



In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch

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[1] A growing body of evidence demonstrates the importance of in-stream processing in regulating nutrient export, yet the influence of temporal variability in stream metabolism on net nutrient uptake has not been explicitly addressed. Stream water DIN and SRP concentrations in Walker Branch, a first-order deciduous forest stream in eastern Tennessee, show a repeated pattern of annual maxima in summer and biannual minima in spring and autumn. Temporal variations in catchment hydrologic flow paths result in lower winter and higher summer nutrient concentrations, but do not explain the spring and autumn nutrient minima. Ambient nutrient uptake rates were measured 2–3 times per week over an 18-month period and compared to daily rates of gross primary production (GPP) and ecosystem respiration (ER) to examine the influence of in-stream biotic activity on nutrient export. GPP and ER rates explained 81% of the variation in net DIN retention with high net NO_3^- uptake (and lower net NH_4^+ release) rates occurring during spring and autumn and net DIN release in summer. Diel nutrient concentration patterns were examined several times throughout the year to determine the relative importance of autotrophic and heterotrophic activity on net nutrient uptake. High spring GPP corresponded to daily decreases in NO_3^- over the illuminated hours resulting in high diel NO_3^- amplitude which dampened as the canopy closed. GPP explained 91% of the variance in diel NO_3^- amplitude. In contrast, the autumn nutrient minima was largely explained by heterotrophic respiration since GPP remained low and little diel NO_3^- variation was observed during the autumn.

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1. Introduction

[2] Stream water nutrient concentrations reflect the cumulative effects of hydrological, geomorphological, and biological processes occurring in both terrestrial and aquatic environments throughout the catchment. In practice, however, in-stream and near-stream processing of nutrients have often been assumed to be minimal, allowing stream water concentration patterns to be used to infer the nutrient cycling and retention status of terrestrial ecosystems drained by streams [e.g., Vitousek and Reiners, 1975; Likens and Bormann, 1995]. A growing body of evidence demonstrates that headwater streams are important sites of nutrient and organic matter processing and retention, often altering the delivery of these constituents to downstream ecosystems [Alexander et al., 2000; Peterson et al., 2001; Bernhardt et al., 2003, 2005; Mulholland, 2004]. Thus, ignoring in-stream nutrient processing may lead to erroneous conclu-

sions about the role of terrestrial processes in controlling stream water nutrient concentrations.

[3] Stream nutrient uptake is influenced by numerous factors including stream size [Wollheim et al., 2001], transient storage [Grimm and Fisher, 1984; Jones and Holmes, 1996; Valett et al., 1996; Mulholland et al., 1997; Hall et al., 2002], water residence times [Valett et al., 1996], water temperature [Butturini and Sabater, 1998], ambient nutrient concentrations [Dodds et al., 2002; Webster et al., 2003], benthic leaf litter [Mulholland et al., 1985], coarse woody debris [Roberts et al., 2007a], riparian vegetation [Sabater et al., 2000], and periphyton biomass [Martí et al., 1997]. While the above stream attributes explain some of the variation in nutrient uptake rates, they only indirectly affect the capacity of stream biota to directly take up nutrients. As a result, metrics of biological activity (i.e., ecosystem metabolism rates) should be more predictive of nutrient uptake than physical variables [Hall and Tank, 2003].

[4] Few previous studies have directly linked ecosystem metabolism with nutrient uptake in streams. Mulholland et al. [1997] showed that a stream with high ecosystem respiration (ER) rates had higher phosphorus uptake rates than a low ER stream. Hall and Tank [2003] found that ~82% of the variation in NH_4^+ uptake was explained by rates of gross primary production (GPP) and ER while NO_3^-

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uptake was controlled by GPP alone (explaining ~75% of the variation) in several streams in Grand Tetons National Park. NO_3^- uptake has also been shown to be correlated with GPP in the West Fork of Walker Branch [Mulholland et al., 2006]. PO_4^{3-} uptake (but not NH_4^+) was correlated with GPP in streams in Spain [Sabater et al., 2000]. Additionally, several studies have provided indirect evidence of biotic control on nutrient uptake rates. DIN uptake increased during algal regrowth after flash flooding in a desert stream, Sycamore Creek [Grimm, 1987]. Similarly, streams with abundant algal mats were found to have higher nutrient uptake rates by algae and lower stream water N and P concentrations in a study of Antarctic streams [McKnight et al., 2004].

[5] This body of evidence suggests that ecosystem metabolism is important in controlling nutrient uptake in streams. However, previous studies were largely based on only a few measurements in each stream. Roberts et al. [2007b] showed that multiple scales of temporal variability (day-to-day, seasonal, episodic, and interannual) in ecosystem metabolism rates can occur within a single stream. Similarly, nutrient uptake rates have been shown to exhibit seasonal variability in an eastern U.S. stream (PO_4^{3-} and NO_3^- [Mulholland et al., 1985; also unpublished data]), 2 Mediterranean streams (NH_4^+ and PO_4^{3-} [Martí and Sabater, 1996]), and 2 New Zealand streams (NO_3^- , NH_4^+ , and PO_4^{3-} [Simon et al., 2005]). None of these studies explicitly examined the relationship between ecosystem metabolism and nutrient uptake. Therefore, in order to truly assess nutrient export and retention for a given stream, temporal variability of both nutrient uptake and ecosystem metabolism rates should be examined in a more comprehensive manner.

[6] Much attention has been focused on quantifying gross rates of nutrient uptake in streams. While this metric is important for understanding the magnitude of biotic activity of a given ecosystem (and the capacity of biota to take up nutrients), net nutrient uptake may be more relevant to understanding controls on nutrient export since changes in nutrient concentrations as water moves downstream reflect the net result of both uptake and release processes (i.e., nutrient retention).

[7] In this study, we examined temporal variability in both net nutrient uptake rates and ecosystem metabolism in a well-studied forested headwater stream, the West Fork of Walker Branch (hereafter referred to as Walker Branch), in eastern Tennessee. Stream water concentrations of NO_3^- and soluble reactive phosphorus (SRP) in Walker Branch show a repeated pattern of annual maxima in summer and biannual minima in spring and autumn [Mulholland and Hill, 1997]. Stream water in Walker Branch arrives through three different flow paths (shallow soil vadose zone flow, deep saturated soil zone flow, and bedrock zone flow) that differ in nutrient concentrations [Mulholland, 1993]. The relative contribution of each flow path to stream water can be determined via an end-member mixing approach using $[\text{Ca}_2^+]$ and $[\text{SO}_4^{2-}]$ [Mulholland and Hill, 1997; Mulholland, 2004]. This approach allowed the authors to determine whether temporal variations in catchment hydrologic processes could explain seasonal nutrient patterns in Walker Branch. Differences in dominant hydrologic flow paths alone explained higher summer and lower winter nutrient concentrations, but predicted concentrations based solely on

flow path variations were higher than observed NO_3^- and SRP concentrations during spring algal bloom and autumn leaf fall periods [Mulholland and Hill, 1997; Mulholland, 2004]. These results suggested that nutrient uptake by stream biota might be responsible for lower concentrations during these periods.

[8] We report results from an 18-month study of ambient net nutrient uptake rates in conjunction with continuous ecosystem metabolism measurements to examine the relationship between ecosystem metabolism and nutrient retention in Walker Branch. In addition, we examined diel stream water nutrient concentration patterns on 13 dates throughout 2004 to examine the relative importance of autotrophic and heterotrophic activity on nutrient retention.

