Influence of dual nitrogen and phosphorus additions on nutrient uptake and saturation kinetics in a forested headwater stream

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Abstract: Nitrogen (N) and phosphorus (P) can limit autotrophic and heterotrophic metabolism in lotic ecosystems, yet most studies that evaluate biotic responses to colimitation focus on patch-scale (e.g., nutrient diffusing substrata) rather than stream-scale responses. In this study, we evaluated the effects of single and dual N and P additions on ambient nutrient uptake rates and saturation kinetics during two biologically contrasting seasons (spring, autumn) in Walker Branch, a temperate forested headwater stream in Tennessee, USA. In each season, we used separate instantaneous pulse additions to quantify nutrient uptake rates and saturation kinetics of N (nitrate) and P (phosphate). We then used steady-state injections to elevate background stream water concentrations (to low and then high background concentrations) of one nutrient (e.g., N) and released instantaneous pulses of the other nutrient (e.g., P). We predicted that elevating the background concentration of one nutrient would result in a lower ambient uptake length and a higher maximum areal uptake rate of the other nutrient in this colimited stream. Our prediction held true in spring, as maximum areal uptake rate of N increased with elevated P concentrations from 185 $\mu g\ m^{-2}\ min^{-1}$ (no added P) to 354 $\mu g\ m^{-2}\ min^{-1}$ (high P). This pattern was not observed in autumn, as uptake rates of N were not measurable when P was elevated. Further, elevating background N concentration in either season did not significantly increase P uptake rates, likely because adsorption rather than biotic uptake dominated P dynamics. Laboratory P sorption assays demonstrated that Walker Branch sediments had a high adsorption capacity and were likely a sink for P during most pulse nutrient additions. Therefore, it may be difficult to use coupled pulse nutrient additions to evaluate biotic uptake of N and P in streams with strong P adsorption potential. Future efforts should use dual nutrient addition techniques to investigate reach-scale coupled biogeochemical cycles (C–N–P, and other elemental cycles [e.g., Fe, Mo]) across seasons, biomes, and land-use types and over longer time periods.

Key words: nitrate, phosphate, uptake length, maximum areal uptake rate, Tracer Additions for Spiraling Curve Characterization, steady-state addition, adsorption, coupled biogeochemical cycles

Nitrogen (N) and phosphorus (P) can limit the production of autotrophs and heterotrophic microbes in stream ecosystems (Elwood et al. 1981, Tank and Webster 1998, Francoeur 2001, Slavik et al. 2004, Johnson et al. 2009, Rosemond et al. 2015). However, in excess, these nutrients can result in eutrophication and negatively affect downstream

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water quality (Rabalais et al. 2002, Royer et al. 2004). The nutrient spiraling framework (Stream Solute Workshop 1990) can be used to assess the ability of organisms to take up and transform streamwater nutrients via assimilatory and dissimilatory processes at the stream-reach scale. Specifically, nutrient addition methods are used to measure nutrient uptake length, which is defined as the downstream distance a nutrient molecule travels before being taken up by biota (e.g., Tank et al. 2006), and additional uptake metrics (uptake velocity, areal uptake rate) are then calculated. Several nutrient addition methods can be used to measure uptake rates (Trentman et al. 2015), including steady-state injections with nutrients or stable isotopes (Peterson et al. 2001, Webster et al. 2003, Mulholland et al. 2008), pulse nutrient additions (Tank et al. 2008), and saturating pulse nutrient additions (Covino et al. 2010a, b, Diemer et al. 2015). The saturating pulse nutrient addition method (Tracer Additions for Spiraling Curve Characterization; TASCC) is advantageous in that both nutrient uptake rates and saturation kinetic parameters (i.e., maximum areal uptake rate, half-saturation constant) can be deduced from a single pulse addition (Covino et al. 2010a, b).

A Michaelis–Menten (MM) kinetic model is often used to describe saturation dynamics of nutrient uptake in streams (Dodds et al. 2002, Covino et al. 2010b, O'Brien and Dodds 2010), although this model may not be applicable to all streams (Earl et al. 2006, O'Brien et al. 2007, Trentman et al. 2015). In the MM model, uptake rate of a limiting nutrient increases hyperbolically with concentration until uptake rate reaches a plateau and becomes saturated. Comparison of saturation kinetic parameters to nutrient uptake metrics can determine if a stream is close to saturation (O'Brien and Dodds 2010). For instance, a stream may be close to saturation if the ambient nutrient concentration is similar to the half-saturation constant (K_m), and if ambient areal uptake rate is similar to the maximum areal uptake rate (U_{max}) (i.e., uptake rate at plateau). Saturation kinetics may also differ for different limiting nutrients depending on the degree of biotic vs physical/chemical uptake. For instance, nitrate uptake, which is primarily biotic, may saturate at lower concentrations than phosphorus uptake, which is also driven by abiotic adsorption to sediments. During a phosphorus release in Walker Branch, a forested stream in Tennessee, USA, soluble reactive phosphorus (SRP) uptake plateaued at low SRP concentrations (<5 μg P/L), likely because of saturation of biotic uptake. However, after this initial plateau, uptake rates continued to increase linearly with concentration, suggesting that higher uptake rates were caused by abiotic adsorption (Mulholland et al. 1990).

Considerable research has examined the controls on nutrient uptake rates in stream ecosystems (e.g., Simon et al. 2005, Hoellein et al. 2007, von Schiller et al. 2008). In some streams, there are strong seasonal controls on nutrient uptake. For instance, nutrient uptake rates in temperate forest streams can vary seasonally because of changes in riparian tree phenology. Nitrate and phosphate uptake rates are often high in the spring open-canopy period because of enhanced autotrophic activity, and high in autumn when leaf-fall stimulates heterotrophic microbial activity (Mulholland et al. 1985, Roberts and Mulholland 2007, Claessens et al. 2010). Nutrient uptake rates in Mediterranean streams also exhibit seasonality, with ammonium uptake controlled by temperature and phosphate uptake controlled by algal dynamics (Martí and Sabater 1996). Nutrient uptake rates are often correlated with biotic processes, especially gross primary production (GPP) and ecosystem respiration (ER) (Hall and Tank 2003, Hoellein et al. 2007, Heffernan and Cohen 2010). Nutrient uptake rates are also higher in streams with high nutrient concentrations (Arango et al. 2008), but removal efficiency decreases as uptake becomes saturated (Bernot et al. 2006, O'Brien et al. 2007, Mulholland et al. 2008, Hall et al. 2009).

Studies of nutrient uptake have greatly improved our understanding of the processes that control nutrient cycling and affect streamwater nutrient concentrations (Mulholland and Webster 2010), but most studies focus on a single nutrient despite the fact that streams can be colimited for N and P (Francoeur 2001, Johnson et al. 2009). Studies that have manipulated nutrient concentrations to examine coupled biogeochemical cycles at stream-reach scales primarily focused on coupled carbon–nutrient dynamics rather than coupled N and P dynamics. For instance, increased concentrations of dissolved organic carbon (DOC) can increase N uptake (Bernhardt and Likens 2002, Johnson et al. 2012, Rodríguez-Cardona et al. 2016), and increased N and P concentrations can increase C mineralization rates (Mineau et al. 2013, Rosemond et al. 2015). Increased DOC concentrations also stimulated P uptake in a high-nitrate stream, but only after ammonium concentrations were simultaneously increased (Ovideo-Vargas et al. 2013). Some studies have measured both N and P uptake in streams (e.g., Bernot et al. 2006, Hoellein et al. 2007, Martí et al. 2009, Diemer et al. 2015) or across streams with different N and P concentrations and ratios (Gibson et al. 2015), but few have manipulated N and P simultaneously in a stream to examine how uptake responds to changing nutrient availability. Based on single and dual N and P TASCC releases, Piper et al. (2017) found that the addition of both nutrients to colimited streams increased N and P uptake rates relative to single nutrient additions. Similarly, Schade et al. (2011) applied steady-state injections of N and P to N-limited streams and found that P uptake lengths were shorter with N addition (but not vice versa). Further, P uptake responses differed between autotrophic and heterotrophic streams (Schade et al. 2011), suggesting that N and P uptake responses may vary in colimited streams that exhibit seasonality (e.g., temperate forested streams).

The objective of this study was to examine how N and P interact to influence nutrient uptake and saturation kinetics. Previous research showed that our study stream (Walker

Branch) can be colimited for N and P (Rosemond et al. 1993, Mulholland et al. 2000), and we predicted that both ambient nutrient demand and maximum areal uptake rate of one nutrient would increase when the concentration of the 2nd nutrient is elevated. To determine if dual N and P uptake responses vary seasonally, we carried out nutrient release experiments in 2 biologically contrasting seasons: 1) autumn, when nutrient demand by heterotrophs is high because of large in-stream standing stocks of leaf litter, and 2) in early spring, when nutrient demand by autotrophs is high because of an open canopy and warmer temperature (Roberts et al. 2007, Lutz et al. 2012).

METHODS

General approach

The main focus of our study was to examine how N and P interact to influence nutrient uptake and saturation kinetics. We addressed this objective by conducting a series of single and dual nutrient additions in 2 contrasting seasons (autumn and spring) in the West Fork of Walker Branch, a forested headwater stream in eastern Tennessee, USA. However, we first wanted to confirm the nutrient limitation status of Walker Branch. Therefore, we deployed nutrient diffusing substrata (NDS) in autumn and spring to determine the potential nutrient limitation of stream biofilms (i.e., N-limited, P-limited, colimited, or neither N- nor P-limited). We then conducted a total of 6 single and dual N and P releases in Walker Branch in each season. We used separate instantaneous pulse (TASCC) additions to quantify nutrient uptake rates and saturation kinetics of N and P individually (Covino et al. 2010b). We then used steady-state injections to increase background streamwater concentrations of 1 nutrient (e.g., N) and released instantaneous pulses of the other nutrient (e.g., P). Background streamwater nutrient concentrations were elevated to low and high levels, and we characterized nutrient uptake rates and kinetics of N and P from the TASCC releases conducted at background and elevated (low and high) P and N concentrations. We will refer to these releases for P uptake as 'P alone', 'P with N low', and 'P with N high', and for N uptake as 'N alone', 'N with P low', and 'N with P high'.

Study site

The West Fork of Walker Branch is a forested headwater stream located on the US Department of Energy's Oak Ridge Reservation (lat 35°57′32″N, long 84°16′47″W) in eastern Tennessee, USA. Walker Branch watershed is underlain by dolomite (Knox Group), and areas of exposed bedrock are present in the streambed (Lietzke 1994). Otherwise, the streambed is composed of cobble, gravel, and fine-grained sediments. Four perennial springs result in relatively constant base flow (5–10 L/s; Mulholland et al. 1997), and surface water flows for approximately 300 m before reaching a 120° v-notch weir where water level is recorded every 15 min. Stream water is generally basic and alkaline, and has low concentrations of inorganic N and SRP (Mulholland 2004, Lutz et al. 2012, Table 1).

