Walker Branch Watershed: Effect of Dual Nitrogen and Phosphorus Additions on Nutrient Uptake and Saturation Kinetics, 2011-2012

ORNL TERRESTRIAL ECOSYSTEM SCIENCE
SCIENTIFIC FOCUS AREA
Walker Branch Watershed

Summary:

This data set reports the results of field experiments investigating the effect of dual nutrient (nitrogen and phosphorus) additions on nutrient uptake and saturation kinetics in the West Fork of Walker Branch Watershed (WBW; Figure 1), a forested headwater stream on the Oak Ridge Reservation in east Tennessee. The experiments were carried out in the autumn of 2011 and spring of 2012 to examine seasonality in nutrient dynamics. The nutrient limitation status (N-limited, P-limited, co-limited for N and P, or neither N- nor P-limited) of Walker Branch was examined using nutrient diffusing substrata (NDS) in both autumn and spring. Next, a combination of instantaneous and steady-state nutrient releases were used to examine the effect of dual N and P releases on nutrient dynamics and saturation kinetics. Lastly, because the nutrient release results from autumn suggested a strong role of P sorption in P dynamics, laboratory assays were carried out in spring to quantify the importance of P sorption to sediments in Walker Branch.

Nutrient Diffusing Substrata Data:

- Measurements include heterotrophic microbial respiration on NDS with no added nutrients (control), or N, P, and N+P added.

Nutrient Release Data:

- Measurements include stream water nutrient (nitrate or soluble reactive phosphorus [SRP]) concentrations and specific conductivity during the instantaneous nutrient releases, nutrient uptake and saturation kinetic metrics calculated from the nutrient releases, nutrient concentration and specific conductivity of the nutrient injectate solutions, and stream discharge, velocity, width, and depth during the nutrient releases.

Phosphorus Sorption Data:

- Measurements include P removed or released during the P sorption assays, and the measurements used to calculate that metric (i.e., sediment dry mass, equilibrium SRP concentration, measured SRP concentration of the standards).

Figure 1 – The West Fork of Walker Branch Watershed in autumn.

These data have been analyzed and reported on in the following paper:

Griffiths, N.A., and L.T. Johnson. 2018. Influence of dual nitrogen and phosphorus additions on nutrient uptake and saturation kinetics in a forested headwater stream. Freshwater Science 37:810-825. https://doi.org/10.1086/700700

Data Citation:

Cite this dataset as follows:

Griffiths, N.A., and L.T. Johnson. 2018. Walker Branch Watershed: Effect of Dual Nitrogen and Phosphorus Additions on Nutrient Uptake and Saturation Kinetics, 2011-2012. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee, U.S.A. https://doi.org/10.25581/ornlsfa.015/1484490

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Data and Documentation Access:

For public access to WBW data please visit the ORNL TES-SFA Web Site:
http://tes-sfa.ornl.gov/

Description and Links to Supplemental Information: A manuscript published in *Freshwater Science* describes the WBW nutrient uptake methods and results (Griffiths and Johnson 2018).

Walker Branch Watershed website: http://walkerbranch.ornl.gov

Data Policy - Sharing, Access, and Use Recommendations: ORNL TES-SFA Data Policy - Data Policy and Fair-Use Statement

Related Data Sets: Historical precipitation, stream discharge, and stream chemistry data are available at http://walkerbranch.ornl.gov/data.shtml

Walker Branch Watershed (WBW) Project Description:

Walker Branch Watershed (WBW) is a forested watershed on the Oak Ridge Reservation and has been the site of long-term environmental research since the 1960s. Hydrological, biogeochemical, and ecological studies in WBW have made important contributions to our understanding of the effects of changes in atmospheric deposition and climate variability and change in this region (see http://walkerbranch.ornl.gov/publications.shtml for list of publications).

Objectives of the WBW long-term observations have been to:

1. Quantify responses of an eastern upland oak forest ecosystem to inter-annual and long-term variations in climate and atmospheric deposition of sulfur and nitrogen, and
2. Provide integrated, long-term data on climate, forest vegetation, soil chemistry, and hydrologic and chemical fluxes at the catchment scale to support other focused research projects on the Oak Ridge Reservation and elsewhere in the region.