2. Site Description

[9] This study was conducted in the West Fork of Walker Branch, a first order, forested stream draining a 38.4 ha catchment on the U. S. Department of Energy's Oak Ridge National Environmental Research Park (35°58'N, 84°17'W) in the Ridge and Valley province of eastern Tennessee. The catchment is underlain by siliceous dolomite that has weathered to develop deep soils abundant in chert [McMaster, 1963]. The Walker Branch watershed is a second-growth deciduous forest dominated by chestnut oak (*Quercus prinus*), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), white oak (*Q. alba*), and American beech (*Fagus granifolia*) [Johnson and Van Hook, 1989]. The climate is typical of the humid Appalachian region of the southeastern United States, with mean annual precipitation of ~135 cm (distributed relatively evenly throughout the year) and a mean annual temperature of ~14.5°C [Mulholland, 2004].

[10] Ambient nutrient uptake rates and ecosystem metabolism rates were measured in a 62 m reach (located ~30 m downstream from two perennial springs, ~180 m downstream from the headwaters, and ~120 m upstream from a weir) used in previous metabolism studies [e.g., Roberts et al., 2007b]. Stream discharge is monitored at a 120° v-notch weir with 15-min stage recordings. Discharge is highly seasonal with higher baseflows and more frequent spates during winter and early spring when evapotranspiration rates are low [Mulholland, 2004]. However, discharge regime is more stable than for southern Appalachian streams in other areas due to the importance of relatively constant discharge in springs that maintain baseflow during dry periods and due to a large storage capacity in deep soils that buffers the impact of small storms. Stream water chemistry is dominated by calcium, magnesium, and bicarbonate, and the pH is moderately basic (usually 8.0–8.3) [Mulholland, 1992, 2004]. The channel gradient is relatively gentle (~0.035 m m⁻¹). The streambed in the study reach is composed of bedrock outcrops, gravel and cobble in shallow (<10 cm deep) riffle-run sections.

3. Methods

3.1. Stream Water Nutrient Sampling

3.1.1. Weekly Water Chemistry

[11] Stream water samples were collected for chemical analyses weekly over a 30-month period (January 2004–

June 2006) at a station 60 m upstream from the weir (~60 m below the study reach) as described in *Mulholland* [2004]. Water samples were collected in well-rinsed polyethylene bottles between 0900–1200 EST on Tuesdays, immediately returned to the laboratory, and filtered (0.4- μm pore size Nucleopore polycarbonate filters) within 3 hours of collection. Filtered water was kept frozen until chemical analyses could be performed.

3.1.2. Net Nutrient Uptake Rates

[12] Net nutrient uptake rates were assessed by collecting stream water samples at the upstream and downstream ends of the study reach between 1200 and 1400 h EST on 165 dates (~2–3 times per week) over an 18-month period (10 January 2005–30 June 2006). On each date, stream water was collected with a 30-mL plastic syringe and immediately passed through Whatman GF/F glass fiber (0.7- μm nominal cut-off) filters into acid-washed, stream-rinsed 60-mL Nalgene sample bottles. Filtered samples were stored frozen prior to nutrient analyses at Oak Ridge National Laboratory. Only dates when $Q < 25$ L/s ($n = 128$) were used to calculate net nutrient uptake rates since in-stream processes are likely to have minimal impact during higher stormflow.

3.1.3. Diel Nutrient Concentration Patterns

[13] The effects of diel cycles on stream water nutrient concentration were determined by collecting stream water samples at hourly intervals over a 24-hour period on 13 dates in 2004 (20 March–20 November) using an autosampler (Teledyne Isco Model 1612) at the location of weekly water sampling (~60 m downstream from the study reach). Stream water samples were passed through Whatman GF/F glass fiber filters into acid-washed, stream-rinsed 60-mL Nalgene sample bottles within 12 hours of collection and kept frozen prior to nutrient analyses. The sampling included 3 days prior to leaf-out (20 March, 4 April, and 6 April), 4 days during canopy closure in April, 3 days during the closed canopy period (May–August), and 2 days after leaf-fall (November). Discharge ranged between 9.8 and 17.4 L/s on March and April sample dates and was slightly lower 4.5–7.4 L/s on other dates. Since all diel cycles occurred during baseflow conditions, observed changes in nutrient concentrations were likely not influenced by discharge.

3.2. Chemical Analyses

[14] Concentrations of soluble reactive phosphorus (SRP) were determined by the ascorbic acid-molybdenum blue method [*APHA*, 1992] using a 10-cm spectrophotometer cell to achieve low detection limits (0.4 $\mu\text{g P/L}$ [*Mulholland and Hill*, 1997]). Concentrations of nitrite (NO_2^-) + nitrate (NO_3^-) were determined by Cu-Cd reduction followed by azo dye colorimetry [*APHA*, 1992] and ammonium (NH_4^+) by phenate colorimetry [*APHA*, 1992], both using an auto-analyzer (Seal Analytical Model AA3). Because stream water was always relatively high in dissolved oxygen concentration (>6 mg/L) and because spot measurements revealed very low NO_2^- concentrations (<2 $\mu\text{g N/L}$), hereafter we refer to $\text{NO}_2^- + \text{NO}_3^-$ as NO_3^- . The method detection limit (MDL) on this instrument for NO_3^- and NH_4^+ were 0.2 and 0.5 $\mu\text{g N/L}$, respectively with the coefficient of variation for the analyses being 0.2% and

0.3%, respectively (Seal Analytical). Dissolved inorganic nitrogen (DIN) concentration is the sum of NO_3^- and NH_4^+ .

3.3. Net Nutrient Uptake Rate Calculations

[15] The net uptake rates of NO_3^- and DIN, expressed as net mass removal rates per unit area (U , $\mu\text{g N m}^{-2} \text{min}^{-1}$), were calculated on each date that upstream and downstream concentrations were measured under non-stormflow conditions ($Q < 25$ L/s, $n = 128$) using the equation:

$$U = \frac{([N_{up}]Q_{up} - [N_{dn}]Q_{dn} + [N_{gw}]Q_{gw})}{lw} \quad (1)$$

where $[N_{up}]$ and $[N_{dn}]$ are the nutrient concentrations (either NO_3^- or DIN) in stream water at the upstream and downstream sampling locations, $[N_{gw}]$ is the nutrient concentration in groundwater, Q_{up} , Q_{dn} , and Q_{gw} are the stream water discharge at the upstream and downstream locations and the discharge in groundwater, l refers to reach length (62 m in this case) and w is the mean wetted channel width. Q_{dn} and Q_{up}/Q_{dn} can be determined for each measurement from previously developed relationships to discharge recorded at the weir (Q_{weir}): $Q_{dn} = 0.7661(Q_{weir}) + 0.929$ ($r^2 = 0.98$) and $Q_{up}/Q_{dn} = 0.0034(Q_{weir}) + 0.965$ ($r^2 = 0.71$) [$n = 31$ in both cases (Roberts, unpublished data)]. Q_{gw} was calculated as the difference between Q_{dn} and Q_{up} . $[N_{gw}]$ was assumed to equal the mean of $[N_{up}]$ and $[N_{dn}]$ for each uptake measurement since two years of monthly measurements in Walker Branch have shown that mean groundwater concentrations of NH_4^+ , NO_3^- , and SRP were not significantly different from stream water concentrations with groundwater concentrations being more consistent over the year and over 90% of the groundwater measurements ranging between 0.5 and 2 times stream water concentrations [*Mulholland*, 1992]. However, in order to test the sensitivity of the observed patterns in net nutrient uptake to differences in $[N_{gw}]$, we also calculated U with $[N_{gw}] = 0.5$ and 2.0 times the mean stream water concentrations. The results of this exercise demonstrated that the observed temporal patterns in net nutrient uptake remained qualitatively similar regardless of which $[N_{gw}]$ value was used (see Results below). w increases with discharge at the weir according to the equation: $w = 0.0139(Q_{weir}) + 1.95$ ($r^2 = 0.85$ [*Roberts et al.*, 2007b]).