The watershed is covered by a 2nd-growth deciduous forest, and forest phenology strongly controls hydrology and in-stream processes (Mulholland 2004, Roberts et al. 2007, Lutz et al. 2012). Stream flow is generally highest in winter and early spring because of low rates of evapotranspiration, and storm flows are more common during this period as well (Mulholland 2004). Streamwater inorganic N and SRP concentrations are generally lowest in spring and autumn because of uptake by stream autotrophs and heterotrophs, respectively (Roberts and Mulholland 2007, Lutz et al. 2012). In early spring, an open canopy alleviates light limitation of periphyton, leading to increased rates of GPP (Hill et al. 2001, Roberts et al. 2007) and nutrient uptake (Roberts and Mulholland 2007). In autumn, leaf fall results in large standing stocks of organic matter (Comiskey 1978) that fuels high rates of ER (Roberts et al. 2007) and nutrient uptake (Mulholland et al. 1985, Roberts and Mulholland 2007). Therefore, we chose these 2 seasons to carry out nutrient releases to examine how a dominant autotrophic (in spring) and heterotrophic (in autumn) community responds to differing concentrations of N and P.

Nutrient diffusing substrata

NDS consisted of 50-mL centrifuge tubes filled with a 2% agar solution containing either 0.05 mol/L $NaNO_3$,

Table 1. Characteristics of the West Fork of Walker Branch during the autumn and spring sampling periods. Discharge, water temperature (°C), photosynthetically active radiation (PAR), gross primary production (GPP), and ecosystem respiration (ER) are mean values calculated from daily measurements collected during the sampling periods (i.e., over 2–3 days/season). Coarse particulate organic matter (CPOM) standing stock and water chemistry (specific conductivity, alkalinity, and ammonium (NH_4), nitrate (NO_3), and soluble reactive phosphorus (SRP) concentrations) were measured once during the sampling period.

	Autumn	Spring
Discharge (L/s)	4	18
Water temperature (°C)	12.2	14.2
Specific conductivity (μS/cm)	250.7	130.6
Alkalinity (mg/L $CaCO_3$)	126	62
NH_4 (μg N/L)	9.8	5.3
NO_3 (μg N/L)	14.1	22.5
SRP (μg P/L)	2.1	2.7
CPOM standing stock (g DM/m^2)	391	45
PAR (mol m^{-2} d^{-1})	1.6	10.8
GPP (g O_2 m^{-2} d^{-1})	0.4	2.4
ER (g O_2 m^{-2} d^{-1})	−5.3	−3.9

0.05 mol/L KH_2PO_4, both (N + P), or no added nutrients as a control ($n = 5$ for each treatment). Results from the autumn NDS found the strongest response with a lower concentration of nutrients (0.05 mol/L) compared with the standard concentration used for NDS experiments (0.5 mol/L; as in Tank et al. 2006). NDS were topped with an organic cellulose sponge cloth to select for heterotrophic constituents of stream biofilms (Johnson et al. 2009). NDS were deployed for 28 d after which the organic substrata were removed from the tubes and respiration was measured on each sample in the field. As described in Johnson et al. (2009), respiration was measured from the consumption of dissolved oxygen after a 4-h incubation in dark centrifuge tubes filled with unfiltered stream water (no air bubbles). Centrifuge tubes containing unfiltered stream water only ($n = 5$) were included to account for background changes in dissolved oxygen concentration. Tubes were placed within a shaded section of the stream during the respiration incubation to keep substrata at ambient temperature. The change in oxygen from the beginning to the end of the incubation was measured with an oxygen probe (YSI-85 probe; Yellow Springs Instruments, Yellow Springs, Ohio).

Nutrient additions

Nutrient additions were conducted in autumn (1–3 November 2011) and spring (20–22 March 2012). To examine nutrient uptake and saturation kinetics of NO_3-N and PO_4-P (hereafter referred to as N and P, respectively), we used the TASCC method detailed in Covino et al. (2010b). For each addition, we added a 10-L solution of either N or P with NaCl as a conservative tracer to the stream. We measured the pulse of nutrient and conservative tracer 74 m downstream from the addition point (and approximately 130 m upstream of the weir). Specific conductivity was continuously measured with a handheld conductivity probe (YSI Model 30; Yellow Springs Instruments). Water samples were collected every min once specific conductivity started to increase and sampling continued until specific conductivity returned to background levels. The autumn nutrient solutions consisted of either 25 g KNO_3 or 2.5 g KH_2PO_4 with 400 g NaCl, and the spring nutrient solutions consisted of either 50 g KNO_3 or 2.75 g KH_2PO_4 with 450 g NaCl. We increased the nutrient concentration in the spring release solutions, because spring discharge was higher than autumn discharge (Table 1). We collected samples of the nutrient release solutions and diluted 5 analytical replicates (1 : 1000) for analysis of N, P, and specific conductivity to confirm nutrient : conservative tracer ratios for uptake calculations (see below).

For the dual nutrient additions, we used steady-state injections to elevate either N or P concentrations throughout the reach. In both seasons, we used a pump (3CKC pump head; Fluid Metering, Inc., Syosset, New York) to add nutrients at a constant rate of 24 mL/min for the low nutrient concentration and 48 mL/min for the high nutrient concentration. In autumn, the nutrient solution contained either 34 g KNO_3 or 3.2 g KH_2PO_4. Thus, the low and high target concentrations were 5 and 10 μg P/L and 32 and 64 μg N/L above ambient concentrations. In the spring, the more concentrated N and P solutions contained 303 g KNO_3 and 41 g KH_2PO_4, respectively. Spring target concentrations for N were the same as in autumn, but we increased target P concentrations to 10 and 20 μg P/L above ambient concentrations, because we noticed high P adsorption in the autumn. During each steady-state release, we collected water samples at 5 stations longitudinally throughout the reach both before the addition and after steady state (i.e., plateau) was reached at the farthest downstream station (74 m). We estimated plateau as the time to peak conductivity from the pulse addition multiplied by 2 (autumn = 60 min; spring = 30 min), because the median travel time determined by peak conductivity is estimated to be ½ the time to plateau (Runkel 2002). We estimated the nutrient concentrations in stream water during the low and high nutrient additions as the geometric means of nutrient concentrations from the 5 longitudinal samples.

After plateau samples were collected, we then conducted a pulse nutrient release while the stream was enriched at a low concentration of the other nutrient. We collected water samples and measured specific conductivity at the farthest downstream station (74 m) during the breakthrough curve as described above. Once specific conductivity from the pulse release returned to baseline, the drip rate was increased to achieve the high nutrient concentration. After the high nutrient concentration plateau was reached, we collected water samples at the 5 stations. We then conducted a second pulse release while the stream was enriched at a high concentration of the other nutrient.

In autumn, we conducted single nutrient additions (pulses) for N and P on the first day of field work, P pulses with low and high N concentrations on the 2nd day, and N pulses with low and high P concentrations on the 3rd day. In the spring, we conducted all P pulses on the 1st day of field work (P alone, P with N low, P with N high) and all N pulses on the 3rd day (N alone, N with P low, N with P high) with a day in between when no nutrient releases were conducted. It is possible that conducting multiple nutrient releases in a single day could have introduced artefacts that affected nutrient uptake rates (e.g., the first nutrient release may have alleviated nutrient limitation during subsequent nutrient releases). However, we chose to conduct multiple releases within a short period of time to minimize effects of changing environmental conditions (e.g., changes in canopy cover due to leafout, storm events) on nutrient uptake.

All water samples were filtered in the field through Whatman GF/F filters (0.7-μm nominal pore size; Maidstone, UK), put on ice in the field, and then frozen in the laboratory until analysis. We used a DIONEX ICS-2000 ion chromatograph with an AS11-HC column (Dionex, Sunnyvale,

California) to quantify NO_3^--N concentrations. We used molybdate-blue colorimetry (APHA 2005) on an autoanalyzer (AA3; Seal Analytical Inc., Mequon, Wisconsin) to quantify concentrations of PO_4-P as SRP. For all water chemistry analyses, blanks and certified commercial standards were analyzed in each run to check for data quality.

From the TASCC pulse additions, we calculated ambient uptake length ($S_{w\text{-}amb}$; m), uptake velocity ($V_{f\text{-}amb}$; mm/min), areal uptake rate (U_{amb}; μg m^{-2} min^{-1}), maximum areal uptake rate (U_{max}; μg m^{-2} min^{-1}), and the half-saturation constant (K_m; μg/L) (described in detail in Covino et al. 2010b). Uptake length ($S_{w\text{-}add\text{-}dyn}$, or the distance in m traveled by the added nutrient prior to uptake) was calculated for each sample as the negative inverse of the difference in the natural log of the injectate nutrient : specific conductivity ratio and each grab sample's nutrient : specific conductivity ratio (background corrected) over reach length. Only data on the falling limb of the pulse addition were analyzed to avoid effects of hysteresis (Trentman et al. 2015). Ambient metrics were calculated as the *y*-intercept of the linear regression of $S_{w\text{-}add\text{-}dyn}$ vs the total nutrient concentration (total [nutrient]):

$$S_{w\text{-}add\text{-}dyn} = m(total[nutrient]) + (S_{w\text{-}amb}) \qquad \text{[Eq. 1]}$$

where m is the slope of the regression, and total [nutrient] is calculated as the geometric mean of the total observed [nutrient] and the total expected [nutrient] given the conservative tracer (Covino et al. 2010b). The regression statistics for each nutrient release are reported in Fig. 4 and the fits were good to excellent. Ambient uptake velocity ($V_{f\text{-}amb}$; mm/min) and ambient areal uptake rate (U_{amb}; μg m^{-2} min^{-1}) were estimated from ambient uptake length ($S_{w\text{-}amb}$), where U_{amb} was calculated by multiplying discharge over width (Q/w) by the ambient streamwater nutrient concentration (ambient [nutrient]), and $V_{f\text{-}amb}$ was calculated as U_{amb}/ambient [nutrient]. We estimated reach discharge (Q) by integrating under the conductivity pulse of each release and calculated mean stream width (w) from stream-width measurements taken every ~2 m along the 74-m study reach.