DOE-BER funded WBW research has ended, and long-term monitoring of WBW is continuing through the National Ecological Observatory Network (NEON; http://www.neoninc.org/). This experiment on nutrient uptake dynamics is one of several studies focused on understanding biogeochemical dynamics at the terrestrial-aquatic interface.

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1. Data Set Overview:

This data set reports the results of field experiments investigating the effect of dual nutrient (nitrogen and phosphorus) additions on nutrient uptake and saturation kinetics in the West Fork of Walker Branch Watershed (WBW; Figure 1), a forested headwater stream on the Oak Ridge Reservation in east Tennessee.

Three types of experiments are briefly described below:

1. The first experiment examined the response of heterotrophic microbial respiration to nitrogen, phosphorus, and nitrogen + phosphorus additions (relative to a control with no added nutrients) using nutrient diffusing substrata (NDS). NDS were deployed in two seasons (autumn and spring) in the West Fork of WBW.

2. The second experiment examined how dual nutrient additions affected nutrient uptake and saturation kinetics. A total of 6 single and dual N and P releases were conducted in the West Fork of WBW in each season (autumn, spring). Separate instantaneous pulse additions were used to measure nutrient uptake rates and saturation kinetics of N and P individually (Covino et al. 2010). Steady-state injections were then used to increase background stream water concentrations of one nutrient (e.g., N) to low and then high concentrations, and instantaneous pulses of the other nutrient (e.g., P) were then released to quantify nutrient uptake and saturation kinetics at these elevated nutrient concentrations.

3. The third experiment examined the role of stream sediments in P sorption dynamics. Two laboratory assays were carried out on sediments collected in spring to measure P isotherms (McDaniel et al. 2009) and the phosphorus sorption index (PSI; Bache and Williams 1971).

2. Data Characteristics:

Spatial Coverage:

The research was conducted along a 74.4-m-long reach in the West Fork of Walker Branch. The West Fork is approximately 300 m in length from the headwaters to the location where the East and West Forks meet (which is just downstream of the West Fork weir). These nutrient addition experiments took place ~200 m (upstream end of the nutrient release reach) to ~125 m (downstream end of the nutrient release reach) upstream of the West Fork weir.