3.4. Ecosystem Metabolism Rates

[16] Daily whole-stream rates of gross primary production (GPP) and ecosystem respiration (ER) were determined using an open system, single station diel dissolved O_2 change approach [*Roberts et al.*, 2007b]. Measurements of dissolved O_2 (DO) and water temperature were made at 15-min intervals from 28 January 2004 to 30 June 2006 using YSI model 6920 sondes equipped with model 6562 DO probes at the same downstream location (~120 m upstream from the weir) of the reach used in previous metabolism studies in Walker Branch [*Marzolf et al.*, 1994; *Mulholland et al.*, 1997, 2000, 2006; *Hill et al.*, 2001; *Roberts et al.*, 2007b]. Percent saturation of DO was determined from the measurement of DO concentration, water temperature, and barometric pressure (measured with a Vaisala Model PTB101B analog barometer and recorded at 15-min inter-

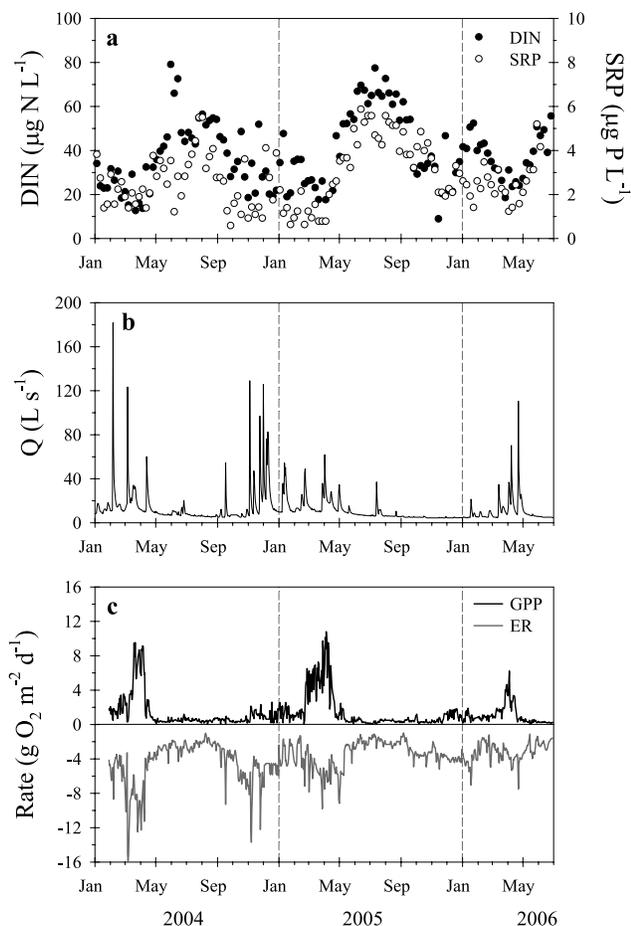


Figure 1. (a) Weekly dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) concentrations ($\mu\text{g L}^{-1}$), (b) daily mean discharge (Q , L s^{-1}), and (c) daily rates of gross primary production (GPP: positive values, black line) and ecosystem respiration (ER: negative values, gray line) in Walker Branch from January 2004 through June 2006. Vertical lines separate years.

vals with a Campbell Scientific Model CR10WP datalogger at a streamside site located ~ 10 m upstream from the sonde).

[17] Ecosystem metabolism rates were determined from the rate of change in DO concentration over 15-min intervals using the equation:

$$\Delta\text{DO} = \text{GPP} - \text{ER} + \text{E} \quad (2)$$

where ΔDO is the change in DO concentration ($\text{g O}_2 \text{ m}^{-3}$), GPP is volumetric gross primary production ($\text{g O}_2 \text{ m}^{-3}$), ER is volumetric ecosystem respiration ($\text{g O}_2 \text{ m}^{-3}$), and E is net exchange of O_2 with the atmosphere ($\text{g O}_2 \text{ m}^{-3}$) between consecutive measurements. The net exchange of O_2 with the atmosphere is the product of the O_2 reaeration coefficient (k_{O_2}) and the average DO deficit (DO concentration at 100% saturation minus the DO concentration in stream water) over the measurement interval. O_2 reaeration coefficient (k_{O_2}) increases with stream discharge (Q) in Walker Branch, according to the equation $k_{\text{O}_2} = 0.0009(Q_{\text{weir}}) + 0.08$ ($r^2 = 0.91$ [Roberts et al., 2007b]).

[18] The net metabolism flux for a given measurement interval is equal to $\Delta\text{DO} - \text{E}$. During the night, GPP is zero, so the net metabolism flux is equal to ER. During the day, ER was determined by interpolating ER averaged over the hour before dawn and the first hour after dusk [Roberts et al., 2007b]. GPP for each daytime interval was the difference between the net metabolism flux and interpolated ER. Daily volumetric GPP and ER rates ($\text{g O}_2 \text{ m}^{-3} \text{ d}^{-1}$) were calculated as the sum of the 15 min rates over each 24 h period (from 0000 h to 2400 h). These volumetric rates were converted to areal units ($\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$) by dividing by the mean water depth (Z_{mean}) of the stream reach (mean water depth increases with discharge according the equation $Z_{\text{mean}} = 0.0006(Q_{\text{weir}}) + 0.05$, $r^2 = 0.74$ [Roberts et al., 2007b]).

4. Results

4.1. Seasonal Patterns in Stream Water Chemistry, Discharge, and Ecosystem Metabolism

[19] Concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) were highest during the summer and lowest during spring and autumn (Figure 1a) as has been seen in previous years in Walker Branch [Mulholland and Hill, 1997; Mulholland, 2004; Roberts et al., 2007b]. DIN was almost all in the form of NO_3^- in Walker Branch as NH_4^+ concentrations were low (typically $< 5 \mu\text{g N L}^{-1}$) and did not vary seasonally.

[20] Daily discharge exhibited a similar seasonal pattern as has been observed in previous studies in Walker Branch [Mulholland and Hill, 1997; Mulholland, 2004] with highest baseflows during winter and spring and lowest flows occurring prior to leaf fall (September and October) (Figure 1b). Low baseflows in summer and early autumn were the result of high rates of evapotranspiration during the forest growing season.

[21] Ecosystem metabolism rates showed distinct seasonal patterns in Walker Branch (Figure 1c). Daily ER rates were highest during spring and autumn [when [DIN] and [SRP] were lowest (Figure 1a)] and lowest in summer [when [DIN] and [SRP] were highest (Figure 1a)] of each year (Figure 1c). Daily GPP rates were highest during the open-canopy spring period, peaked in late March–early April, and declined as the light availability declined during canopy closure (Figure 1c). Daily GPP was lowest during the closed-canopy summer period and exhibited a slight increase after the canopy re-opened in early November of each year. In addition to seasonal variability, both GPP and ER rates exhibited high day-to-day variability particularly during times when rates were generally high (Figure 1c). Daily GPP was lower in 2006 than in previous years as a result of persistent leaf coverage of the stream surface throughout the winter and early spring period (e.g., $> 50\%$ of the stream surface was still covered in leaves as late as 6 March 2006). This extensive leaf coverage in late winter and early spring 2006, resulting from a lack of large spates after leaf fall (Figure 1b), both reduced light availability to the autotrophic community and repressed the development of the macroalgal bloom observed in 2004 and 2005.