To estimate saturation kinetic metrics, we calculated areal uptake for each sample ($U_{add\text{-}dyn}$) on the falling limb by multiplying Q/w by the measured and expected geometric mean nutrient concentrations given the conservative tracer concentration (Covino et al. 2010b). We then calculated total areal uptake (U_{total}) for each sample by summing U_{amb} and $U_{add\text{-}dyn}$. Saturation kinetics (U_{max}, K_m) were calculated by fitting a MM model to U_{total} vs total [nutrient] with the Dynamic Fit Wizard in SigmaPlot 11 (Systat Software Inc., San Jose, California):

$$U_{total} = \frac{U_{max} \times total[nutrient]}{K_m + total[nutrient]}. \qquad \text{[Eq. 2]}$$

The regression statistics for the MM fits are reported in Fig. 5 and the fits were good to excellent.

We also calculated the mass of nutrient from the pulse addition that was retained within the reach by subtracting the nutrient mass exported at 74 m from the nutrient mass added in the pulse addition. The mass exported was calculated by integrating the area under the curve for the pulse and multiplying by Q (Tank et al. 2008).

Phosphorus sorption assays

In the spring, we used P isotherms (McDaniel et al. 2009) and the phosphorus sorption index (PSI; Bache and Williams 1971) to measure adsorption of P in the sediments. We collected 5 cores (6 cm wide × 3 cm deep) from 6 stations along the 74-m reach (total $n = 30$) in areas of gravel and fine benthic organic matter (FBOM) accumulation (the remaining area was cobble or bedrock and these substrates were not sampled) and composited these 30 cores into 1 sample in the field. A subsample of the sediment was wet sieved in the laboratory to produce a <8-mm size fraction for adsorption assays. Smaller sediments are often the primary locations of P adsorption (Lottig and Stanley 2007). A 2nd subsample was used to determine organic matter content and classify particle sizes.

For the P isotherms, seven 40 mL standards (0–2000 μg P/L) made with KH_2PO_4 and stream water were added to 5 mL of wet sediment (~8 g dry mass) with 3 replicates per standard ($n = 21$) to measure both biotic uptake and abiotic sorption. A 2nd set ($n = 21$) was prepared to measure abiotic sorption by killing biota on sediments (killed sediments) with 1 mL $HgCl_2$ (0.2%) for a minimum of 15 min prior to adding standards. Samples were shaken for 16 h and then centrifuged. The supernatant was filtered and analyzed for SRP. We also used a similar method to measure the PSI on both live and killed sediments, but only used 3 standards (0, 50, and 2000 μg P/L) and shook samples for 2 to 3 h prior to filtering and analyzing for SRP.

The P isotherms and PSI were expressed per g dry mass (DM) of sediment in the reach. The sediment remaining after subsampling for adsorption isotherms (64% of the total sample) was wet sieved in the laboratory into 5 categories: coarse gravel (22.6–60 mm), medium gravel (8–22.6 mm), fine gravel (2–8 mm), sand (0.063–2 mm), and silt/clay (<0.063 mm) (Wentworth 1922). Each size class was then dried at 60°C to calculate DM. In the field, we also conducted a visual survey to assess the proportion of gravel, fine and coarse benthic organic matter, boulders and bedrock, and cobbles along ten 5-m lengths of streambed along the reach. To estimate the g DM/m^2 of substrate <8 mm on the streambed, we divided the g DM of sediments <8 mm (corrected for the material previously removed and used to produce adsorption isotherms) by the area of streambed sampled from the 30 cores and multiplied by the proportion of the reach that was gravel and FBOM.

From the P isotherms, we calculated the equilibrium P concentration at zero release or retention (EPC_0), where P

is neither adsorbed nor desorbed from the sediments. We regressed the increase or decrease in P during the assay scaled to g DM of sediment (μg P/g DM) for each sample against the final equilibrium P concentration in the sample and calculated EPC_0 as the *x*-intercept of this relationship (Froelich 1988, McDaniel et al. 2009). These data were fit to a non-linear (logarithmic) model with the Dynamic Fit Wizard. Values of EPC_0 > ambient streamwater SRP concentrations indicate that the sediments were a source of P to the water column, whereas EPC_0 < ambient streamwater SRP concentrations indicate that sediments were a sink for water column P.

Additionally, we calculated the phosphorus sorption index (PSI) with the 2000 μg P/L standard as the amount of P adsorbed by the sediments (μg P/g DM) relative to the natural log of the P concentration remaining (μg P/L) after the assay (Bache and Williams 1971, Meyer 1979). This index is commonly used in lieu of the full P isotherm as an indicator of P adsorption capacity in sediments (Reddy et al. 1999, McDaniel et al. 2009, Marton and Roberts 2014). Finally, we used the 50 μg P/L standard from the PSI assay with live sediments to estimate the capacity for the sediments to remove P at a concentration and time-scale similar to that of the pulse nutrient additions. We scaled the 50 μg P/L PSI assay to the stream by multiplying the P adsorbed during the assay (μg P g^{-1} DM h^{-1}) by the amount of sediment <8 mm on the streambed (g DM/m^2) to assess the potential for biotic and abiotic uptake of P during the nutrient additions.

Environmental measurements

We measured a suite of physical, chemical, and biological attributes during the spring and autumn releases to characterize the factors that could affect nutrient uptake dynamics. Specific conductivity, alkalinity, ammonium, nitrate, and SRP concentration reported in Table 1 were measured as part of the weekly water-chemistry sampling in Walker Branch (Mulholland 2004, Lutz et al. 2012). Specific conductivity was measured with a hand-held conductivity meter; alkalinity was measured via titration to pH 4.5; and concentrations of ammonium (NH_4^+-N), nitrate, and SRP were determined from phenolate colorimetry, cadmium reduction, and molybdate-blue methods, respectively (APHA 2005) on either an autoanalyzer (AA3; Seal Analytical Inc., Mequon, Wisconsin) or spectrophotometer with a 10-cm cell to achieve low detection limits for SRP (Mulholland and Hill 1997).

We used the 1-station, open-channel method to measure GPP and ER rates on the nutrient release dates (Odum 1956). Briefly, we placed a data-logging sonde (YSI Model 600 OMS with an optical dissolved oxygen sensor, Yellow Springs Instruments) at the bottom of the study reach, and logged dissolved oxygen (DO) concentration and streamwater temperature every 15 min. Rates of GPP and ER were calculated from the rate of change in DO over time accounting for reaeration (see Roberts et al. 2007 for more details). We measured photosynthetically active radiation (PAR) every 15 min with a PAR sensor (S-LIA-M003 model; Onset Computer Corporation, Bourne, Massachusetts) that was placed ~20 cm above the stream.

We measured the standing stock of coarse particulate organic matter (CPOM) after the nutrient releases in autumn and spring by randomly sampling the streambed at 10 locations throughout the reach. At each sampling location, we used a Surber sampler to collect CPOM from a 780-cm^2 area. Samples were returned to the laboratory, rinsed to remove any remaining fine particles, and dried at 60°C for 1 wk to determine g DM per unit area.

Statistics

We first analyzed nutrient limitation of biofilm respiration on NDS in autumn and spring. We were interested in analyzing nutrient limitation only, so we used 2 separate 1-way analysis of variance (ANOVA) tests, with nutrient type as the main factor. Significant ANOVAs were followed by Tukey's pairwise comparisons tests to examine which nutrient treatments were significantly different from one another. NDS respiration rates were log(*x*)-transformed prior to analysis to meet the assumptions of normality and equal variance.

We next analyzed the effects of nutrient additions on uptake rates and saturation kinetics. Because of the lack of replication in uptake rates and saturation kinetics (i.e., $n = 1$), we were unable to use traditional statistical analyses. Instead, we used 95% confidence intervals (CIs) to assess significant differences, with distinct 95% CIs for a given nutrient metric considered to be significantly different. We calculated the 95% CIs for the modeled TASCC metrics ($S_{w\text{-}amb}$, U_{max}, K_m) from the standard error in the intercepts and model coefficients. To calculate the standard error and 95% CI in $V_{f\text{-}amb}$ and U_{amb}, which were not directly modeled but rather calculated from $S_{w\text{-}amb}$, we used the relative error in $S_{w\text{-}amb}$ (error divided by the mean) and then multiplied this value by either $V_{f\text{-}amb}$ or U_{amb}(Hage and Carr 2011). We also used 95% CIs to compare MM modeled parameters (U_{max}, K_m) across nutrient addition treatments and seasons (spring vs autumn). We note that using 95% CIs to assess significance is a conservative approach, as there can be cases when there may be a significant difference even though the 95% CIs overlap. However, we also note that these 95% CIs likely underestimate uncertainty because of the lack of independence in estimates (i.e., temporal autocorrelation) and because additional sources of uncertainty are not accounted for (e.g., error in nutrient and specific conductivity measurements) (Brooks et al. 2017).

Statistical tests were carried out in SigmaPlot 11. All statistical tests were considered significant at the $\alpha = 0.05$ level, and we ensured each test followed parametric assumptions.

RESULTS

Stream characteristics and nutrient availability and limitation

Physiochemical characteristics differed by season (Table 1). Ambient nutrient concentrations were low in both autumn and spring. In autumn, ambient nitrate and SRP concentrations (from the weekly chemistry sampling) were 14.1 μg N/L and 2.1 μg P/L, respectively. In the spring, ambient nitrate concentration was slightly higher than in autumn (22.5 μg N/L), and SRP concentration was similar (2.7 μg P/L). Discharge in autumn was about 4× higher than in the spring, and alkalinity and specific conductivity were lower in the spring than the autumn. PAR was almost an order of magnitude higher in the spring compared with the autumn leading to a 6× higher rate of GPP. ER was slightly higher in autumn associated with the higher CPOM standing stock from leaf-litter inputs.

Respiration on cellulose disks from NDS's was strongly colimited by N and P in both autumn and spring (Fig. 1; 1-way ANOVAs, $F_{autumn} = 93$, $F_{spring} = 158$, df = 3, $p < 0.001$). On average, respiration was 6 to 7.5× higher on N+P treatments relative to all other treatments (Tukey's Honestly Significant Difference [HSD] test, $p < 0.001$), and respiration on N and P treatments alone were not significantly different than the controls ($p > 0.05$).

Nutrient releases

In autumn, we increased background nitrate and SRP concentrations (Fig. 2), although SRP did not reach our target enrichments of 5 μg P/L and 10 μg P/L above ambient concentrations (Fig. 2D–F). Background nitrate was elevated from 15 μg N/L (P alone, Fig. 2A) to 44 μg N/L (P with N low, Fig. 2B) and 72 μg N/L (P with N high, Fig. 2C), and background SRP was elevated from 3 μg P/L (N alone, Fig. 2D) to 5 μg P/L (N with P low, Fig. 2E) and 7 μg P/L (N with P high, Fig. 2F) (Table S1). In spring, background SRP concentrations were increased to higher concentrations than in autumn (Fig. 3), but we still did not achieve the higher target enrichments of 10 and 20 μg P/L above ambient concentrations (Fig. 3D–F). However, we increased background nitrate concentrations (Fig. 3A–C) to almost the same concentrations achieved in autumn. Background nitrate was elevated from 12 μg N/L (P alone, Fig. 3A) to 43 μg N/L (P with N low, Fig. 3B) and 86 μg N/L (P with N high, Fig. 3C), and background SRP was elevated from 2 μg P/L (N alone, Fig. 3D) to 9 μg P/L (N with P low, Fig. 3E) and 13 μg P/L (N with P high, Fig. 3F) (Table S1).