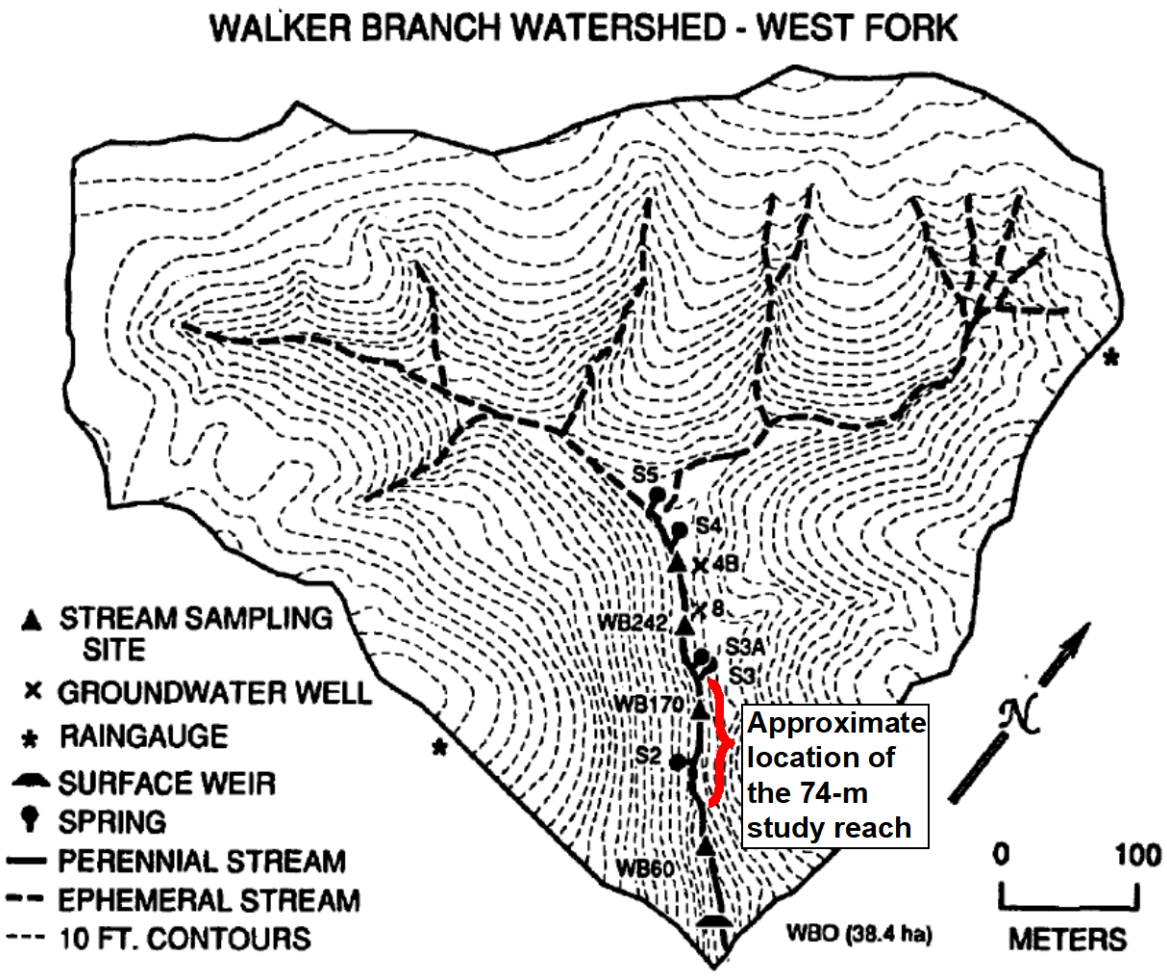

Figure 2 – Map of the West Fork of Walker Branch with the approximate location of the 74-m study reach shown. Map is from Genereux et al. 1993.

Site boundaries: Latitude and longitude given in decimal degrees. Source Google Earth.

Site (Region)	Westernmost Longitude	Easternmost Longitude	Northernmost Latitude	Southernmost Latitude	Elevation (meters amsl)	Geodetic Datum
West Fork of Walker Branch Watershed	-84.27981	-84.27858	35.96071	35.95949	265	WGS84

Temporal Coverage:

Nutrient releases and microbial respiration measurements on Nutrient Diffusing Substrata (NDS) were carried out in autumn and spring. The autumn nutrient releases were conducted on November 1-3, 2011, and the spring nutrient releases were conducted on March 20-22, 2012. Phosphorus sorption measurements occurred on sediments collected after the spring releases only.

Time period: The data set covers the period from November 2011 to March 2012.

Data File Description:

All of the data are contained in 5 comma separated (*.csv) files.

Nutrient Diffusing Substrata Data:

File #1: WBW_NDS_respiration.csv

Nutrient Release Data:

File #2: WBW_Nutrient_Release_Data.csv
File #3: WBW_Nutrient_Uptake_Data.csv
File #4: WBW_Nutrient_Injection_Data.csv

Phosphorus Sorption Data:

File #5: WBW_P_Sorption_Data.csv

Data Dictionary:

Nutrient Diffusing Substrata Data

File #1: WBW_NDS_respiration.csv

Column	Heading	Units/ Format	Description	Measurement Method
1	SEASON		Season of measurement (autumn, spring).	
2	TREATMENT		Nutrient treatment in the NDS (control = no nutrients (2% agar solution only), N = nitrogen added, P = phosphorus added, N+P = nitrogen and phosphorus added).	
3	RESPIRATION	mg $O_2/m^2/h$	Microbial respiration on cellulose disks placed on top of NDS.	Cellulose disks were removed after a 28-d incubation period. Respiration was measured as the change in dissolved oxygen concentration after cellulose disks were placed in dark centrifuge tubes filled unfiltered stream water and incubated in the stream at ambient water temperature for 4 hours (as in Johnson et al. 2009).

