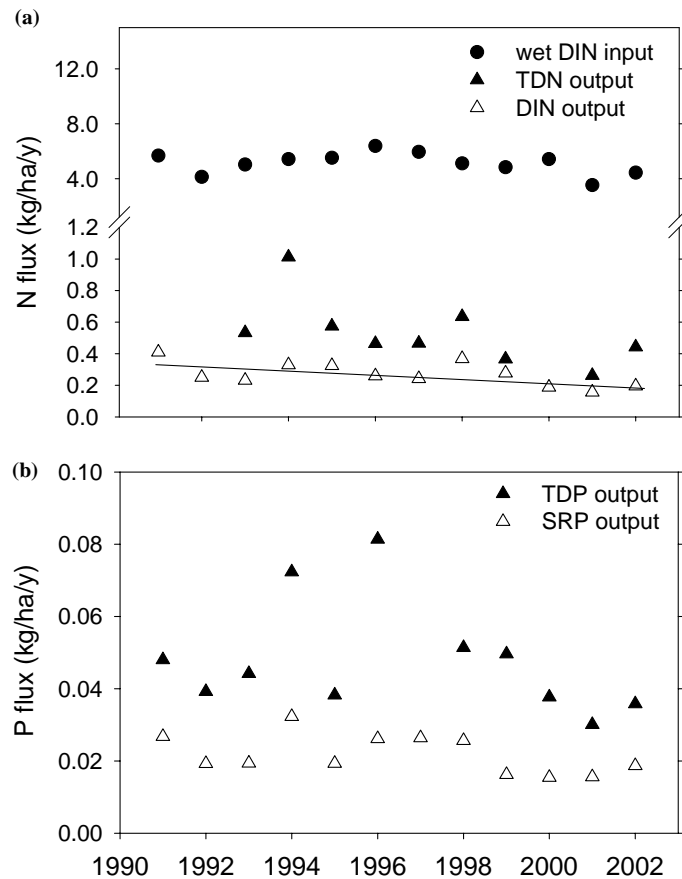


Figure 3. Annual watershed input and stream output fluxes of (a) N and (b) P in the West Fork of Walker Branch. There was a significant declining trend in DIN output (-0.013 kg/ha/y, $p = 0.037$).

In addition, the March/April NO_3^- minima occurred when discharge was highest. In contrast to NO_3^- , average monthly SRP concentrations remained relatively low throughout the winter and early spring months. During the summer months NO_3^- concentrations were considerably greater than DON concentrations; at other times of the year DON concentrations were similar or slightly greater than NO_3^- concentrations. The seasonal pattern in DIN:SRP ratio (Figure 5d) appears to be controlled primarily by increases in NO_3^- concentrations during December/January and declines in NO_3^- concentrations in March/April and again in November.

Concentrations of NO_3^- and SRP predicted by the end-member mixing model were both greater than and considerably less than observed concentrations depending on the time of year, suggesting effects of in-stream processes on concentrations. Observed/predicted ratios greater than 1.0 indicate