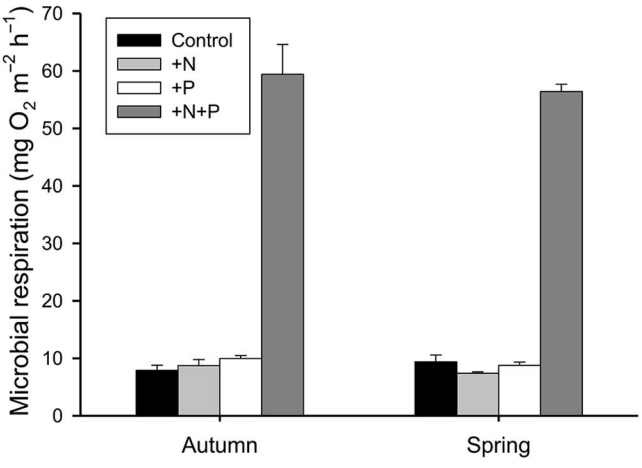

Figure 1. Respiration rates (mg O_2 m^{-2} h^{-1}) (±1 SE) on cellulose disks overlaying nutrient diffusing substrata containing agar only (control) or added nutrients (+N, +P, +N+P) in autumn and spring.

Ambient uptake of N

Elevated P did not affect ambient N uptake metrics in either autumn or spring. In autumn, $S_{w\text{-}amb}$ for N alone was 49.2 m, but when background P was elevated (both low and high P), N uptake was no longer measurable (Fig. 4A). The percentage of added nitrate that was removed was low for both the N alone (8%) and N with P low (7%) releases and was negative during the N with P high release (Table S2), corroborating the lack of measurable nitrate uptake for that release. In spring, $S_{w\text{-}amb}$ for N alone was 80.2 m and $S_{w\text{-}amb}$ decreased slightly to 66.4 and 29.0 m when P was elevated to low and high P concentrations, respectively (Fig. 4B). Yet the percentage of added N that was consumed was low for all releases (2–7%; Table S2), and variation in $S_{w\text{-}amb}$ was high (95% CI ranged from ±29.6 to ±75.3 m, Table S2). The elevated P did not, therefore, significantly change $S_{w\text{-}amb}$ of N in spring (based on overlapping 95% CIs). Although $V_{f\text{-}amb}$ and U_{amb} of N also increased with increasing background concentrations of P in spring, these metrics were highly variable leading to no significant differences between experiments. Finally, ambient metrics for N alone were similar in autumn and spring (Table S2).

Ambient uptake of P

Similar to ambient N uptake, elevated N did not affect ambient P uptake metrics in either autumn or spring. In autumn, ambient P uptake metrics were similar with differing N concentrations, as $S_{w\text{-}amb}$ for P alone was 47.5 m, and was 50.7 and 56.1 m for low and high N, respectively (Fig. 4C, Table S2). The percentage of added P that was retained was high for all releases (62–70%) (Table S2). In spring, $S_{w\text{-}amb}$ decreased from 87.2 m when P was added alone, to 44.4 and 35.8 m for low and high N, respectively (Fig. 4D). Yet, variation in the intercept was high (Table S2), and these differences were not significant. $V_{f\text{-}amb}$ and U_{amb} of P were not significantly different across N experiments (Table S2). In spring, the percentage of added P that was removed was high (35–45%; Table S2), but lower than for added P in autumn. Finally, there were no differences in

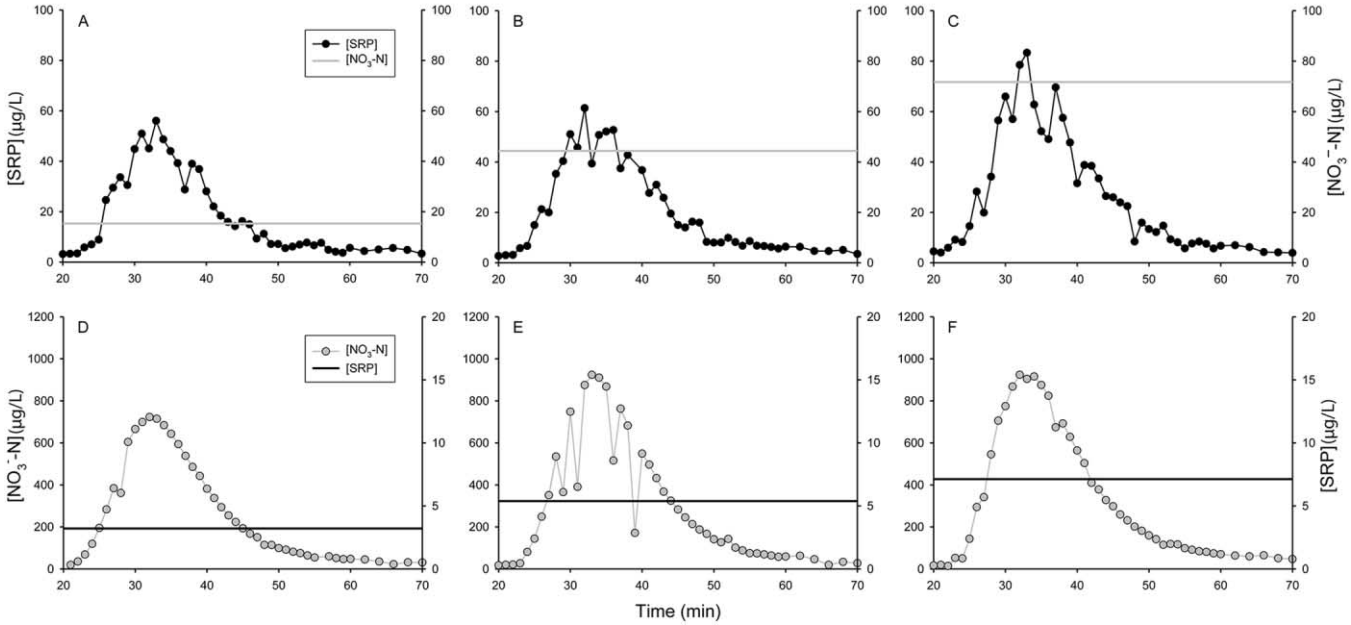

Figure 2. Nitrate and soluble reactive phosphorus (SRP) concentrations (µg/L) during the saturating pulse and steady-state nutrient releases in autumn. Straight lines indicate either background (A, D) or elevated (B, C, E, F) concentrations during steady-state additions. Concentrations were calculated as geometric means for the study reach.

ambient P uptake metrics between autumn and spring (Table S2).

Saturation kinetics of N

Elevated background P concentrations increased maximum areal uptake rates (U_{max}) of N, but only in spring (Figs 5B, 6B). The highest U_{max} was observed when background P was high (N with P high, 354 µg m^{-2} min^{-1}). The lowest (based on distinct 95% CIs) U_{max} occurred without added P (N alone, 185 µg m^{-2} min^{-1}), and moderate U_{max} occurred under low P concentrations (N with P low, 234 µg m^{-2} min^{-1}; Figs. 5B, 6B). There was no change in the K_m of N with increasing P in spring (Fig. 6A). In autumn, U_{max} for the N alone treatment was 109 µg m^{-2} min^{-1}, and K_m was 40 µg/L (Fig. 5A, Table S3). However, N uptake kinetics could not be calculated when background concentrations of P were elevated, because N uptake was not measurable. U_{max} for the N alone treatment was higher in the spring than autumn, but K_m did not differ between spring and autumn (Table S3).

Saturation kinetics of P

Elevating background N concentrations did not affect U_{max} of P in either spring or autumn. There was a trend of increasing U_{max} and K_m for P with increasing background concentrations of N in autumn (Fig. 6C, D), but the relationships between U_{total} and total [SRP] did not reach a plateau. The U_{max} and K_m parameters estimated through MM fits were highly uncertain (Fig. 5C) as indicated by their large 95% CIs, which ranged from ±182 to ±879 µg m^{-2} min^{-1} for U_{max} and ±114 to ±683 µg/L for K_m (Table S3). In spring, U_{max} and K_m of P were similar across all experiments (Table S3). The U_{total} vs total [SRP] relationships approached plateaus in spring (Fig. 5D) more closely than in autumn (Fig. 5C), but variation in estimated parameters was still high (95% CI were ±61 to ±140 µg m^{-2} min^{-1} for U_{max} and ±9 to ±47µg/L for K_m, Table S3). There were no differences between autumn and spring U_{max} or K_m (Fig. 6A–D).