4.2. Downstream Changes in Stream Water Nutrient Concentrations

[22] Distinct seasonal patterns in the net change in DIN concentrations along the 62 m study reach were observed in

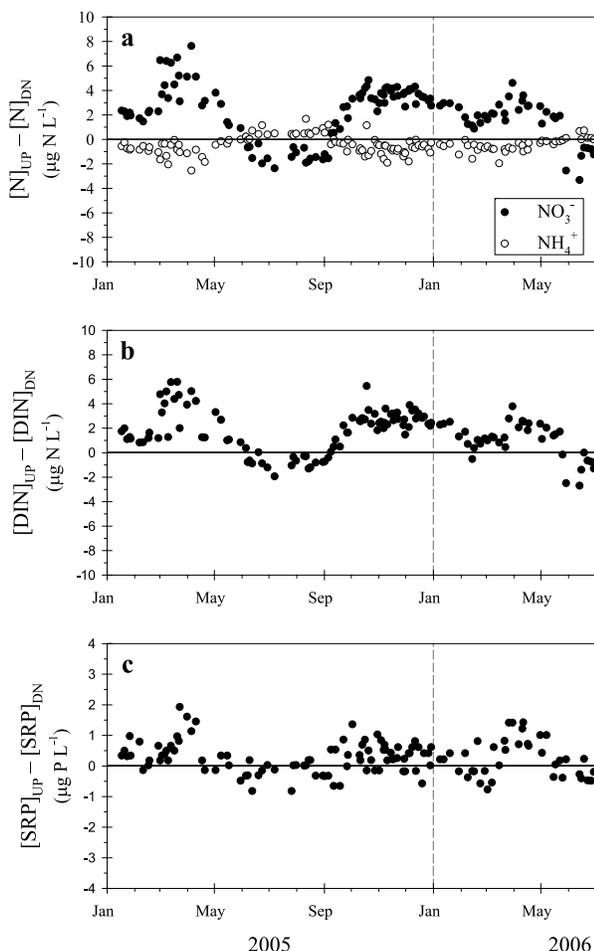


Figure 2. Net downstream changes ($[N]_{up} - [N]_{dn}$) in NO_3^- (solid symbols) and NH_4^+ (open symbols) (a), dissolved inorganic nitrogen (DIN) (b), and SRP (c) concentrations along a 62 m reach in Walker Branch from January 2005 through June 2006. Vertical lines separate years.

Walker Branch over the 18-month study with $[\text{NO}_3^-]_{up} - [\text{NO}_3^-]_{dn}$ ranging between -3.4 and $+7.6 \mu\text{g N L}^{-1}$ (Figure 2a, solid symbols) and $[\text{NH}_4^+]_{up} - [\text{NH}_4^+]_{dn}$ ranging between -2.6 and $+1.6 \mu\text{g N L}^{-1}$ (Figure 2a, open symbols). The net longitudinal change in $[\text{NO}_3^-]$ was positive (indicating a decline in concentration as water moved downstream) throughout the year with the exception of the closed-canopy summer (June–mid-September) when $[\text{NO}_3^-]$ increased as water moved downstream (Figure 1a). The greatest downstream declines were observed in March and April 2005 (when GPP was highest, Figure 1c), with secondary peaks in downstream $[\text{NO}_3^-]$ decline being observed after leaf-fall (October–December) as well as in March and April 2006 (Figure 2a, solid symbols). The net longitudinal change in $[\text{NH}_4^+]$ exhibited the opposite pattern, with downstream increases in $[\text{NH}_4^+]$ in spring and autumn and downstream decreases during summer (Figure 2a, open symbols). Downstream changes in $[\text{NH}_4^+]$ were significantly negatively related to downstream changes in $[\text{NO}_3^-]$ (i.e., $[\text{NH}_4^+]$ declined when $[\text{NO}_3^-]$ increased) with $[\text{NO}_3^-]_{up} -$

$[\text{NO}_3^-]_{dn}$ explaining 51% of the variation in $[\text{NH}_4^+]_{up} - [\text{NH}_4^+]_{dn}$ (Figure 3a). The net result of the contrasting longitudinal patterns in NO_3^- and NH_4^+ was a net longitudinal change in DIN (Figure 2b) that was a similar pattern but of a lesser amplitude than that observed for NO_3^- (Figure 2a). Interestingly, only a weak seasonal pattern in downstream $[\text{SRP}]$ changes was detected in this study (Figure 2c), but these changes were significantly positively related to downstream changes in $[\text{NO}_3^-]$ with $[\text{NO}_3^-]_{up} - [\text{NO}_3^-]_{dn}$ explaining 29% of the variation in $[\text{SRP}]_{up} - [\text{SRP}]_{dn}$ (Figure 3b).

4.3. Net Nutrient Uptake Rates

[23] Net uptake rates for NO_3^- ($U_{\text{NO}_3^-}$) and DIN (U_{DIN}) both displayed distinct seasonal patterns, with $U_{\text{NO}_3^-}$ and U_{DIN} ranging between -7.6 and $+54.8 \mu\text{g NO}_3^- \text{-N m}^{-2} \text{ min}^{-1}$ and -6.2 and $+36.0 \mu\text{g DIN-N m}^{-2} \text{ min}^{-1}$, respectively (Figures 4a and 4d). Both $U_{\text{NO}_3^-}$ and U_{DIN} were highest during March and April 2005 (Figures 4a and 4d) when GPP was highest during the study (Figure 1c). Both rates were negative, indicating a net release of NO_3^- and DIN over the study reach, during the closed-canopy summer (June–early September). The spring peak in U rates during 2006 was lower than in 2005, but comprised the second highest values in the study followed by autumn and early winter when both values were also positive. U_{DIN} rates were consistently lower than $U_{\text{NO}_3^-}$ rates during September–May and higher during June–August periods (Figures 4a and 4d) as a result of NH_4^+ typically being released to the water column when NO_3^- was being taken up by biota and

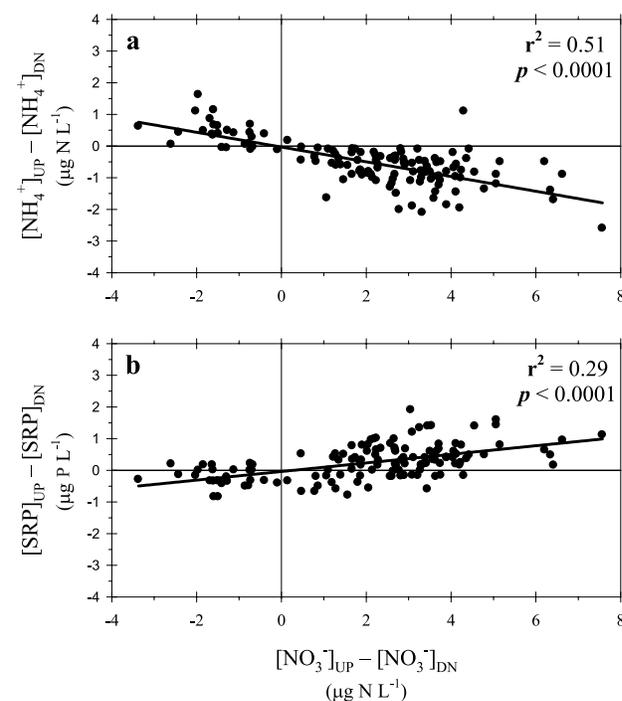


Figure 3. Relationships between net downstream changes ($[N]_{up} - [N]_{dn}$) in NH_4^+ (a) and SRP (b) concentrations with net downstream changes in NO_3^- concentrations ($n = 128$ for both regressions). Solid lines indicate highly significant linear regressions ($p < 0.0001$).