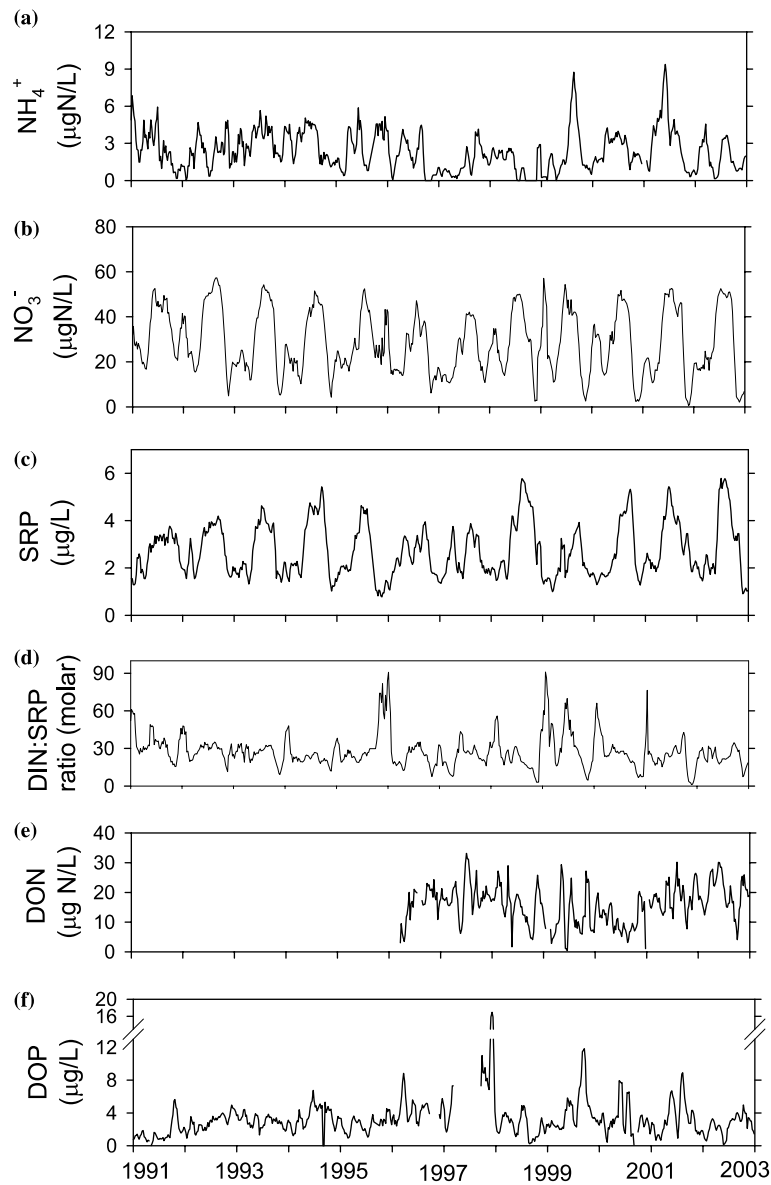


Figure 4. Plot of 4-week running averages for stream N and P concentrations and DIN:SRP ratios in the West Fork of Walker Branch.

in-stream net release, whereas ratios less than 1.0 suggest in-stream net uptake. Long-term trends in observed/predicted concentration ratios for both NO_3^- and SRP showed a distinct seasonal pattern with values near or above 1.0 generally during the summer months and values considerably less than 1.0