Phosphorus sorption

The results of the laboratory experiments highlighted the dominant role of P adsorption to Walker Branch sediments. The size class of sediments <8 mm in diameter used in the P sorption experiments corresponded to 50% of the sediments (by DM) in Walker Branch. In just 2.5 h, 48% of the 50 µg/L P standard was adsorbed to sediments, corresponding to an areal uptake rate of 58 mg P m^{-2} min^{-1}. The PSI, and thus P removal potential, for the live sediments was slightly lower (1.45) than for killed sediments (1.82). One would expect the live sediments to have higher sorption (biotic uptake and abiotic sorption), but this result has been found by others (e.g., Lottig and Stanley 2007). It is possible that $HgCl_2$ influenced adsorption processes by affecting pH. However, EPC_0 was similar between live and killed sediments (11 vs 13 µg P/L; Fig. 7). The EPC_0 was higher than ambient SRP concentrations (2–3 µg P/L; Table 1), suggesting that sediments were a source of P to the water column under ambient conditions. However, the peak SRP concen-

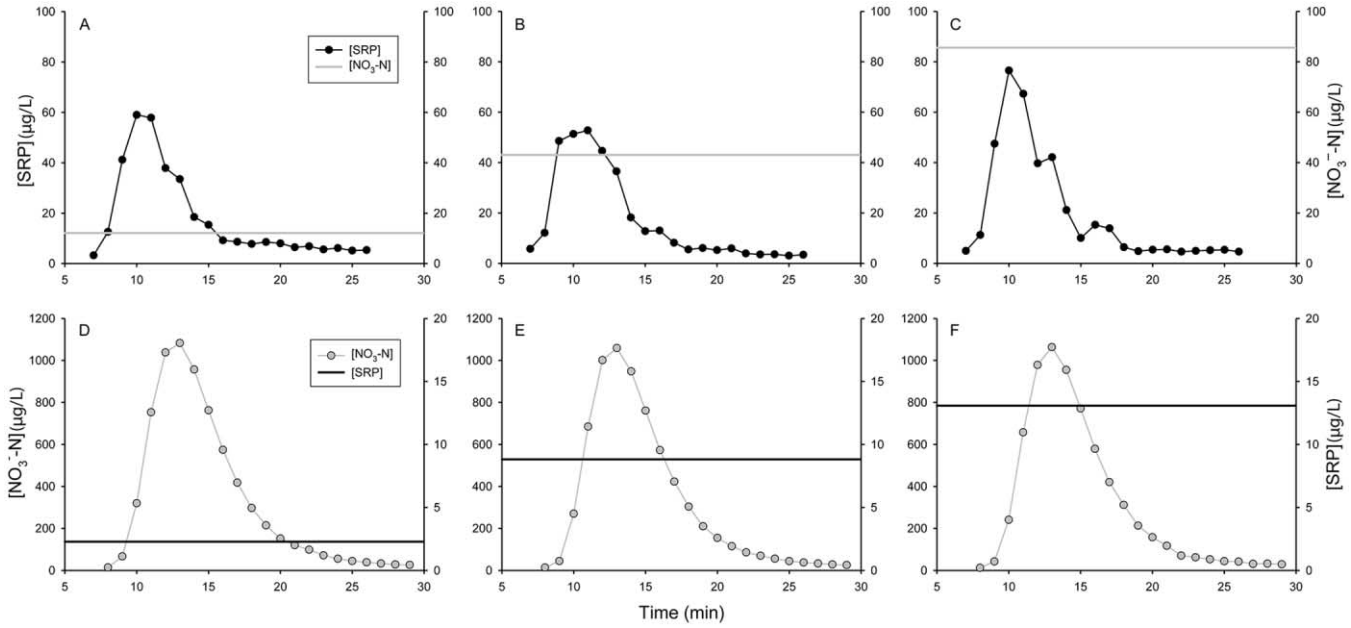

Figure 3. Nitrate and soluble reactive phosphorus (SRP) concentrations (µg/L) during the saturating pulse and steady-state nutrient releases in spring. Straight lines indicate either background (A, D) or elevated (B, C, E, F) concentrations during steady-state additions. Concentrations were calculated as geometric means for the study reach.

trations (~60–80 µg P/L) during the pulse additions likely resulted in sorption of P to stream sediments.

DISCUSSION

Ambient N and P uptake

Walker Branch was strongly colimited for N and P in spring and autumn based on NDS responses, as has been found by others in this well-studied stream (Rosemond et al. 1993, Mulholland et al. 2000). Thus, we predicted that ambient uptake length of one nutrient would decrease (i.e., increased nutrient demand) when the concentration of the other nutrient was elevated. Specifically, dual nutrient addition would result in a shorter $S_{w\text{-}add\text{-}dyn}$ for a given total nutrient concentration than for N or P alone, and the shorter $S_{w\text{-}add\text{-}dyn}$ along the TASCC breakthrough curve would result in a smaller y-intercept ($S_{w\text{-}amb}$). A pattern of decreasing $S_{w\text{-}amb}$ with elevated N and P was observed in spring (for both N with elevated P and P with elevated N); however, these changes were not significant as there was large error associated with the relationships between $S_{w\text{-}add\text{-}dyn}$ and total nutrient concentration and thus $S_{w\text{-}amb}$. These error estimates may be even larger when additional sources of uncertainty (e.g., error in individual nutrient concentration measurements, specific conductivity measurements) are accounted for (Brooks et al. 2017). Thus, large error estimates may make it difficult to determine significant responses when using these techniques. Overall, the lack of change in ambient nutrient uptake metrics with dual nutrient additions suggests that either ambient uptake rates of N and P are not colimited at the stream-reach scale, the high variation associated with $S_{w\text{-}amb}$ estimates precludes the ability to determine significant differences, or that ambient uptake rates are not affected by short-term changes in nutrient concentrations. For instance, it is possible that these short-term (i.e., ~1-h long) nutrient releases were not long enough to elicit an ecosystem response, and thus, longer-duration (e.g., days to weeks to months) steady-state additions may be needed.

$S_{w\text{-}amb}$ for both N and P was measurable in spring, but N uptake was not measureable in autumn when stream water P was experimentally elevated. Our inference that uptake was nonmeasurable is supported by the low percentage removal of the added nutrient based on mass–balance calculations, and suggests that N was primarily transported downstream with little uptake. The lack of measurable N uptake suggests that N may not have been limiting at the stream-reach scale during this time, or uptake was inhibited by increased P concentrations.

The ambient P uptake rates estimated from the TASCC method were within the range of P uptake metrics previously reported for the West Fork of Walker Branch (Newbold et al. 1983, Mulholland et al. 1985, 1990, 1997). For the P alone releases, U_{amb} in autumn and spring (7 and 11 µg P m^{-2} min^{-1}, respectively) was similar to rates estimated primarily from radioisotope labeling methods of P (range = 1.3 to 15.5 µg P m^{-2} min^{-1}, mean = 6.6 µg P m^{-2} min^{-1} in Newbold et al. 1983, Mulholland et al. 1985, 1997). However, ambient areal nitrate uptake rates in our study (33 and 48 µg N m^{-2} min^{-1} in autumn and spring, respectively)

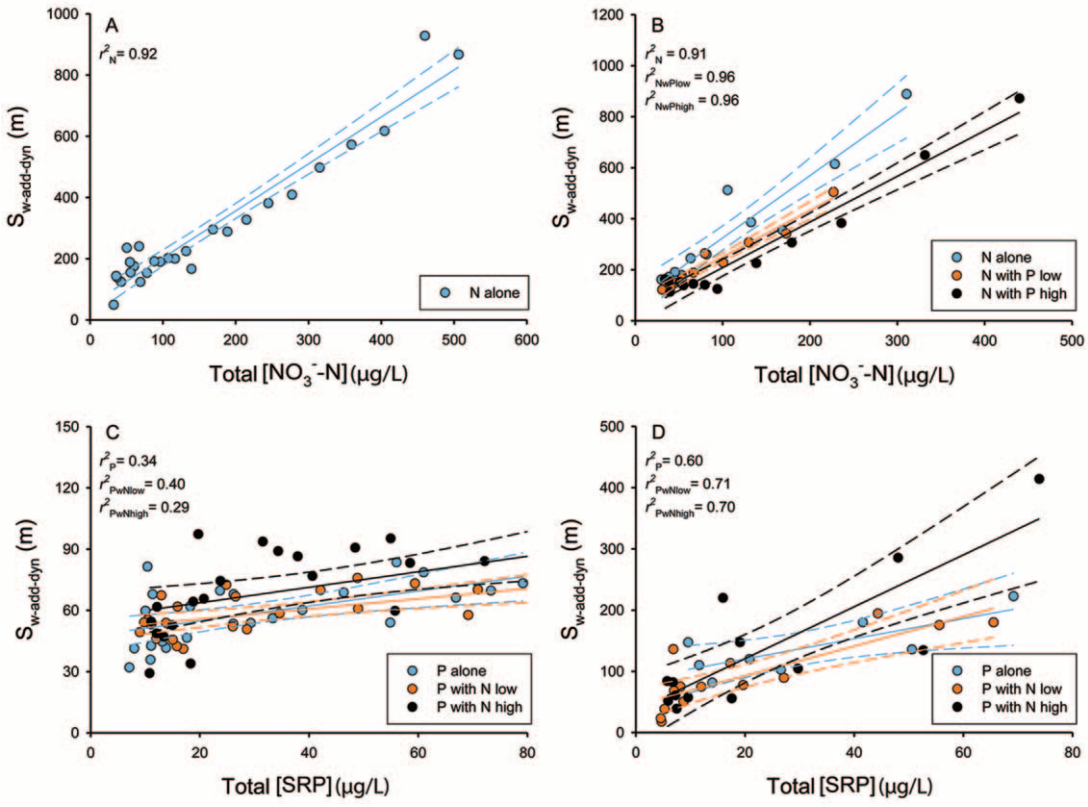

Figure 4. Nitrate (A, B) and phosphate (C, D) uptake lengths ($S_{w\text{-}add\text{-}dyn}$; m) vs total nitrate or total soluble reactive phosphorus (SRP) concentrations (µg/L) in autumn (A, C) and spring (B, D) when N or P was added alone (blue) or in combination with low (orange) or high (black) concentrations of the other nutrient. Note that N uptake was not measurable when P was added in autumn (A). Dotted lines represent the 95% confidence interval (CI) for each release. Linear regression statistics are reported for the relationships between uptake length vs total nutrient concentration.

were higher than previously published nitrate (^{15}N-NO_3) areal uptake rates (range = 0 to 29 µg N m^{-2} min^{-1}, mean = 4 µg N m^{-2} min^{-1} in Mulholland et al. 2000, 2006). This difference could possibly be due to differing methods or environmental conditions. Rates of nitrate U_{amb} from the TASCC method were more similar to previously published ammonium uptake rates (range = 7 to 37 µg N m^{-2} min^{-1}, mean = 23 µg N m^{-2} min^{-1} in Mulholland et al. 2000, Griffiths and Hill 2014). The similarity in areal uptake rates suggests that the TASCC method may be appropriate for estimating ambient uptake metrics in Walker Branch. However, the high variability in estimates of $S_{w\text{-}amb}$ (Table S2) makes comparisons (e.g., among nutrient releases and seasons) difficult.