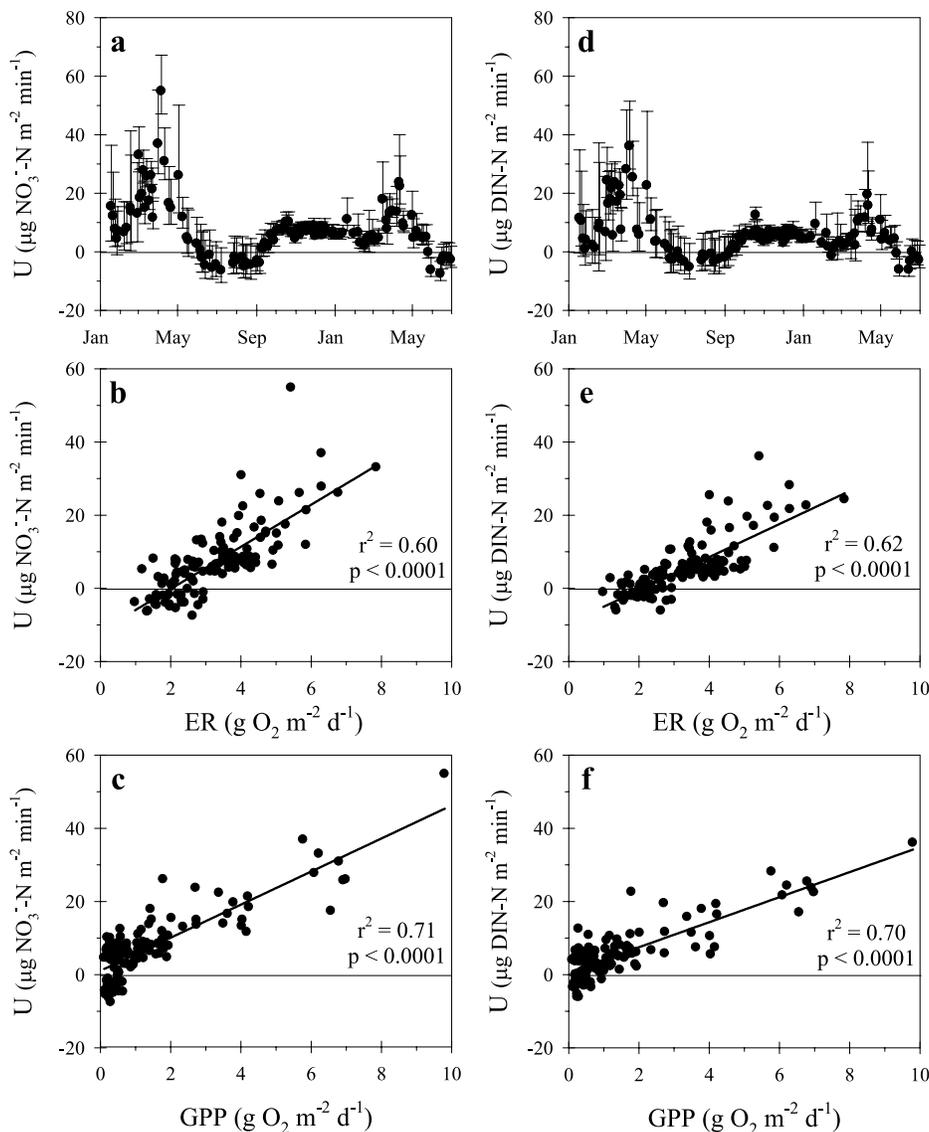


Figure 4. Net uptake rates (U) for NO_3^- (a) and DIN (d) calculated from downstream concentration changes in Walker Branch from January 2005 through June 2006. Points indicate U rates calculated assuming $[\text{N}_{\text{gw}}]$ was equal to the mean stream water concentration ($[\text{N}_{\text{sw}}] = [\text{N}_{\text{up}}] - [\text{N}_{\text{dn}}]/2$). Error bars indicate U rates when $[\text{N}_{\text{gw}}]$ ranged between 0.5 and 2.0 times mean $[\text{N}_{\text{sw}}]$. Relationships between $U_{\text{NO}_3^-}$ (b, c) and U_{DIN} (e, f) with ER (b, e) and GPP (c, f) ($n = 128$ for all regressions). Solid lines indicate highly significant linear regressions ($p < 0.0001$). Horizontal line indicates $U = 0$ in each panel.

NH_4^+ being taken up when NO_3^- was being released to the water column (Figure 2a). In addition to high seasonal variability, both $U_{\text{NO}_3^-}$ and U_{DIN} (Figures 4a and 4d) exhibited high day-to-day variability that appeared to coincide with variability in daily GPP and ER rates (Figure 1c).

[24] $U_{\text{NO}_3^-}$ and U_{DIN} were positively related to ecosystem metabolism rates over the study period, with ER explaining 60% and 62% and GPP explaining 71% and 70% of the variance in $U_{\text{NO}_3^-}$ and U_{DIN} , respectively (Figures 4b, 4c, 4e, and 4f). These relationships were robust with respect to $[\text{N}_{\text{gw}}]$, since even a four-fold variation in assumed $[\text{N}_{\text{gw}}]$ resulted in the slopes of the linear regressions of $U_{\text{NO}_3^-}$ with GPP and ER only varying from 4.1–5.3

and 5.4–6.4, respectively and the slopes of U_{DIN} with GPP and ER only ranging from 2.9–4.4 and 4.2–5.2, respectively ($p < 0.0001$ for all regressions). Using multiple regression analysis, GPP and ER explained 81% of the variation in both $U_{\text{NO}_3^-}$ and U_{DIN} ($U_{\text{NO}_3^-} = 3.16(\text{GPP}) + 2.97(\text{ER}) - 6.91$ and $U_{\text{DIN}} = 2.27(\text{GPP}) + 2.51(\text{ER}) - 6.11$, $p < 0.0001$ for both regressions). When allochthonous carbon inputs from leaf fall were low (February–August), GPP explained an even greater percentage of the variance in U rates, explaining 79% and 78% of the variance in $U_{\text{NO}_3^-}$ and U_{DIN} , respectively ($n = 72$, $p < 0.0001$; data not shown). Using multiple regression analysis, GPP and ER explained 85% of the variation in $U_{\text{NO}_3^-}$ and 86% of the variation in