Example Data Records:

```
SEASON,TREATMENT,RESPIRATION
Spring,Control,12.33
Spring,Control,11.56
Spring,Control,9.50
Spring,Control,7.50
Spring,Control,6.03
….
Autumn,N+P,43.85
Autumn,N+P,55.51
Autumn,N+P,69.13
Autumn,N+P,72.79
Autumn,N+P,55.72
```

Nutrient Release Data

File #2: WBW_Nutrient_Release_Data.csv

Column	Heading	Units/ Format	Description	Measurement Method
1	SEASON		Season of measurement (autumn, spring).	
2	TREATMENT		The type of nutrient release (N alone, N with P low, N with P high, P alone, P with N low, P with N high)	
3	TIME	min	Time of measurement in minutes after the start of the instantaneous pulse nutrient release.	Stop watch.
4	SP_COND	µS/cm	Specific conductivity of stream water.	Hand-held conductivity probe.
5	BKD_CORR_SP_COND	µS/cm	Background-corrected specific conductivity of stream water.	Calculated based on background (i.e., pre-nutrient and conservative tracer release) specific conductivity of stream water.
6	NUT_CONC	µg NO_3-N/L for N releases; µg SRP-P/L for P releases	Nitrate or SRP concentration measured in stream water. For N releases (N alone, N with P low, N with P high), the reported nutrient is nitrate. For P releases (P alone, P with N low, P with N high), the reported nutrient is SRP.	Nitrate-N concentrations were measured using the cadmium reduction method. SRP concentrations were measured using the molybdate-antimony method.
7	BKD_CORR_NUT_CONC	µg NO_3-N/L for N releases; µg SRP-P/L for P releases	Background-corrected nitrate or SRP concentration of stream water.	Calculated based on background (i.e., pre-nutrient and conservative tracer release) nitrate or SRP concentration of stream water.

Example Data Records:

```
SEASON,TREATMENT,TIME,SP_COND,BKD_CORR_SP_COND,NUT_CONC,BKD_CORR_NUT_CONC
Autumn,N_alone,21,253.6,2.4,19.4,4.1
Autumn,N_alone,22,257.8,6.6,35.4,20.1
Autumn,N_alone,23,266.7,15.5,68.7,53.4
Autumn,N_alone,24,282.7,31.5,119.5,104.2
Autumn,N_alone,25,305.4,54.2,195.4,180.1
….
Spring,P_with_N_high,22,132.8,5.7,4.6,0.7
Spring,P_with_N_high,23,131.5,4.4,4.9,1.1
Spring,P_with_N_high,24,130.8,3.7,5.2,1.3
Spring,P_with_N_high,25,130.2,3.1,5.4,1.5
Spring,P_with_N_high,26,129.8,2.7,4.6,0.7
```

File #3 name: WBW_Nutrient_Uptake_Data.csv

Column	Heading	Units/ Format	Description	Measurement Method
1	SEASON		Season of measurement (autumn, spring).	
2	TREATMENT		The type of nutrient release (N alone, N with P low, N with P high, P alone, P with N low, P with N high)	
3	TIME	min	Time of measurement in minutes after the start of the instantaneous pulse nutrient release.	Stop watch.
4	NUT_TOTAL	µg N/L or µg P/L	Total nutrient concentration. For N releases (N alone, N with P low, N with P high), the reported nutrient is nitrate. For P releases (P alone, P with N low, P with N high), the reported nutrient is SRP.	Total nutrient concentration was calculated as the geometric mean of the total observed nutrient concentration and the total expected nutrient concentration given the conservative tracer (as in Covino et al. 2010). Only data from the falling limb are reported.
5	SW_ADD_DYN	m	Uptake length of the added nutrient.	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 (as in Covino et al. 2010). Only data from the falling limb are reported.
6	U_TOTAL	µg N or P/m^2/min	Total areal nutrient uptake.	Calculated total areal uptake for each sample by summing ambient areal uptake and areal uptake of the added nutrient. Areal uptake of the added nutrient was calculated as the product of discharge over width and the measured and expected geometric mean nutrient concentrations given the conservative tracer concentration over the uptake length of the added nutrient (as in Covino et al. 2010). Only data from the falling limb are reported.