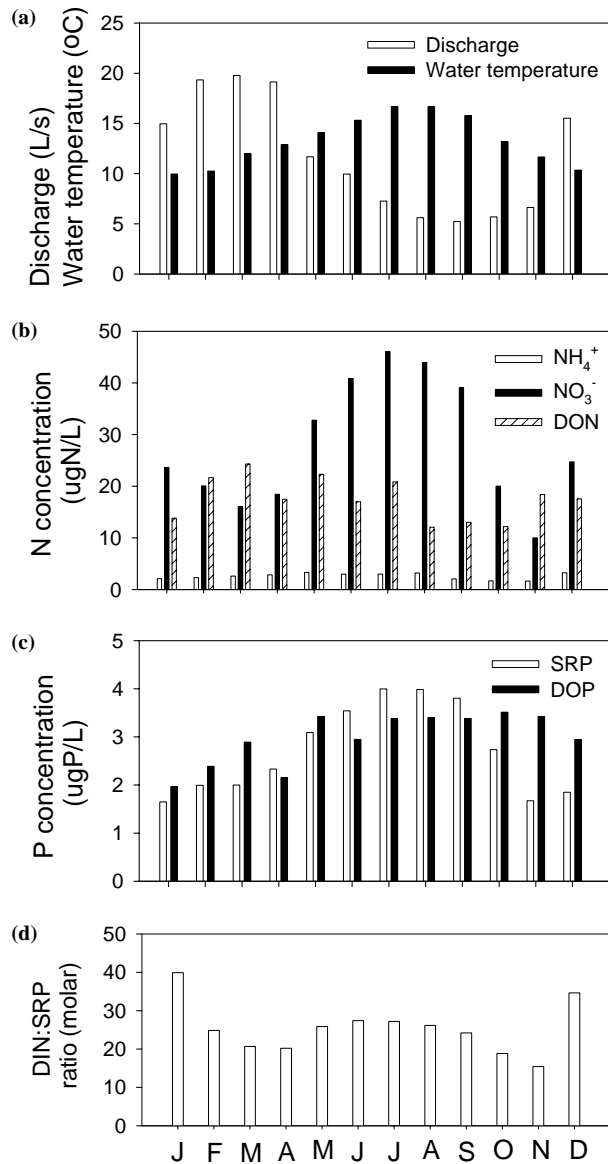


Figure 5. Average monthly (a) discharge and water temperature, (b) N concentrations, (c) P concentrations, and (d) DIN:SRP ratios in the West Fork of Walker Branch.

during early spring and autumn (Figure 6). There was often a secondary peak in observed/predicted ratios in winter. The relatively high variability in the vadose zone end-member NO_3^- and SRP concentrations had little impact on the analysis because these concentrations were very low and vadose zone water usually comprised < 10% of stream discharge.

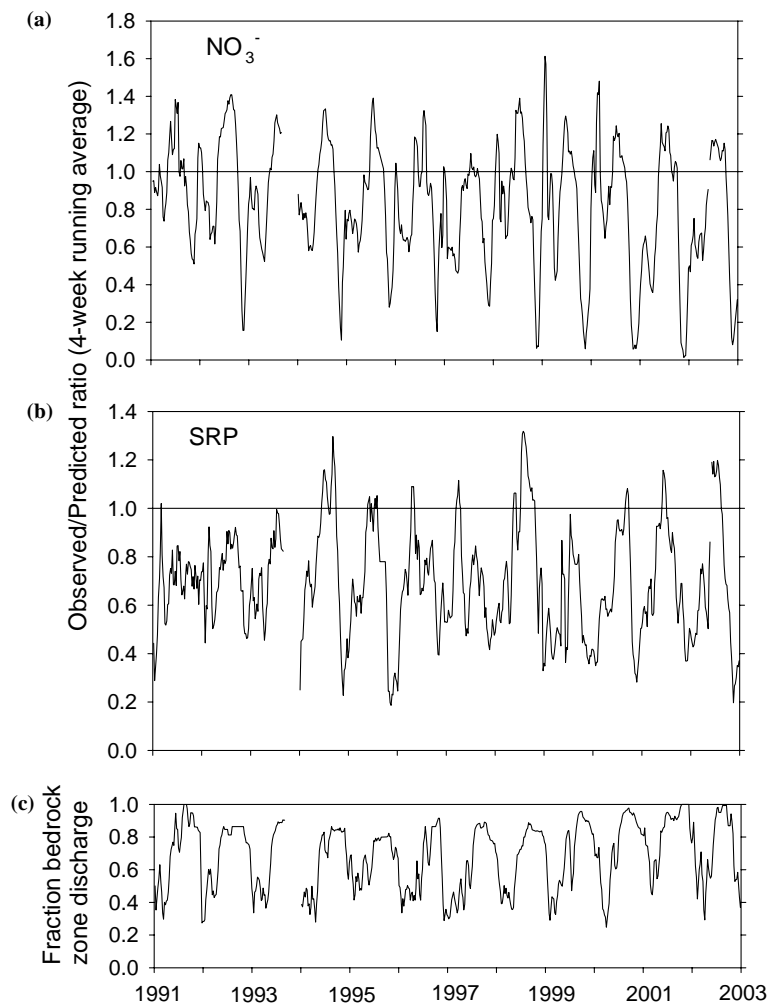


Figure 6. Four-week running averages of observed/predicted concentration ratios of (a) NO_3^- and (b) SRP, and (c) fraction of bedrock zone contribution to stream discharge. Predicted NO_3^- and SRP concentrations were determined using an end-member mixing model to compute contributions of vadose zone, saturated zone, and bedrock zone to stream discharge and average NO_3^- and SRP concentrations in these flowpaths.