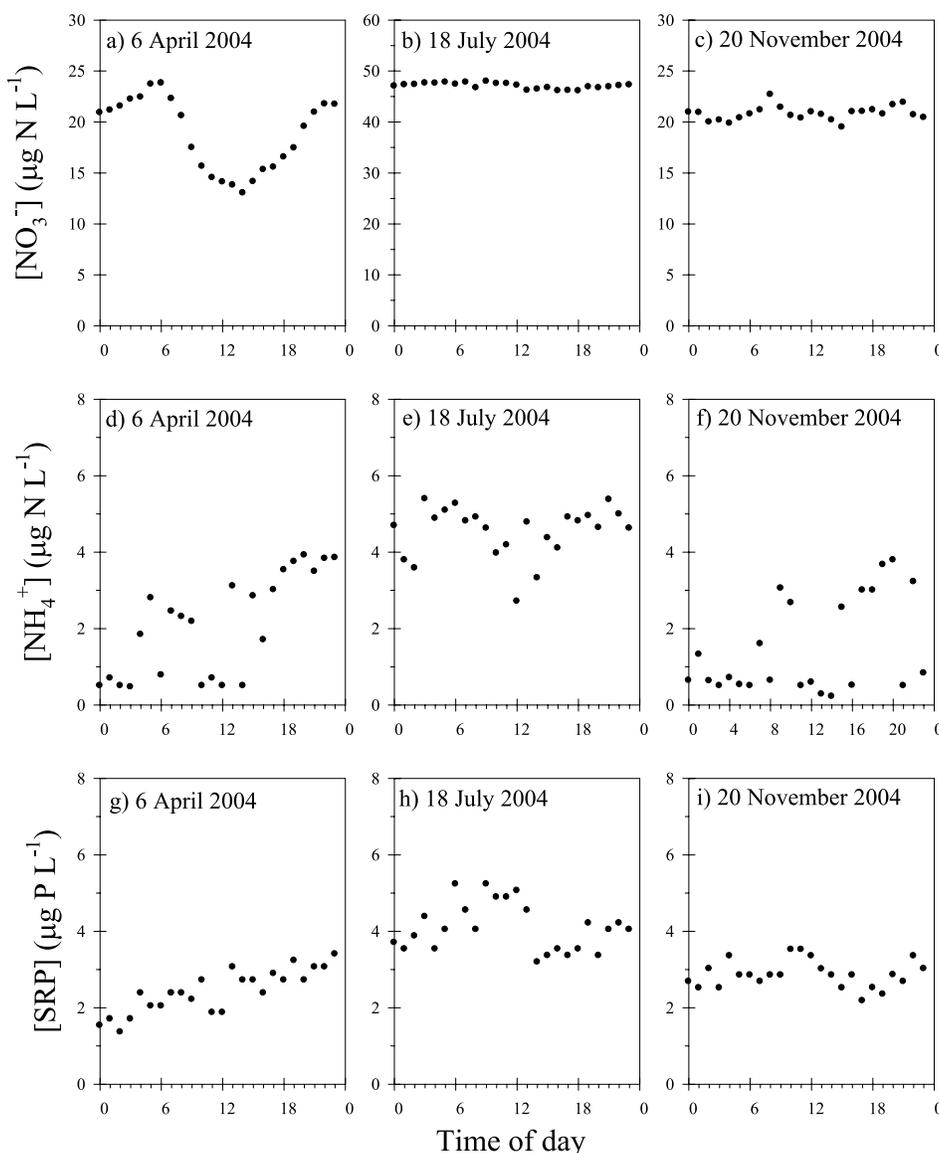


Figure 5. Hourly Walker Branch stream water concentrations for NO_3^- (a, d, c), NH_4^+ (d, e, f), and SRP (g, h, i) over a 24-hour period on 6 April (a, c, g), 18 July (b, e, h), and 20 November (c, f, i) 2004.

U_{DIN} during the February–August period ($U_{\text{NO}_3^-} = 3.45(\text{GPP}) + 2.95(\text{ER}) - 7.91$ and $U_{DIN} = 2.54(\text{GPP}) + 2.57(\text{ER}) - 7.10$, $p < 0.0001$ for both regressions).

4.4. Diel Stream Water Nutrient Concentrations

[25] Diel patterns in stream water nutrients differed seasonally and among constituents (i.e., NO_3^- , NH_4^+ , and SRP). Diel patterns for $[\text{NO}_3^-]$, $[\text{NH}_4^+]$, and $[\text{SRP}]$ for dates from 3 different seasons [open-canopy spring (6 April), mid-summer (18 July) and after leaf-fall (20 November)] in 2004 are depicted in Figure 5. Ecosystem metabolism rates were highest in April, lowest in July, and intermediate in November (GPP and ER rates were $+9.0$ and -6.5 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, $+0.48$ and -2.41 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, and $+0.34$ and -3.36 $\text{g O}_2 \text{ m}^{-2} \text{ d}^{-1}$, respectively). Walker Branch stream water displayed the strongest seasonal differences in $[\text{NO}_3^-]$ (Figures 5a–5c). Mean \pm SE $[\text{NO}_3^-]$ were higher in July (47.0 ± 0.6 $\mu\text{g NO}_3^- \text{ N L}^{-1}$) than in April (18.7 ± 3.6 μg

$\text{NO}_3^- \text{ N L}^{-1}$) or November (20.8 ± 0.7 $\mu\text{g NO}_3^- \text{ N L}^{-1}$). In April, $[\text{NO}_3^-]$ had a diel amplitude of 10.8 $\mu\text{g NO}_3^- \text{ N L}^{-1}$ or 58% of the daily mean (Figure 4a). In contrast, $[\text{NO}_3^-]$ in July and November had significantly lower diel amplitudes (1.8 and 3.1 $\mu\text{g NO}_3^- \text{ N L}^{-1}$, respectively) (Figures 5b and 5c). Neither $[\text{NH}_4^+]$ nor $[\text{SRP}]$ exhibited diel patterns in any season, but concentrations were higher in July (4.5 ± 0.7 $\mu\text{g NH}_4^+ \text{ N L}^{-1}$ and 4.1 ± 0.6 $\mu\text{g SRP L}^{-1}$) than in April (2.15 ± 1.3 $\mu\text{g NH}_4^+ \text{ N L}^{-1}$ and 2.4 ± 0.6 $\mu\text{g SRP L}^{-1}$) or November (1.5 ± 1.2 $\mu\text{g NH}_4^+ \text{ N L}^{-1}$ and 2.9 ± 0.4 $\mu\text{g SRP L}^{-1}$) for both nutrients.

[26] Diel amplitudes in stream water $[\text{NO}_3^-]$ in Walker Branch were maximal during the open-canopy period (March–early April), decreased concurrently with leaf emergence (sharp decline through April) reaching a minima in mid-summer (Figure 6a). Diel amplitudes in early April were 5–6 times greater than in June and July. 91% of the variance in $[\text{NO}_3^-]$ diel amplitude was explained by daily

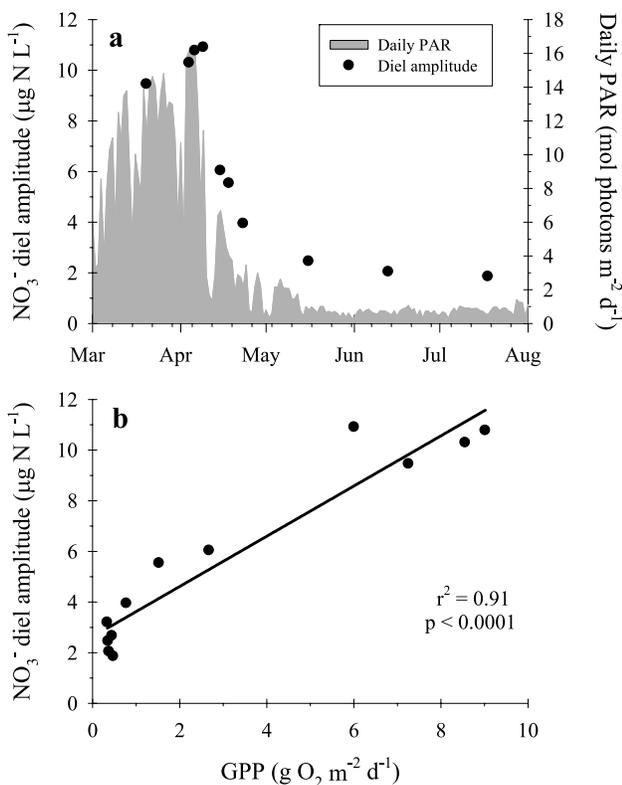


Figure 6. (a) NO_3^- diel amplitude (points) and daily PAR (gray shading) in Walker Branch from March through July 2004. Daily PAR values were taken from Roberts *et al.* [2007b]. (b) Relationship between NO_3^- diel amplitude and GPP measured on the same day in Walker Branch. Solid line indicates a highly significant linear regression ($p < 0.0001$).