Example Data Records:

```
SEASON,TREATMENT,TIME,NUT_TOTAL,SW_ADD_DYN,U_TOTAL
Autumn,N_alone,38,506.3,867.1,92.3
Autumn,N_alone,39,459.8,928.2,83.1
Autumn,N_alone,40,404.5,617.3,99.0
Autumn,N_alone,41,359.3,572.4,95.9
Autumn,N_alone,42,315.4,497.5,96.2
….
Spring,P_with_N_high,19,7.5,39.0,75.6
Spring,P_with_N_high,20,7.3,60.6,67.3
Spring,P_with_N_high,21,6.8,82.1,60.3
Spring,P_with_N_high,22,5.9,51.6,59.8
Spring,P_with_N_high,23,5.8,84.1,54.9
```

File #4: WBW_Nutrient_Injection_Data.csv

Column	Heading	Units/ Format	Description	Measurement Method
1	SEASON		Season of measurement (autumn, spring).	
2	TREATMENT		The type of nutrient release (N alone, N with P low, N with P high, P alone, P with N low, P with N high)	
3	INJ_SP_COND	mS/cm	Specific conductivity of the nutrient + conservative tracer injectate used in the instantaneous pulse release.	Specific conductivity of 5 replicate samples diluted 1:1000 measured using a hand-held conductivity probe. Mean of the 5 replicate samples reported here.
4	INJ_NUT_CONC	mg N or P/L	Nitrate or SRP concentration of the nutrient + conservative tracer injectate used in the instantaneous pulse release.	Nitrate or SRP concentration of 5 replicate samples diluted 1:1000. Nitrate-N concentrations were measured using the cadmium reduction method. SRP

Column	Heading	Units/ Format	Description	Measurement Method
				concentrations were measured using the molybdate-antimony method. Mean of the 5 replicate samples reported here.
5	DISCHARGE	L/s	Stream discharge during the nutrient + conservative tracer instantaneous pulse release.	Calculated as the product of the volume of the instantaneous pulse solution and the mean specific conductivity of the pulse solution, divided by the area under the specific conductivity curve.
6	VELOCITY	m/s	Mean stream velocity during the nutrient + conservative tracer instantaneous pulse release.	Calculated as the time to peak specific conductivity during the pulse release divided by the stream reach length (74.4 m).
7	WIDTH	m	Mean stream wetted width during the nutrient + conservative tracer instantaneous pulse release.	Calculated as the mean wetted width based on widths measured every ~2 m along the 74.4-m-long reach.
8	DEPTH	m	Mean water depth during the nutrient + conservative tracer instantaneous pulse release.	Calculated from discharge divided by the product of velocity and width.

Example Data Records:

```
SEASON,TREATMENT,INJ_SP_COND,INJ_NUT_CONC,DISCHARGE,VELOCITY,WIDTH,DEPTH
Autumn,N_alone,79.5,304.1,4.3,0.04,2.46,0.05
Autumn,N_with_P_low,81.4,322.2,4.1,0.04,2.46,0.05
Autumn,N_with_P_high,80.8,333.7,4.2,0.04,2.46,0.05
Autumn,P_alone,77.0,54.1,4.2,0.04,2.46,0.05
Autumn,P_with_N_low,80.0,63.3,4.3,0.04,2.46,0.05
….
Spring,N_with_P_low,99.5,623.5,15.2,0.11,2.90,0.05
Spring,N_with_P_high,104.9,678.9,15.9,0.10,2.90,0.05
Spring,P_alone,88.4,61.0,19.8,0.13,2.90,0.05
Spring,P_with_N_low,88.9,58.6,19.6,0.13,2.90,0.05
Spring,P_with_N_high,93.4,60.3,20.6,0.13,2.90,0.06
```

Phosphorus Sorption Data

File #5: WBW_P_Sorption_Data.csv

Column	Heading	Units/ Format	Description	Measurement Method
1	ASSAY		The type of P sorption assay. EPC = P sorption isotherms from which the equilibrium P concentration at zero release (EPC_0) was determined. PSI = phosphorus sorption index assay.	
2	TREATMENT		Treatment = live or killed.	Killed = biota on sediments killed with 1 mL $HgCl_2$ (0.2%) for at least 15 min before standards were added.
3	CALC_STD_CONC	µg P/L	Calculated concentration of each standard if made in DI water.	
4	MEASURED_STD_CONC	µg P/L	Measured concentration of each standard that was made in stream water.	SRP concentrations were measured using the molybdate-antimony method.
5	REP		Replicate (1, 2, or 3).	
6	EQUILIBRIUM_P_CONC	µg P/L	Measured SRP concentration after the desired incubation time.	SRP concentrations were measured using the molybdate-antimony method.
7	SED_DM	g DM	Dry mass (DM) of the sediments used in the sorption assay.	After the sorption assay, sediment was dried at 60°C and then weighed.
8	P_REMOVED_OR_RELEASED	mg P/kg DM	Mass of P removed or released by sediments per dry mass of sediment	Calculated from the measured standard P concentration and equilibrium P concentration and the dry mass of sediments. Negative values = released. Positive values = removed (sorbed).