The long-term average monthly values of observed/predicted ratios of NO_3^- and SRP concentrations showed the bimodal annual pattern more clearly, with a strong minima in November immediately after peak input of senescent leaves and a secondary minima for NO_3^- in March/April during the period of maximum light availability and maximum algal productivity in the stream (Figure 7a). While observed/predicted ratios indicated the relative magnitude of in-stream effects on streamwater concentrations, the difference between

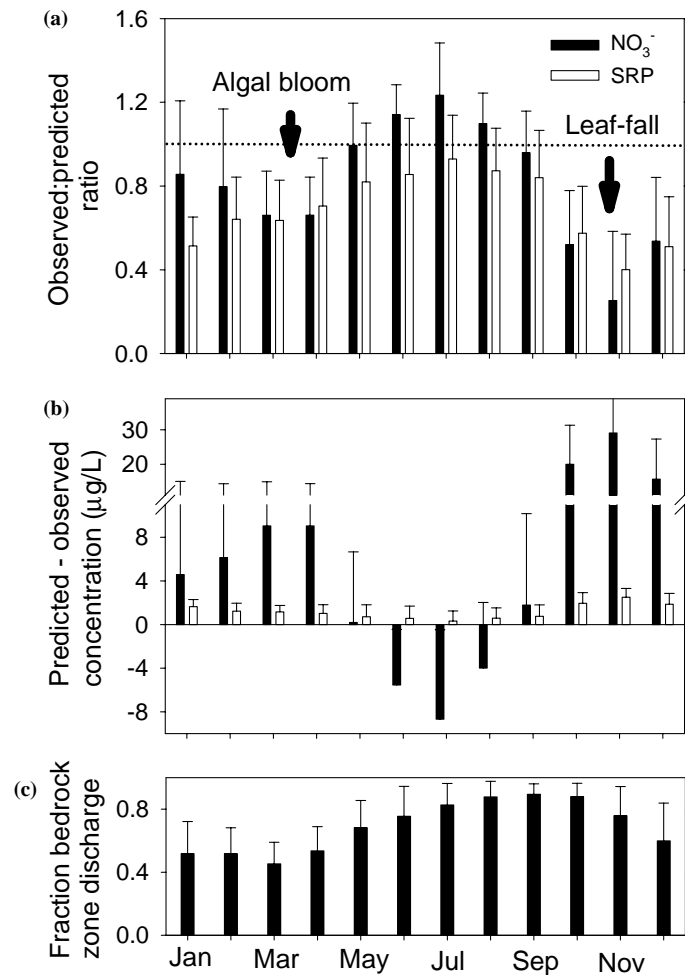


Figure 7. Average monthly values of (a) observed/predicted ratios for NO_3^- and SRP concentrations, (b) differences between predicted and observed concentrations for NO_3^- and SRP, and (c) fraction of bedrock zone contribution to stream discharge.

predicted and observed concentrations showed the absolute magnitude of in-stream effects on concentrations (Figure 7b). Long-term average monthly values of observed/predicted concentrations indicated that the reduction of streamwater NO_3^- concentration in autumn (maximum of $29 \mu\text{g N/l}$ in November) was considerably higher than the reduction in early spring (maximum of $9 \mu\text{g N/l}$ in March and April). In-stream reduction of SRP concentration also was greatest in autumn (peak of $2.5 \mu\text{g P/l}$ in November). In summer, there was a net release of NO_3^- to streamwater, resulting in a maximum increase of about $9 \mu\text{g N/l}$ in July. For SRP in-stream processes had very little net effect on streamwater concentrations in summer.