Saturation kinetics of N and P

The maximum areal uptake rate (U_{max}) of N increased as P concentrations increased in spring, but no other significant changes were apparent (i.e., U_{max} of P in both seasons, U_{max} of N in autumn). The increased U_{max} rate for N with elevated background P concentrations followed our prediction, and suggested that when P limitation was alleviated, the biotic capacity to take up N increased. However, we did not see a similar pattern for N in autumn as uptake was not measurable when P was elevated to low and high concentrations. It is possible that the difference in N uptake with added P between spring and autumn was due to the autotroph-dominant community (in spring) being more flexible in taking up nutrients with variable stoichiometric ratios than the heterotroph-dominated community in autumn (Schade et al. 2011). Whether this pattern was also present for U_{max} of P with added N could not be determined because abiotic adsorption dominated the removal of P (described in detail below). It is also possible that the increase in U_{max} of N with added P was a response to the 3 consecutive nutrient releases that were conducted in 1 day in spring; however, we did not see the same response in U_{max} of P with added N despite the same nutrient release schedule. Further, the U_{total} vs total (SRP) relationships (MM curves) did not reach plateau (especially in autumn), resulting in large 95% CI estimates for both K_m and U_{max}. The importance of P adsorption in Walker Branch was demonstrated in a previous experiment when the addition of ammonium and phosphate to-

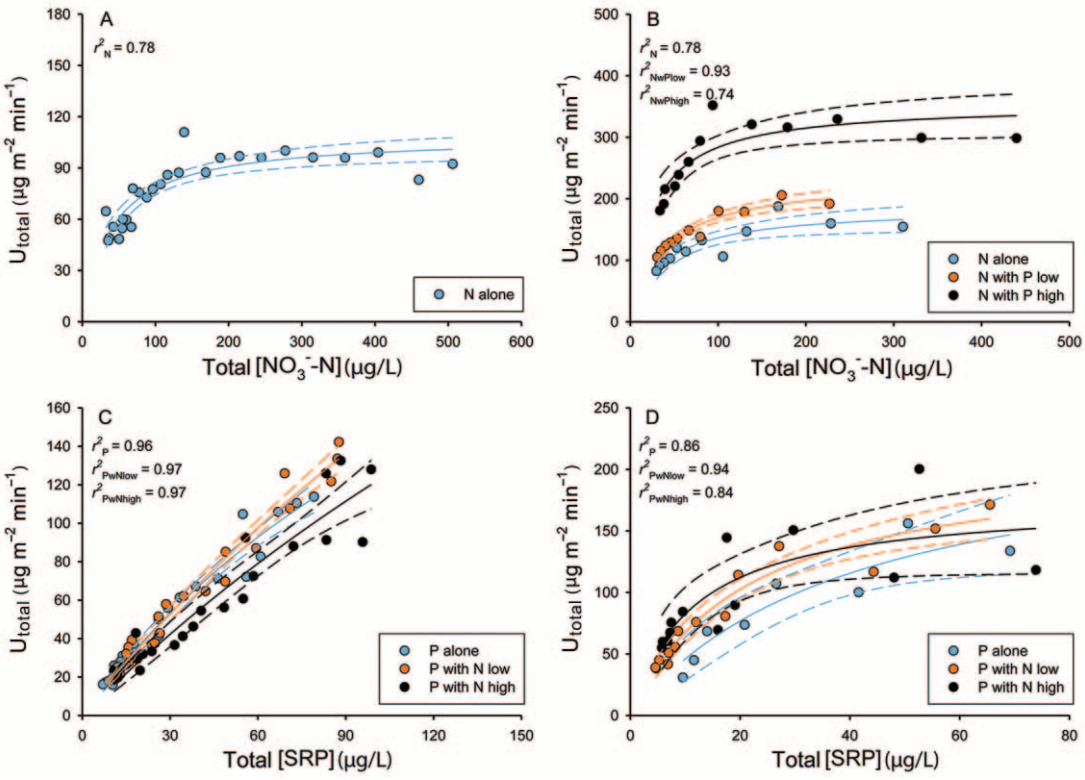

Figure 5. Total areal uptake rates (U_{total}; µg m^{-2} min^{-1}) vs total nitrate (A, B) or total soluble reactive phosphorus (SRP) (C, D) concentrations (µg/L) in autumn (A, C) and spring (B, D) when N or P was added alone (blue) or in combination with low (orange) or high (black) concentrations of the other nutrient. Note that N uptake was not measurable when P was added in autumn (A). Dotted lines represent the 95% CI for each release. Non-linear regression statistics are reported for the Michaelis-Menten relationships between areal uptake rate vs total nutrient concentration.

gether did not increase uptake relative to P alone likely because abiotic adsorption dominated uptake (Mulholland et al. 1990).

K_m may be a fairly consistent value (if accurately estimated from the MM curves) given that there were no differences in K_m for either N or P across treatments and seasons. The estimates of nitrate K_m (25–40 µg N/L across experiments) in Walker Branch fell within the ranges reported for forested streams in Virginia and North Carolina, USA (3–330 µg N/L; Earl et al. 2006), and were higher than K_m for a mountain stream in Idaho (4.2–14.4 µg N/L; Covino et al. 2010a) and for a grassland stream in New Zealand (1.2–1.4 µg N/L; Simon et al. 2005). The minimum and maximum nitrate K_m values for Walker Branch corresponded to the 53rd and 78th percentiles from all nitrate concentrations measured in the stream (weekly from 1989–2012), suggesting that Walker Branch may be approaching N saturation 22 to 47% of the time. These estimates are based only on nitrate, but ammonium concentrations are fairly low in Walker Branch (Lutz et al. 2012). Similarly, the low ratio of U_{amb}/U_{max} for nitrate suggests that ambient areal uptake was not N saturated (U_{amb} was 25–29% of U_{max}). Estimates of P K_m in Walker Branch were much higher than that of nitrate, likely influenced by the dominance of abiotic adsorption.

Abiotic adsorption of P

Abiotic adsorption was an important fate of P in Walker Branch, and likely complicated the ability to examine how biotic uptake responded to dual nutrient additions. Five lines of evidence pointed to the dominance of adsorption in P removal. First, laboratory sorption experiments estimated that areal uptake of P by sediments was 58 mg P m^{-2} min^{-1} over a 2.5-hr period. This uptake rate was two orders of magnitude higher than U_{max} estimated from the TASCC releases, suggesting that the sediments in Walker Branch have a high capacity for adsorption, and higher than measured via pulse releases in the field. However, incubation times (2.5-h adsorption experiments vs 0.2–0.5 h to reach the breakthrough curve peak) could also explain the difference in P uptake measured in the field vs P adsorption measured in the laboratory. Second, the EPC_0 was similar in live (11 µg P/L) vs killed (13 µg P/L) sediments, suggesting a small role of biotic uptake in influencing streamwater SRP concentrations. Sandy sediments in a headwater stream in

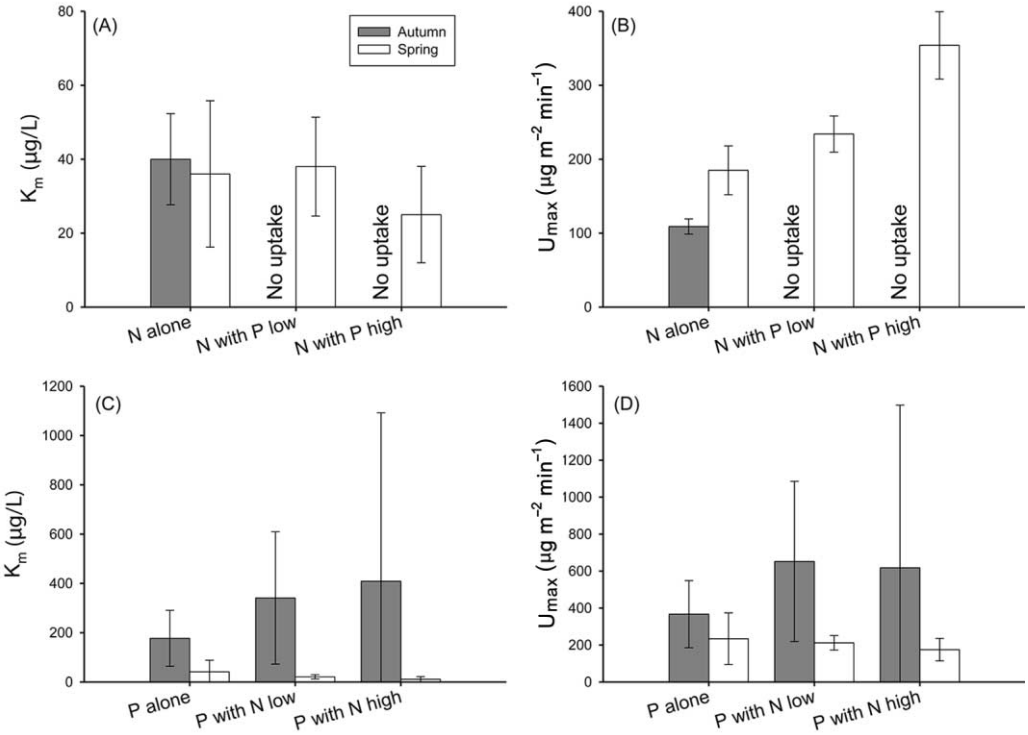

Figure 6. Half-saturation constants ± 95% confidence interval (CI) for K_m; µg/L (A, C) and maximum areal uptake rates ± 95% CI for U_{max}; µg m^{-2} min^{-1} (B, D) when N (A, B) or P (C, D) was added alone or in combination with low or high concentrations of the other nutrient in autumn (gray bars) and spring (white bars).

Wisconsin, USA also had a similar EPC_0 in live and killed sediments (10 µg/L for both; Lottig and Stanley 2007). Third, EPC_0 in Walker Branch was lower than the peak SRP concentrations (~60–80 µg P/L) measured during the pulse additions, suggesting that the sediments were a sink for P during the majority of the nutrient pulse. Fourth, relationships between U_{total} and total (SRP), from which MM kinetic parameters were calculated, also did not often reach plateau (especially in autumn), suggesting that abiotic sorption sites were not saturated. The lack of plateau resulted in highly variable estimates of U_{max} and K_m, with higher values of both also suggestive of adsorption dominance. Last, adsorption of P likely contributed to the difficulty in elevating background P concentrations with steady-state additions and the high percentage removal of P calculated via mass balance from the pulse additions (compared to the much lower percentage removal for N). The percentage removal for P was also likely higher in autumn than spring because of lower stream discharge in autumn (Meals et al. 1999).