Example Data Records:

```
ASSAY,TREATMENT,STD_CONC,MEASURED_STD_CONC,REPLICATE,EQUILIBRIUM_SRP_CONC,SED_DM,P_REMOVED_OR_REL
EASED
EPC,Live,2.5,6.375,1,11.5,9.1492,-0.022
EPC,Live,2.5,6.375,2,10.2,6.7262,-0.023
EPC,Live,2.5,6.375,3,12.1,9.1792,-0.025
EPC,Live,27.5,30.05,1,11.7,8.2759,0.089
EPC,Live,27.5,30.05,2,12.2,7.4398,0.096
….
PSI,Killed,52.5,52.15,2,16,6.1617,0.235
PSI,Killed,52.5,52.15,3,19.9,9.7446,0.132
PSI,Killed,2002.5,2350,1,595,9.6978,7.239
PSI,Killed,2002.5,2350,2,1050,7.6642,6.785
PSI,Killed,2002.5,2350,3,827,9.3449,6.519
```

3. Data Application and Derivation:

The goal of this project was to examine how nitrogen and phosphorus interact to influence nutrient uptake and saturation kinetics in a headwater forested stream. A manuscript published in *Freshwater Science* describes the WBW nutrient uptake methods and results (Griffiths and Johnson 2018).

4. Quality Assessment:

These data are considered at Level 2. Level 2 indicates that, in addition to the Level 1 checks, the product is a complete, externally consistent data product that has undergone interpretative and diagnostic analyses and can be shared with the public. Level 1 indicates an internally consistent data product that has been subjected to quality checks and data management procedures. Instrument calibrations were carried out following the manufacturer's instructions and analyses followed published procedures.

5. Data Acquisition Materials and Methods:

Site Description:
Walker Branch Watershed (WBW) is a 97.5 ha second-growth forest on the U.S. Department of Energy's Oak Ridge Reservation in east Tennessee, USA. There are two headwaters streams that drain the watershed: the West Fork drains 38.4 ha (Figure 2) and the East Fork drains 59.1 ha (Curlin and Nelson 1968). The watershed is underlain by bedrock (Knox Dolomite) with deep soils, primarily Utisols. Vegetation is primarily oaks (*Quercus prinus, Quercus alba*), tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), and American beech (*Fagus grandifolia*) (Johnson 1989, Kardol et al. 2010). The climate is typical of the southern Appalachian region, with a mean annual temperature of 14.5ºC and mean annual precipitation of 135 cm (Curlin and Nelson 1968, Johnson 1989). Long-term records have documented a 1.8ºC increase in mean annual air temperature over the past 40 years (Lutz et al. 2012). More detailed site descriptions are available in Curlin and Nelson (1968) and Johnson (1989).

The nutrient uptake experiments were carried out in the West Fork of WBW. The West Fork is approximately 300 m in length from the headwaters to the location where the East and West Forks meet. Four perennial springs discharge to the West Fork. The S3 spring that is located approximately 160 m downstream from the headwater springs provides the majority of baseflow compared to the other springs (Curlin and Nelson 1968, Genereux et al. 1993, Figure 2). The stream bed is composed primarily of bedrock outcrops, areas of cobble and gravel, and deposits of organic matter. Stream water nutrient concentrations are low (Mulholland 2004, Lutz et al. 2012) and the snail, *Elimia clavaeformis*, is the dominant invertebrate in the stream (Newbold et al. 1983, Rosemond et al. 1993, Griffiths and Hill 2014).