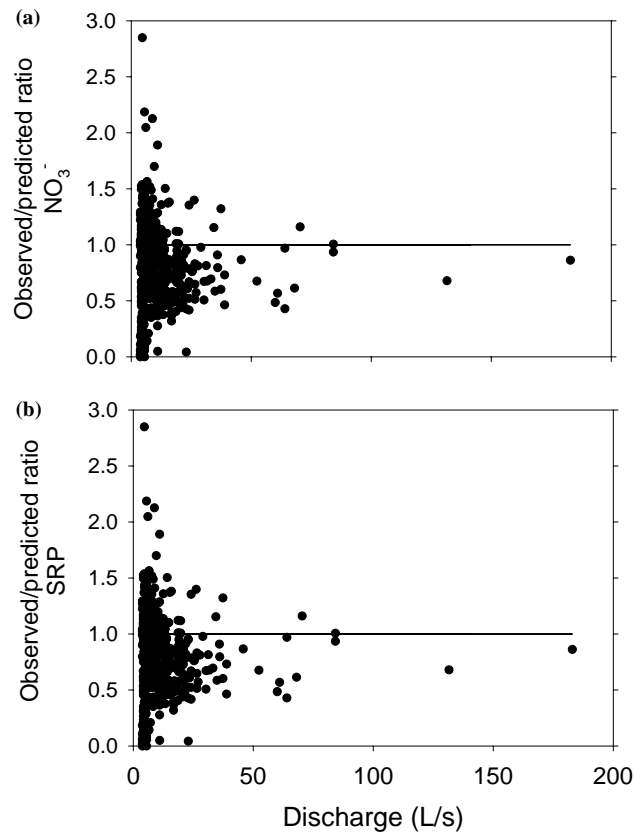


Figure 8. Relationship between observed/predicted ratios of (a) NO_3^- and (b) SRP and stream discharge. A line representing a ratio of 1.0 is shown.

Stream discharge was dominated by bedrock zone water during most times of the year, although the bedrock zone fraction was strongly seasonal with minima in winter and early spring and maxima in summer and autumn (Figure 7c). High rates of net in-stream uptake occurred during periods of relatively high bedrock zone discharge fraction (October and November) and during periods of lower bedrock zone fraction (March and April), suggesting that in-stream uptake was not related to catchment flowpath variations.

There was evidence that at higher discharges the effect of in-stream processes was reduced. Observed/predicted ratios of NO_3^- and SRP concentrations were generally closer to 1.0 at high discharges, particularly discharges > 75 l/s (Figure 8). However, there is greater uncertainty in the end-member mixing analysis of NO_3^- and SRP concentrations during large storms because vadose zone water can comprise 50% or more of the total stream discharge at these times and variability in vadose zone end-member concentrations was greater than for the other end-members.

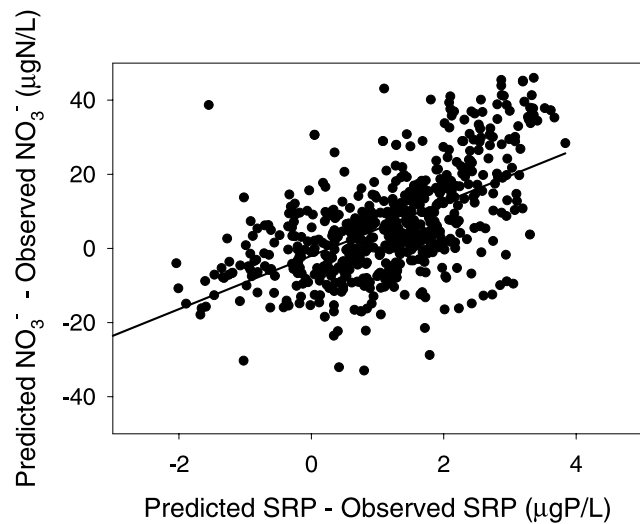


Figure 9. Relationship between predicted – observed concentrations for NO_3^- and SRP ($r = 0.57$, $p < 0.0001$). The regression line has a slope of 7.0 (15.4 in molar terms).

The discharge-weighted annual average observed/predicted concentration ratios were 0.78 for NO_3^- and 0.67 for SRP. Thus, in-stream processes resulted in a net removal of about 22% of the NO_3^- and 33% of the SRP entering the stream from the catchment. These net removal percentages were computed using only data for stream discharges < 50 l/s. The annual average net removal fractions probably would be somewhat lower than these values if we had accounted for reduction of in-stream uptake at high discharge, as suggested by Figure 8. However, only about 4% of the annual discharge during the study period occurred at discharges > 50 l/s, and therefore these in-stream uptake fractions are likely only slight overestimates. Based on the uncertainty analysis (uncertainty in both the flowpath fractions and the flowpath nutrient concentrations), the in-stream net removal percentages ranged from 10 to 31% for NO_3^- and from 14 to 44% for SRP.