Previous studies in Walker Branch have also highlighted the importance of abiotic processes in affecting P dynamics. For instance, multiple P additions in Walker Branch suggested that biotic uptake likely saturated at low SRP concentrations (5 µg P/L), after which abiotic processes dominated P uptake (Mulholland et al. 1990). High alkalinity and pH in Walker Branch may also result in the coprecipitation of P with $CaCO_3$ (Mulholland et al. 1985), which is likely another abiotic fate of P in this stream. The importance of P adsorption has also been identified in other streams (Meyer 1979, Davis and Minshall 1999, Lottig and Stanley 2007). Overall, the dominance of physical and chemical processes

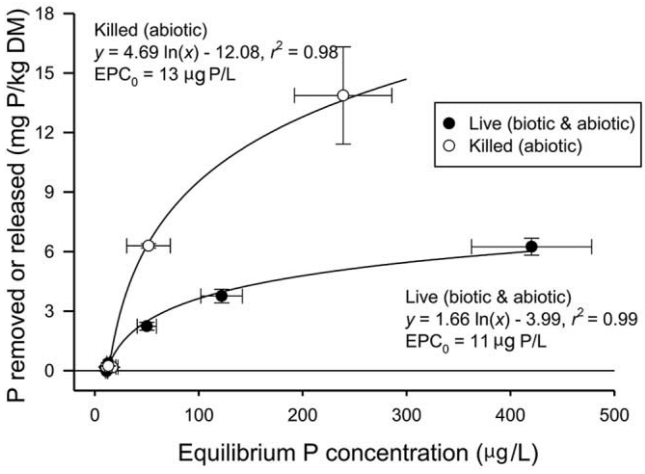

Figure 7. Phosphorus isotherms for live (closed circles) and killed (open circles) sediments (±SD; n = 3 replicates) and the equilibrium P concentrations (µg/L) at zero release or retention (EPC_0). The logarithmic regressions are based on the mean values of P removed or released vs equilibrium P concentration.

in affecting P uptake at higher concentrations suggests that the saturating pulse nutrient addition method and the MM model (derived for biotic processes; i.e., enzyme kinetics) may not be appropriate for investigating the biotic controls of P in Walker Branch and similar stream ecosystems.

Concluding remarks

The cycling of individual elements in the environment does not occur in isolation, and there is a growing need to advance understanding of coupled biogeochemical cycles in both terrestrial and aquatic ecosystems (Finzi et al. 2011). By conducting dual nutrient additions in a nutrient-limited stream, we found evidence of N and P colimitation at the stream-reach scale. This colimitation would not have been observed if only single nutrient additions were carried out. However, the responses of N and P uptake to dual nutrient additions did not always follow our predictions across seasons, nutrients, or uptake metrics. Some of this variability was due to high parameter error estimates. However, the disparate response of N vs P uptake to dual nutrient additions primarily reflects the dominant role of P sorption in driving P uptake dynamics.

Phosphorus adsorption was a complicating factor in evaluating biotic N and P uptake from dual nutrient addition experiments. However, examined from a different perspective, the dual nutrient addition technique was useful in that it revealed the strong role of adsorption in P dynamics. Thus, dual nutrient addition techniques may reveal important insights into the potentially disparate drivers of multiple nutrients (i.e., dominant biotic vs abiotic uptake). The importance of P adsorption vs biotic uptake of P is known to vary across streams and sediment types (e.g., Davis and Minshall 1999, Haggard and Stanley 1999, Lottig and Stanley 2007). Future efforts that use whole-stream nutrient additions to examine coupled N and P cycling in stream ecosystems will need to use a combination of laboratory assays and field experiments to better tease apart the roles of abiotic sorption vs biotic uptake of P (Stutter et al. 2010).

Building on the rich literature on single element dynamics in streams, we suggest that future efforts use dual nutrient addition techniques to determine how coupled biogeochemical cycles (C–N–P, and other important elemental cycles [e.g., Fe, Mo]) vary across seasons, biomes, and land-use types. The length of time that these dual nutrient addition experiments are carried out will be an important consideration for future studies. It is possible that the lack of consistent responses to dual nutrient additions in this study was caused, in part, by the short-term nature of these experiments. Longer-term nutrient additions (e.g., weeks to years) may result in much different responses to dual N and P additions associated with changes in the autotrophic and heterotrophic communities involved in nutrient uptake (e.g., Slavik et al. 2004). Overall, examining multiple nutrients in concert at various time-scales and conditions will help us better understand how to manage aquatic ecosystems in the future.

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Author contributions: NAG and LTJ designed the study, performed the work, analyzed and interpreted the data, and wrote the manuscript.

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LITERATURE CITED

APHA (American Public Health Association). 2005. Standard methods for the examination of water and wastewater, 21st edition. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Arango, C. P., J. L. Tank, L. T. Johnson, and S. K. Hamilton. 2008. Assimilatory uptake rather than nitrification and denitrification determines nitrogen removal patterns in streams of varying land use. Limnology and Oceanography 53:2558–2572.

Bache, B. W., and E. G. Williams. 1971. Phosphate sorption index for soils. Journal of Soil Science 22:289–301.

Bernhardt, E. S., and G. E. Likens. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology 83:1689–1700.

Bernot, M. J., J. L. Tank, T. V. Royer, and M. B. David. 2006. Nutrient uptake in streams draining agricultural catchments of the Midwestern United States 51:499–506.

Brooks, S. C., C. C. Brandt, and N. A. Griffiths. 2017. Estimating uncertainty in ambient and saturation nutrient uptake metrics from nutrient pulse releases in stream ecosystems. Limnology and Oceanography: Methods 15:22–37.

Claessens, L., C. L. Tague, P. M. Groffman, and J. M. Melack. 2010. Longitudinal and seasonal variation of stream N uptake in an urbanizing watershed: effect of organic matter, stream size, transient storage and debris dams. Biogeochemistry 98: 45–62.

Comiskey, C. E. 1978. Aspects of the organic carbon cycle on Walker Branch Watershed: a study of land/water interaction. PhD Dissertation, University of Tennessee, Knoxville, Tennessee.

Covino, T., B. McGlynn, and M. Baker. 2010a. Separating physical and biological nutrient retention and quantifying uptake kinetics from ambient to saturation in successive mountain stream reaches. Journal of Geophysical Research 115:G04010.

Covino, T. P., B. L. McGlynn, and R. A. McNamara. 2010b. Tracer Additions for Spiraling Curve Characterization (TASCC):

quantifying stream nutrient uptake kinetics from ambient to saturation. Limnology and Oceanography: Methods 8:484–498.

Davis, J. C., and G. W. Minshall. 1999. Nitrogen and phosphorus uptake in two Idaho (USA) headwater wilderness streams. Oecologia 119:247–255.

Diemer, L. A., W. H. McDowell, A. S. Wymore, and A. S. Prokushkin. 2015. Nutrient uptake along a fire gradient in boreal streams of Central Siberia. Freshwater Science 34:1443–1456.

Dodds, W. K., A. J. López, W. B. Bowden, S. Gregory, N. B. Grimm, S. K. Hamilton, A. E. Hershey, E. Martí, W. H. McDowell, J. L. Meyer, D. Morrall, P. J. Mulholland, B. J. Peterson, J. L. Tank, H. M. Valett, J. R. Webster, and W. Wollheim. 2002. N uptake as a function of concentration in streams. Journal of the North American Benthological Society 21:206–220.

Earl, S. R., H. M. Valett, and J. R. Webster. 2006. Nitrogen saturation in streams. Ecology 87:3140–3151.

Elwood, J. W., J. D. Newbold, A. F. Trimble, and R. W. Stark. 1981. The limiting role of phosphorus in a woodland stream ecosystem: effects of P enrichment on leaf decomposition and primary producers. Ecology 62:146–158.

Finzi, A. C., J. J. Cole, S. C. Doney, E. A. Holland, and R. B. Jackson. 2011. Research frontiers in the analysis of coupled biogeochemical cycles. Frontiers in Ecology and the Environment 9:74–80.

Francoeur, S. N. 2001. Meta-analysis of lotic nutrient amendment experiments: detecting and quantifying subtle responses. Journal of the North American Benthological Society 20:358–368.

Froelich, P. N. 1988. Kinetic control of dissolved phosphate in natural rivers and estuaries: a primer on the phosphate buffer mechanism. Limnology and Oceanography 33:649–668.

Gibson, C. A., C. M. O'Reilly, A. L. Conine, and S. M. Lipshutz. 2015. Nutrient uptake dynamics across a gradient of nutrient concentrations and ratios at the landscape scale. Journal of Geophysical Research: Biogeosciences 120:326–340.

Griffiths, N. A., and W. R. Hill. 2014. Temporal variation in the importance of a dominant consumer to stream nutrient cycling. Ecosystems 17:1169–1185.

Hage, D. S., and J. D. Carr. 2011. Analytical chemistry and quantitative analysis. 1st edition. Prentice Hall, Upper Saddle River, New Jersey.

Haggard, B. E., and E. H. Stanley. 1999. Sediment-phosphorus relationships in three northcentral Oklahoma streams. Transactions of the American Society of Agricultural Engineers 42:1709–1714.

Hall, R. O., Jr., and J. L. Tank. 2003. Ecosystem metabolism controls nitrogen uptake in streams in Grand Teton National Park, Wyoming. Limnology and Oceanography 48:1120–1128.

Hall, R. O., Jr., J. L. Tank, D. J. Sobota, P. J. Mulholland, J. M. O'Brien, W. K. Dodds, J. R. Webster, H. M. Valett, G. C. Poole, B. J. Peterson, J. L. Meyer, W. H. McDowell, S. L. Johnson, S. K. Hamilton, N. B. Grimm, S. V. Gregory, C. N. Dahm, L. W. Cooper, L. R. Ashkenas, S. M. Thomas, R. W. Sheibley, J. D. Potter, B. R. Neiderlehner, L. T. Johnson, A. M. Helton, C. M. Crenshaw, A. J. Burgin, M. J. Bernot, J. J. Beaulieu, and C. P. Arango. 2009. Nitrate removal in stream ecosystems measured by ^{15}N addition experiments: total uptake. Limnology and Oceanography 54:653–665.

Heffernan, J. B., and M. J. Cohen. 2010. Direct and indirect coupling of primary production and diel nitrate dynamics in a subtropical spring-fed river. Limnology and Oceanography 55:677–688.

Hill, W. R., P. J. Mulholland, and E. R. Marzolf. 2001. Stream ecosystem responses to forest leaf emergence in spring. Ecology 82:2306–2319.

Hoellein, T. J., J. L. Tank, E. J. Rosi-Marshall, S. A. Entrekin, and G. A. Lamberti. 2007. Controls on spatial and temporal variation of nutrient uptake in three Michigan headwater streams 52:1964–1977.

Johnson, L. T., T. V. Royer, J. M. Edgerton, and L. G. Leff. 2012. Manipulation of the dissolved organic carbon pool in an agricultural stream: responses in microbial community structure, denitrification, and assimilatory nitrogen uptake. Ecosystems 15:1027–1038.

Johnson, L. T., J. L. Tank, and W. K. Dodds. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Canadian Journal of Fisheries and Aquatic Sciences 66:1081–1094.

Lietzke, D. A. 1994. Soils of Walker Branch Watershed. ORNL-TM-11606, Oak Ridge National Laboratory, Oak Ridge, Tennessee.

Lottig, N. R., and E. H. Stanley. 2007. Benthic sediment influence on dissolved phosphorus concentrations in a headwater stream. Biogeochemistry 84:297–309.

Lutz, B. D., P. J. Mulholland, and E. S. Bernhardt. 2012. Long-term data reveal patterns and controls on stream water chemistry in a forested stream: Walker Branch, Tennessee. Ecological Monographs 82:367–387.