GPP rates in Walker Branch [Figure 6b; Diel amplitude = $0.99(\text{GPP}) + 2.63$, $p < 0.0001$].

5. Discussion

5.1. Temporal Patterns in Stream Water Nutrient Concentrations and In-Stream Nutrient Uptake

[27] Seasonal patterns in stream water DIN and SRP concentrations during the study period (2004–2006) were similar to those observed in previous years [Mulholland and Hill, 1997; Mulholland, 2004] with highest concentrations occurring in summer, intermediate in winter, and lowest in spring and autumn. Upstream-downstream patterns of nutrient decline also exhibited distinct seasonal patterns, with large downstream declines in $[\text{NO}_3^-]$ occurring during spring and autumn and downstream increases during summer. Stream water $[\text{NH}_4^+]$ exhibited the opposite pattern, with downstream increases in $[\text{NH}_4^+]$ during both spring and autumn and downstream decreases during summer. Longitudinal declines in $[\text{NO}_3^-]$ and [SRP] were observed during the period from November to May in an earlier study in Walker Branch [Mulholland and Rosemond, 1992]. One possible explanation for the weak longitudinal pattern in [SRP] observed in the current study was that we were attempting to discern small changes (typically $< 1 \mu\text{g P L}^{-1}$; Figure 2c) against a small SRP pool ($1\text{--}6 \mu\text{g P L}^{-1}$; Figure 1a) over a

much shorter reach (62 m versus 140 m) than the one studied in *Mulholland and Rosemond* [1992].

[28] The observed downstream declines in $[\text{NO}_3^-]$ and increases in $[\text{NH}_4^+]$ were not likely the result of groundwater input, since $[N_{\text{gw}}]$ have been previously shown to be similar to stream water concentrations [Mulholland, 1992]. Additionally, groundwater inflow is very low in the study reach, with inflow typically being less than 6% under baseflow conditions (B. J. Roberts, unpublished data). Further, the distinct temporal patterns observed in $U_{\text{NO}_3^-}$ and U_{DIN} were robust with respect to $[N_{\text{gw}}]$ since even a four-fold variation in assumed $[N_{\text{gw}}]$ values (from 0.5 to 2.0 times stream water concentrations) did not strongly influence temporal patterns of either $U_{\text{NO}_3^-}$ (Figure 4a) or U_{DIN} (Figure 4d).

[29] Previous studies in Walker Branch have shown that temporal variation in the dominant hydrologic flow paths through soil result in lower observed winter and higher summer nutrient concentrations, but do not explain the spring and autumn DIN and SRP concentration minima [Mulholland and Hill, 1997]. The lower observed than predicted (based on soil flow paths alone) stream concentrations were attributed to in-stream biotic uptake since an algal bloom occurs in the spring and leaf fall increases organic matter availability in autumn [Mulholland, 2004]. This assertion is further supported by results of two intensive studies that have shown that as light availability declines during closure of the forest canopy in spring, GPP rates decline and stream water $[\text{NO}_3^-]$ increase [Hill *et al.*, 2001; Roberts *et al.*, 2007b]. Mulholland [2004] also observed higher stream $[\text{NO}_3^-]$ than predicted from soil flow paths alone during the summer (June through August). The temporal patterns in net nutrient uptake rates we report in the current study, with high net NO_3^- (and DIN) uptake rates during both spring and autumn and a small net release of NO_3^- ($U_{\text{NO}_3^-} < 0$) to stream water in summer, are consistent with these earlier observations.

[30] A large fraction of NH_4^+ uptake is accounted for by nitrification in many streams [e.g., Webster *et al.*, 1991; Peterson *et al.*, 2001; Bernhardt *et al.*, 2002]. In a ^{15}N tracer study, Mulholland *et al.* [2000] demonstrated that nitrification (both direct and indirect [coupled regeneration of biomass N and nitrification]) is an important process in Walker Branch with direct nitrification accounting for $\sim 20\%$ of total NH_4^+ uptake during the spring algal bloom. Nitrification consumed $\sim 3 \mu\text{g N/L}$ of NH_4^+ and produced a similar amount of NO_3^- -N during this period [Mulholland *et al.*, 2000]. We observed net downstream declines in $[\text{NH}_4^+]$ and increases in $[\text{NO}_3^-]$ of a similar magnitude ($\sim 1\text{--}2 \mu\text{g N/L}$) during summer, suggesting that during this period of low assimilatory N demand (see low GPP and ER rates in Figure 1a) nitrification rates likely remained relatively high. Previous studies in Walker Branch have also demonstrated that high assimilatory N demand during the spring algal bloom results in high gross uptake rates of both NH_4^+ [Mulholland *et al.*, 2000] and NO_3^- [Mulholland *et al.*, 2006]. However, in the current study we observed net uptake of NO_3^- but net release of NH_4^+ to the water column during spring and autumn. One reason for this observed release is that ammonium excretion by the dominant grazer in Walker Branch, the snail *Elimia clavaeformis*, peaks during spring and autumn when it can account for over 60% of baseflow $[\text{NH}_4^+]$ ($\sim 2\text{--}3 \mu\text{g NH}_4^+ \text{N/L}$) (B. J. Roberts

et al., unpublished data). Additionally, when algal production is high in spring, regeneration of biomass N as NH_4^+ might be expected to be high.

[31] In summary, NH_4^+ uptake is constrained by the very low stream water concentrations ($<5 \mu\text{g NH}_4^+\text{-N/L}$) at all times in Walker Branch while NH_4^+ regeneration is a function of total DIN uptake by high quality biomass that increases in spring and autumn due to snail grazing on highly productive algae and decomposition of leaf detritus, respectively. In spring and autumn, although NH_4^+ uptake increases due to N demand, uptake is constrained by low concentrations and net release occurs. At these times, NO_3^- increases sharply to meet the N demand that cannot be met by NH_4^+ due to its low concentration [Mulholland et al., 2000].

[32] It should be noted that U rates calculated during the spring were likely overestimated since NO_3^- uptake in Walker Branch varies over diel cycles during this period of the year (as evidenced by diel $[\text{NO}_3^-]$ patterns presented here (Figure 5) and reported in previous studies [Mulholland et al., 2006]) and sampling usually occurred during the time of maximal uptake (1200–1400 EST). However, seasonal U patterns are still likely to be robust since if daytime rates were assumed to be ~ 2 times nighttime rates (the maximal difference observed in Mulholland et al. [2006]) our calculated daily net U rates would only have been overestimated by $\sim 33\%$ during spring.