There was a significant relationship between the predicted – observed concentrations of NO_3^- and SRP ($r = 0.57$, $p < 0.0001$, Figure 9). The slope of this relationship, converted to a molar basis, was 15.4. This is similar to the Redfield ratio expected for biological processes, although it does not include the effects of uptake of NH_4^+ and the organic forms of N and P.

Discussion

The inorganic N and P concentrations measured in Walker Branch are relatively low and typical of streams draining many forested watersheds (Lewis 2002, Smith et al. 2003). Despite moderately high levels of wet N deposition,

stream outputs of N were low (DIN outputs < 10% of wet N deposition) and Walker Branch Watershed remains highly retentive of N.

The long-term records of stream NO_3^- concentrations and outputs in Walker Branch are in contrast to observations of increasing NO_3^- concentrations and outputs in forested streams in other areas of the eastern US (Murdoch and Stoddard 1993; Peterjohn et al. 1996; Swank and Vose 1997). However, recent reports have also documented declines in NO_3^- concentrations in forested streams over the past several decades in the northeastern US (Driscoll et al. 2003; Goodale et al. 2003). While increasing stream nitrate levels have been attributed to high and/or increasing rates of N deposition (Peterjohn et al. 1996; Swank and Vose 1997), other studies have suggested that other factors may be more important for explaining long-term trends. Several studies have shown that climate variations are an important regulator of stream nitrate outputs (Mitchell et al. 1996; Murdoch et al. 1998). Although DIN and SRP outputs in Walker Branch were positively related to annual runoff, there was no trend in runoff over the period of this study. Further, there was no relationship between annual stream DIN output and mean annual air temperature in Walker Branch as has been observed for streams in the Catskill Mountains of New York (Murdoch et al. 1998).

Differences in species composition of the forest can have considerable effects on stream nitrate concentrations (Lovett et al. 2000). The forest drained by Walker Branch is dominated by oaks and streams draining oak forests have been reported to be particularly low in nitrate (Lewis and Likens 2000; Lovett et al. 2000). However, Lewis and Likens (2000) argue that the low nitrate concentrations of oak forest streams are likely due to soil physical characteristics or land use history rather than the direct effect of oaks on nitrate losses.

Several recent studies have suggested that legacy effects of past disturbances may explain current trends in N concentrations and output. Aber et al. (2002), using a model of forest carbon, nitrogen, and water dynamics, found that the declining nitrate concentrations in streams at the Hubbard Brook Experimental Forest in recent decades could be explained as a long-term recovery from temporarily increased outputs after extreme drought and a defoliation event in the 1960s. Goodale et al. (2000) reported considerably lower stream nitrate exports from forests that had been logged and burned a century ago than those from old growth forests. In Walker Branch there have been several insect outbreaks that resulted in considerable mortality of pines and hickory over the past several decades, and a 1967 wildfire burned approximately 40% of the watershed (Johnson 1985; Dale et al. 1990). Long-term recovery from these disturbances may be responsible for the slightly declining trend in annual nitrate output in Walker Branch.

Although stream nutrient concentrations in Walker Branch were low, there was considerable seasonal variation, particularly in NO_3^- and SRP concentrations. Stream concentrations of NO_3^- and SRP were highest in the summer and early autumn and lowest in late autumn, winter, and early spring. This seasonal pattern has also been observed in streams at the Coweeta

Hydrologic Laboratory in North Carolina (Swank and Vose 1997). However, it contrasts with the seasonal pattern reported for streams in the northeastern US and Canada, where NO_3^- concentrations are generally lowest in summer and highest in spring (Murdoch and Stoddard 1993; Creed and Band 1998; Goodale et al. 2003) and probably reflects latitudinal differences in both soil and in-stream processes. At lower latitudes, biological processes in soils and streams do not decline as sharply in the colder months as they do at higher latitudes, and large inputs of carbon-rich leaf litter to soils and streams in autumn can result in high rates of nutrient uptake during winter and early spring. Nutrient concentrations can be higher in summer in low latitude streams due to greater dominance of deeper water flow paths which often have higher nutrient concentrations and the concentrating effects of low discharge rates.