Martí, E., P. Fonollà, D. von Schiller, F. Sabater, A. Argerich, M. Ribot, and J. Lluís Riera. 2009. Variation in stream C, N and P uptake along an altitudinal gradient: a space-for-time analogue to assess potential impacts of climate change. Hydrology Research 40:123–127.

Martí, E., and F. Sabater. 1996. High variability in temporal and spatial nutrient retention in Mediterranean streams. Ecology 77:854–869.

Marton, J. M., and B. J. Roberts. 2014. Spatial variability of phosphorus sorption dynamics in Louisiana salt marshes. Journal of Geophysical Research: Biogeosciences 119:451–465.

McDaniel, M. D., M. B. David, and T. V. Royer. 2009. Relationships between benthic sediments and water column phosphorus in Illinois streams. Journal of Environmental Quality 38:607–617.

Meals, D. W., S. N. Levine, D. Wang, J. P. Hoffmann, E. A. Cassell, J. C. Drake, D. K. Pelton, H. M. Galarneau, and A. B. Brown. 1999. Retention of spike additions of soluble phosphorus in a northern eutrophic stream. Journal of the North American Benthological Society 18:185–198.

Meyer, J. L. 1979. The role of sediments and bryophytes in phosphorus dynamics in a headwater stream ecosystem. Limnology and Oceanography 24:365–375.

Mineau, M. M., C. M. Rigsby, D. T. Ely, I. J. Fernandez, S. A. Norton, T. Ohno, H. M. Valett, and K. S. Simon. 2013. Chronic catchment nitrogen enrichment and stoichiometric constraints on the bioavailability of dissolved organic matter from leaf leachate. Freshwater Biology 58:248–260.

Mulholland, P. J. 2004. 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 rec-

ords for Walker Branch Watershed. Biogeochemistry 70:403–426.

Mulholland, P. J., A. M. Helton, G. C. Poole, R. O. Hall, S. K. Hamilton, B. J. Peterson, J. L. Tank, L. R. Ashkenas, L. W. Cooper, C. N. Dahm, W. K. Dodds, S. E. G. Findlay, S. V. Gregory, N. B. Grimm, S. L. Johnson, W. H. McDowell, J. L. Meyer, H. M. Valett, J. R. Webster, C. P. Arango, J. J. Beaulieu, M. J. Bernot, A. J. Burgin, C. L. Crenshaw, L. T. Johnson, B. R. Niederlehner, J. M. O'Brien, J. D. Potter, R. W. Sheibley, D. J. Sobota, and S. M. Thomas. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature 452:202–205.

Mulholland, P. J., and W. R. Hill. 1997. Seasonal patterns in streamwater nutrient and dissolved organic carbon concentrations: separating catchment flow path and in-stream effects. Water Resources Research 33:1297–1306.

Mulholland, P. J., E. R. Marzolf, J. R. Webster, D. R. Hart, and S. P. Hendricks. 1997. Evidence that hyporheic zones increase heterotrophic metabolism and phosphorus uptake in forest streams. Limnology and Oceanography 42:443–451.

Mulholland, P. J., J. D. Newbold, J. W. Elwood, L. A. Ferren, and J. R. Webster. 1985. Phosphorus spiralling in a woodland stream: seasonal variations. Ecology 66:1012–1023.

Mulholland, P. J., A. D. Steinman, and J. W. Elwood. 1990. Measurement of phosphorus uptake length in streams: comparison of radiotracer and stable PO_4 releases. Canadian Journal of Fisheries and Aquatic Sciences 47:2351–2357.

Mulholland, P. J., J. L. Tank, D. M. Sanzone, W. M. Wollheim, B. J. Peterson, J. R. Webster, and J. L. Meyer. 2000. Nitrogen cycling in a forest stream determined by a ^{15}N tracer addition. Ecological Monographs 70:471–493.

Mulholland, P. J., S. A. Thomas, H. M. Valett, J. R. Webster, and J. Beaulieu. 2006. Effects of light on NO_3^- uptake in small forested streams: diurnal and day-to-day variations. Journal of the North American Benthological Society 25:583–595.

Mulholland, P. J., and J. R. Webster. 2010. Nutrient dynamics in streams and the role of *J-NABS*. Journal of the North American Benthological Society 29:100–117.

Newbold, J. D., J. W. Elwood, R. V. O'Neill, and A. L. Sheldon. 1983. Phosphorus dynamics in a woodland stream ecosystem: a study of nutrient spiralling. Ecology 64:1249–1265.

O'Brien, J. M., and W. K. Dodds. 2010. Saturation of NO_3^- uptake in prairie streams as a function of acute and chronic N exposure. Journal of the North American Benthological Society 29:627–635.

O'Brien, J. M., W. K. Dodds, K. C. Wilson, J. N. Murdock, and J. Eichmiller. 2007. The saturation of ^{15}N cycling in Central Plains streams: N experiments across a broad gradient of nitrate concentrations. Biogeochemistry 84:31–49.

Odum, H. T. 1956. Primary production in flowing waters. Limnology and Oceanography 1:102–117.

Oviedo-Vargas, D., T. V. Royer, and L. T. Johnson. 2013. Dissolved organic carbon manipulation reveals coupled cycling of carbon, nitrogen, and phosphorus in a nitrogen-rich stream. Limnology and Oceanography 58:1196–1206.

Peterson, B. J., W. M. Wollheim, P. J. Mulholland, J. R. Webster, J. L. Meyer, J. L. Tank, E. Martí, W. B. Bowden, H. M. Valett, A. E. Hershey, W. H. McDowell, W. K. Dodds, S. K. Hamilton, S. Gregory, and D. D. Morrall. 2001. Control of nitrogen export from watersheds by headwater streams. Science 292:86–90.

Piper, L. R., W. F. Cross, and B. L. McGlynn. 2017. Colimitation and the coupling of N and P uptake kinetics in oligotrophic mountain streams. Biogeochemistry 132:165–184.

Rabalais, N. N., R. E. Turner, and W. J. Wiseman. 2002. Gulf of Mexico hypoxia, a.k.a. "The Dead Zone". Annual Review of Ecology and Systematics 33:235–263.

Reddy, K. R., R. H. Kadlec, E. Flaig, and P. M. Gale. 1999. Phosphorus retention in streams and wetlands: a review. Critical Reviews in Environmental Science and Technology 29:83–146.

Roberts, B. J., and P. J. Mulholland. 2007. In-stream biotic control on nutrient biogeochemistry in a forested stream, West Fork of Walker Branch. Journal of Geophysical Research 112:G04002.

Roberts, B. J., P. J. Mulholland, and W. R. Hill. 2007. Multiple scales of temporal variability in ecosystem metabolism rates: results from 2 years of continuous monitoring in a forested headwater stream. Ecosystems 10:588–606.

Rodríguez-Cardona, B., A. S. Wymore, and W. H. McDowell. 2016. DOC:NO_3^- ratios and NO_3^- uptake in forested headwater streams. Journal of Geophysical Research: Biogeosciences 121:205–217.

Rosemond, A. D., J. P. Benstead, P. M. Bumpers, V. Gulis, J. S. Kominoski, D. W. P. Manning, K. Suberkropp, and J. B. Wallace. 2015. Experimental nutrient additions accelerate terrestrial carbon loss from stream ecosystems. Science 347:1142–1145.

Rosemond, A. D., P. J. Mulholland, and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264–1280.

Royer, T. V., J. L. Tank, and M. B. David. 2004. Transport and fate of nitrate in headwater agricultural streams in Illinois. Journal of Environmental Quality 33:1296–1304.

Runkel, R. L. 2002. A new metric for determining the importance of transient storage. Journal of the North American Benthological Society 21:529–543.

Schade, J. D., K. MacNeill, S. A. Thomas, F. C. McNeely, J. R. Welter, J. Hood, M. Goodrich, M. E. Power, and J. C. Finlay. 2011. The stoichiometry of nitrogen and phosphorus spiralling in heterotrophic and autotrophic streams. Freshwater Biology 56:424–436.

Simon, K. S., C. R. Townsend, B. J. F. Biggs, and W. B. Bowden. 2005. Temporal variation of N and P uptake in 2 New Zealand streams. Journal of the North American Benthological Society 24:1–18.

Slavik, K., B. J. Peterson, L. A. Deegan, W. B. Bowden, A. E. Hershey, and J. E. Hobbie. 2004. Long-term responses of the Kuparuk River ecosystem to phosphorus fertilization. Ecology 85:939–954.

Stream Solute Workshop. 1990. Concepts and methods for assessing solute dynamics in stream ecosystems. Journal of the North American Benthological Society 9:95–110.

Stutter, M. I., B. O. L. Demars, and S. J. Langan. 2010. River phosphorus cycling: separating biotic and abiotic uptake during short-term changes in sewage effluent loading. Water Research 44:4425–4436.

Tank, J. L., M. J. Bernot, and E. J. Rosi-Marshall. 2006. Nitrogen limitation and uptake. Pages 213–238 *in* F. R. Hauer and G. A.

Lamberti (editors). Methods in Stream Ecology. 2nd edition. Elsevier, New York.

Tank, J. L., E. J. Rosi-Marshall, M. A. Baker, and R. O. Hall Jr. 2008. Are rivers just big streams? A pulse method to quantify nitrogen demand in a large river. Ecology 89:2935–2945.

Tank, J. L., and J. R. Webster. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology 79:2168–2179.

Trentman, M. T., W. K. Dodds, J. S. Fencl, K. Gerber, J. Guarneri, S. M. Hitchman, Z. Peterson, and J. Rüegg. 2015. Quantifying ambient nitrogen uptake and functional relationships of uptake versus concentration in streams: a comparison of stable isotope, pulse, and plateau approaches. Biogeochemistry 125:65–79.

von Schiller D., E. Martí, J. L. Riera, M. Ribot, A. Argerich, P. Fonollà, and F. Sabater. 2008. Inter-annual, annual, and seasonal variation of P and N retention in a perennial and an intermittent stream. Ecosystems 11:670–687.

Webster, J. R., P. J. Mulholland, J. L. Tank, H. M. Valett, W. K. Dodds, B. J. Peterson, W. B. Bowden, C. N. Dahm, S. Findlay, S. V. Gregory, N. B. Grimm, S. K. Hamilton, S. L. Johnson, E. Martí, W. H. McDowell, J. L. Meyer, D. D. Morrall, S. A. Thomas, and W. M. Wollheim. 2003. Factors affecting ammonium uptake in streams – an inter-biome perspective. Freshwater Biology 48:1329–1352.

Wentworth, C. K. 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30:377–392.