5.2. In-Stream Nutrient Uptake and Annual Nutrient Input-Output Budgets

[33] We attempted to examine our net nutrient uptake rates in the broader context of the annual nutrient input-output budget for Walker Branch. In 2005, 77% of the annual water flux occurred under the low flow ($<25 \text{ L/s}$) conditions when net nutrient uptake was measured in this study. This greater importance of low flow compared to high flow water flux is typical of Walker Branch and other stable groundwater streams [Poff, 1996]. By comparing the annual DIN flux (from weekly nutrient sampling and flow-weighted concentrations as in Mulholland [2004]) with our low flow DIN flux calculated from the average weekly DIN concentrations at our upstream station and Q , we determined that $\sim 75\%$ of the 2005 DIN flux occurred under low flow conditions. Since our sampling design accounted for the majority of the annual water and nutrient flux, we attempted to estimate the fraction of DIN delivered to our reach that was retained over the course of the year. We calculated the percentage of DIN loading taken up within our reach by comparing the weekly U_{DIN} (average weekly U_{DIN} (per min) multiplied by 10080 min/wk) with the weekly DIN load (as calculated above). On average, 6.54% of the 2005 DIN load was taken up within our 62 m reach with the maximum weekly average of 25.4% occurring in early April and the minimum average of -4.0% occurring in mid-July. If we ignore further longitudinal DIN inputs and assume there is no reduction in U_{DIN} as concentration decreases, then, on average, 948 m (and only 244 m in early April) of stream length are required to remove all of the DIN loaded from upstream of our reach.

5.3. Metabolism Control on Nutrient Retention

[34] We showed that there is substantial day-to-day and seasonal variability in net DIN uptake rates in Walker

Branch. Several studies have observed substantial monthly variation in nutrient uptake rates [Mulholland et al., 1985; Martí and Sabater, 1996; Simon et al., 2005], but few have explicitly examined if the observed variability was related to ecosystem metabolism. The strong relationships between net NO_3^- and DIN uptake and ecosystem metabolism rates in Walker Branch indicate the important role of in-stream biotic activity in regulating N retention and export in streams. These findings are consistent with other studies that have shown that N uptake is related to metabolic activity in streams. For example, NO_3^- uptake in Walker Branch was related to daily GPP on several dates in early spring 2001 [Mulholland et al., 2006]. Hall and Tank [2003] found that GPP and ER explained 82% of the variance in NH_4^+ uptake velocity, while GPP alone explained 75% of the variance in NO_3^- uptake velocity, indicating that autotrophs used both NO_3^- and NH_4^+ as a N source but heterotrophs only used NH_4^+ , in 11 low-nitrogen streams in Wyoming. These earlier studies were based on either a single measurement of N uptake in 11 streams along a metabolism gradient [Hall and Tank, 2003] or measurements on 3 dates of varying cloud cover in a single stream [Mulholland et al., 2006]. In our study, we were able to show that variability in ecosystem metabolism rates was able to explain a significant amount of both the day-to-day and seasonal variability in net DIN uptake in Walker Branch.

[35] The best predictor of net $U_{\text{NO}_3^-}$ (and net U_{DIN}) in Walker Branch was daily GPP (especially during the February–August period when allochthonous organic matter inputs from leaves was minimal) suggesting that autotrophy plays an important role in regulating DIN, particularly NO_3^- , concentrations in streams. The observed seasonal patterns in net U rates (highest during spring algal bloom, intermediate during autumn leaf fall, and lowest in summer) suggest that algae (which dominate metabolism in spring) are strong net retainers of DIN, while DIN uptake and release is more tightly coupled in bacteria and fungi growing on leaf detritus (which dominate metabolism in autumn). This is consistent with the notion that bacteria and fungi have a lower capacity for long-term net storage of nutrients in biomass.

5.4. Diel Patterns in Stream Water Nutrient Concentrations

[36] We showed that Walker Branch stream water $[\text{NO}_3^-]$ exhibit distinct diel patterns prior to leaf emergence in spring. The observation of lower stream water $[\text{NO}_3^-]$ during daylight hours than at night in spring suggests in-stream NO_3^- uptake is greater during the day, since the $[\text{NO}_3^-]$ in source waters (i.e., springs) does not vary over diel periods. In a previous study in Walker Branch (10–11 April 1991), stream water $[\text{NO}_3^-]$ had a diel amplitude of up to $14 \mu\text{g N L}^{-1}$ or $\sim 50\%$ of the mean concentration on that date. Similar to the current study, neither $[\text{NH}_4^+]$ nor $[\text{SRP}]$ showed any evidence of diel variation during April [Mulholland, 1992]. Since stream water $[\text{NO}_3^-]$ would be relatively constant in the absence of biotic activity, these diel patterns indicate that in-stream NO_3^- uptake increases during the morning (as light availability increases) to a maximum in early afternoon ($\sim 1400 \text{ EST}$). NO_3^- uptake then declines through the afternoon (as light decreases) and

night (as stored photosynthate generated during daylight is depleted) reaching a daily minimum just prior to dawn. This pattern is consistent with a previous study in Walker Branch using ^{15}N tracer additions that showed NO_3^- uptake rates were $\sim 2\text{--}3$ times higher at mid-day than before dawn with rates at midnight being intermediate in early April 2001 [Mulholland *et al.*, 2006].

[37] Other studies also have reported large diel variations in stream water $[\text{NO}_3^-]$ [Manny and Wetzel, 1973; Grimm, 1987; Burns, 1998], often attributing the observed diel patterns to autotrophic activity. In the current study, we were able to directly demonstrate a biological mechanism to explain diel variations in stream water $[\text{NO}_3^-]$ by measuring diel $[\text{NO}_3^-]$ patterns in conjunction with daily GPP rates. The reduction of NO_3^- for use in biosynthesis is an energetically expensive process [Dortch, 1990]. Photosynthesis provides additional energy that can be used to reduce NO_3^- for use in metabolism and biosynthesis [Falkowski and Raven, 1997]. As a result, it is not surprising to observe tight coupling between GPP and diel $[\text{NO}_3^-]$ amplitude (and corresponding diel variation in NO_3^- uptake rates).

[38] The lack of detectable diel $[\text{NO}_3^-]$ amplitude during either summer or autumn suggests that autotrophs play a less important role in NO_3^- retention during these seasons than during the open-canopy spring. When GPP and ER rates were low during the summer, DIN retention was often negative (resulting in a net release of DIN into the water column). When light energy for photosynthesis is low in summer, NO_3^- uptake is a relatively more energetically costly process for autotrophs than during the spring. In autumn no detectable diel amplitude was observed even though stream water $[\text{NO}_3^-]$ were lower and net nutrient uptake was high, suggesting that uptake was largely a result of heterotrophic N demand associated with increased organic matter availability from leaf litter inputs.

6. Implications for Interpreting Stream Water Nutrient Concentrations and Nutrient Export From Catchments

[39] This study adds to the growing body of evidence demonstrating that in-stream processes are important regulators of stream water nutrient concentrations and must be considered in interpreting nutrient exports. The implications of our results are three-fold: (1) if in-stream nutrient processing is ignored, erroneous conclusions may be reached about the role of terrestrial processes in controlling catchment nutrient retention and export, (2) substantial diel and day-to-day variability in in-stream nutrient uptake suggests the potential for significant errors when assessing nutrient retention based on infrequent uptake measurements, and (3) seasonal patterns in stream water N concentrations are often controlled by seasonal variation in rates of in-stream biotic activity in many headwater streams.

[40] Specifically, our research showed that temporal variability in net DIN retention was strongly related to whole ecosystem rates of GPP and ER in Walker Branch. We further showed that temporal variability in DIN retention and export is highly influenced by the relative importance of autotrophic and heterotrophic contributions to ecosystem metabolism. These results suggest that stream metabolism data may be used to estimate in-stream nutrient uptake and

retention rates. These results further indicate that disturbances or other environmental changes that alter stream ecosystem metabolism rates (e.g., changes in substratum characteristics, light regime, organic matter retention capacity) may have large impacts on the ability of stream biota to retain N, thus increasing N losses to downstream ecosystems.

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