The seasonal nutrient concentration patterns in Walker Branch suggest that in-stream processes rather than processes in the terrestrial system may be primarily responsible for this variation. Minima in streamwater NO_3^- and SRP occurred in autumn and in early spring, periods of maximum heterotrophic and autotrophic activity in the stream. Tracer addition experiments have demonstrated that gross rates of N and P uptake by stream biota are considerably higher at these times than at other times of the year (Mulholland et al. 1985, 2000). Large accumulations of leaves in autumn provide a carbon-rich but nutrient poor substrate for microbial growth, stimulating uptake of nutrients from the water. Over the winter these leaves gradually decompose or are flushed from the active stream channel by high flows and nutrient uptake declines. In early spring, increasing light levels reaching the stream stimulate the growth of algae and bryophytes increasing their demand for nutrients from water. As leaves emerge in the riparian forest (usually about mid-April in Walker Branch), light levels drop sharply as does primary production and nutrient demand (Hill et al. 2001). The summer is a period of relatively low biological activity and very low to undetectable nutrient uptake rates in the stream.

Although the seasonal patterns of in-stream biological activity agree with an in-stream mechanism for seasonal variations in streamwater nutrient concentrations, variation in flow paths of water through the catchment might also account for some of the seasonal chemistry variations (Creed and Band 1998). To quantitatively separate the effects of variation in catchment flow paths from in-stream processes we used an end-member mixing analysis. End-member mixing analysis of streamwater chemistry has been used to identify different water pathways as well as the soil and groundwater contributions to variations in streamwater chemistry (Hooper et al. 1990; Mulholland 1993). Here we use it first to identify water flow paths (using Ca^{2+} and SO_4^{2-} concentrations) and then to determine the expected nutrient concentrations in stream water based on conservative mixing of the different water sources.

The results of the comparison between observed nutrient concentrations and those predicted by end-member mixing analysis were consistent with the

temporal patterns expected from changes in in-stream uptake. Minimum observed/predicted ratios and maximum differences between predicted and observed concentrations of NO_3^- and SRP were found during autumn, with secondary minima and maxima during early spring. Thus, streamwater nutrient concentrations were not simply the result of conservative mixing of nutrients from different flow paths through the catchment at these times. Further, the end-member mixing analysis indicated an additional source of streamwater NO_3^- during the summer, consistent with a net release of N within the stream as uptake processes decline at this time of year. These results are also consistent with earlier studies on the same stream showing net uptake of nutrients during spring and autumn and net release during summer (Mulholland 1992; Mulholland and Hill 1997). Finally, the relationship between the differences in predicted and observed concentrations for NO_3^- and SRP suggested that biological processes were the primary mechanism (Figure 9). Previous studies in this stream involving nutrient tracer additions also have indicated that biological processes dominate N and P dynamics (Mulholland et al. 1985; 2000).

There was little evidence of in-stream net release of inorganic N and P back to water during storms in Walker Branch. Concentrations of NO_3^- and SRP usually decline during high discharge events (Mulholland et al. 1990) and end-member mixing analysis indicated that, although net retention is reduced, it is not eliminated at these times. However, it seems likely that some of the net in-stream retention of inorganic N and P is exported as particulate organic forms during storms. Meyer and Likens (1979) found that much of the net retention of SRP measured during prolonged periods of lower flow in a headwater stream at the Hubbard Brook Experimental Forest was exported as fine particulate P during a few storms. Nonetheless, nutrient tracer experiments in Walker Branch have demonstrated some relatively long-term retention of inorganic N removed from water by bryophytes. Results from a $^{15}\text{N}\text{-NH}_4^+$ tracer addition experiment conducted during early spring indicated that about 25% of the uptake of ^{15}N from water by the bryophyte *Porella* sp., which dominated in-stream uptake of NH_4^+ , was retained within its biomass 6 months after the tracer addition was terminated (Mulholland et al. 2000).

Our analysis indicates that in Walker Branch Watershed in-stream processes retain about 20% of the NO_3^- and 30% of the SRP delivered to the stream in drainage water from the terrestrial portions of the catchment. This uptake occurs over a stream length of approximately 300 m and during a surface water travel time of approximately 50–100 min based on average water velocities of 5–10 cm/s determined in subsequent studies (Mulholland et al. 2000). The N retention fraction is within the range of reports from other studies using budget approaches to estimate in-stream N retention. Swank and Caskey (1982) reported that in-stream nitrate retention was >50% of inputs immediately after forest logging, but declined to about 5% 4 years later for a stream at the Coweeta Hydrologic Laboratory in North Carolina, USA. Hill (1983) determined that nitrate retention in two streams in Ontario, Canada, were 13 and

50% of inputs of total N during summer, but declined to < 5% of N inputs on an annual basis. Triska et al. (1984) reported net in-stream retention of 16% of N inputs to a small coniferous forest stream in the Cascade Mountains of Oregon, USA. Sjodin et al. (1997) estimated that denitrification resulted in about 50% retention of nitrate inputs to a plains reach of the South Platte River, USA. Burns (1998) found that in-stream processes removed 10–50% of the dissolved N inputs during baseflow to a forest stream in the Catskill Mountains of New York, USA. Budget approaches, however, have generally provided only approximate estimates of net N retention in streams due to the difficulties of accurately measuring inputs and outputs over long periods of time.

Modeling approaches of stream N dynamics have also suggested considerable in-stream retention of N. Peterson et al. (2001) used a model of stream N dynamics parameterized with data from ^{15}N tracer addition experiments conducted in 11 streams across the USA and found that headwater streams retain approximately 2/3 of their DIN inputs in the first km of length. Using a model of in-stream N retention based on water displacement time, Seitzinger et al. (2002) estimated that about 20–40% of the N inputs to surface waters are retained within 1–4th order streams in 16 large drainage basins in the eastern USA. In their study of N retention in the Mississippi River basin, Alexander et al. (2000) found that in-stream N retention rates were inversely and exponentially related to river depth, probably reflecting the strong influence of surface/volume ratio on biologically mediated processes. These modeling studies suggest that small, headwater streams may be a particularly important component of the drainage network for N retention.

There is considerable interest in the role of in-stream nutrient retention, particularly for N, because regional exports in rivers account for only a small portion of the inputs to the landscape (Howarth et al. 1996; Boyer et al. 2002). In recent decades humans have greatly altered the global cycle of N resulting in increases in acidification, eutrophication, and greenhouse gas emissions (Vitousek et al. 1997). Identification of the major sinks for N inputs and their controls is critically important for future land management.

In Walker Branch Watershed there appear to be two hierarchical controls on stream nitrate. The generally low stream concentrations and outputs of nitrate relative to N deposition inputs are the result mostly of efficient retention within terrestrial portions of the watershed, whereas in-stream processes exert further control by reducing stream nitrate concentrations and outputs during some seasons. It is primarily the in-stream processes that produce the sharp seasonality in stream nitrate concentrations, however. This appears to be in contrast to streams at higher latitudes, such as those in the northeastern US, where the spring peaks and summer lows in stream nitrate concentrations appear to be primarily the result of the seasonality in uptake and remineralization of N in the terrestrial environment.

In conclusion, there is accumulating evidence that terrestrial–aquatic interfaces represented by low-order streams and their riparian zones are hotspots

for N retention in the landscape (McClain et al. 2003). Although both biological assimilation and denitrification result in short-term N retention in streams, particulate organic forms are susceptible to scour and export during storms and thus denitrification is probably the primary mechanism for long-term N retention. Although ecosystem-scale denitrification rates have been difficult to determine for streams, new field ^{15}N tracer addition approaches show much promise for assessing reach-scale denitrification rates (Böhlke et al. 2004; Mulholland et al. 2004). Clearly, in-stream retention of N and other nutrients must be considered in any analysis of watershed outputs or landscape-scale nutrient budgets. This study, as well as other recent work, suggests that in-stream processes are important buffers on stream nutrient concentrations and exports, reducing the effects of changes in inputs and retention in terrestrial portions of watersheds.

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