Nutrient diffusing substrata:
Nutrient diffusing substrata (NDS) were used to measure the response of heterotrophic microbial respiration to added nutrients. The NDS were constructed of 50-mL centrifuge tubes filled with a 2% agar solution containing either 0.05 mol/L $NaNO_3$, 0.05 mol/L KH_2PO_4, both (N + P), or no added nutrients as a control (n=5 for each treatment). NDS were topped with an organic cellulose sponge cloth to select for heterotrophic constituents of stream biofilms (Johnson et al. 2009). NDS were deployed in the stream for 28 days. After this incubation period, the organic substrata were removed from the tubes and placed into dark centrifuge tubes filled with unfiltered stream water (no air bubbles). The centrifuge tubes were then placed in a shaded section of the stream to keep the substrata at ambient temperatures and were left for 4 hours. Microbial respiration was measured as the consumption of dissolved oxygen after the 4-hour incubation period by measuring dissolved oxygen concentration before and after the incubation. Centrifuge tubes containing unfiltered stream water only (n=5) were included to account for background changes in dissolved oxygen concentration. 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). This method follows that described in Johnson et al. (2009).

Nutrient additions:
A total of 6 single and dual N and P releases were carried out in Walker Branch in each season (autumn, spring). Separate instantaneous pulse additions (Tracer Additions for Spiraling Curve Characterization; TASCC method described by Covino et al. 2010) were used to quantify nutrient uptake rates and saturation kinetics of N and P individually. A combination of steady-state nutrient injections (Tank et al. 2006) and TASCC instantaneous pulse nutrient additions were used to examine the interaction between N and P uptake. The steady-state nutrient injections were used to increase background stream water concentrations of one nutrient (e.g., N). After stream water (e.g., N) nutrient concentrations were elevated, an instantaneous pulse of the other nutrient (e.g., P) was released. Nutrient uptake rates and kinetics of N and P from the TASCC releases were conducted at background and elevated (low and high) P and N concentrations. These releases are referred to: 'P alone', 'P with N low', and 'P with N high' for P uptake, and 'N alone', 'N with P low', and 'N with P high' for N uptake. Nutrient additions were conducted on November 1-3, 2011 (autumn) and March 20-22, 2012 (spring). In autumn, single nutrient pulse additions were conducted on the first day of fieldwork, P pulses with low and high N concentrations on the second day, and N pulses with low and high P concentrations on the third day. In the spring, all P pulses were conducted on the first day of fieldwork and all N pulses were conducted on the third day.

The instantaneous pulse (TASCC) nutrient addition method to estimate nutrient uptake rates and saturation kinetics is detailed in Covino et al. (2010) but will be briefly described here. For each TASCC addition, a solution of either N or P with NaCl as a conservative tracer was added to a 74-m reach of stream. The autumn nutrient solutions consisted of either 25 g KNO_3 or 2.5 g KH_2PO_4 with 400 g NaCl dissolved in 10 L of stream water, and the spring nutrient solutions consisted of either 50 g KNO_3 or 2.75 g KH_2PO_4 with 450 g NaCl dissolved in stream water. The nutrient solution was released over a short (~10 s) period, and specific conductivity was measured every minute at a location 74 m downstream from the release point using a handheld conductivity probe (YSI Model 30; Yellow Springs Instruments). Water samples for N and P analysis (described below) were collected every minute starting when specific conductivity began to increase and stopping once specific conductivity returned to background levels. Five replicate samples from the nutrient release solutions were collected for analysis of N, P, and specific conductivity.

For the dual nutrient additions, steady-state nutrient injections were used to increase either N or P concentrations throughout the 74-m stream reach. In autumn and spring, 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. In the spring, the nutrient solution contained either 303 g KNO_3 or 41 g

KH_2PO_4. Water samples were collected at 5 stations longitudinally throughout the 74-m reach both before the steady-state addition and after steady state (i.e., plateau) was reached at the farthest downstream station (74 m). The nutrient concentrations in stream water during the low and high nutrient additions were estimated as the geometric mean of nutrient concentrations from the 5 longitudinal samples at plateau.

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

All water samples were filtered in the field through Whatman GF/F filters (0.7-µm nominal pore size; Maidstone, England), placed on ice, and then frozen upon return to the laboratory (within a few hours of collection). Nitrate-N concentrations were measured on a DIONEX ICS-2000 ion chromatograph with an AS11-HC column (Dionex, Sunnyvale, California) and soluble reactive phosphorus (SRP) concentrations were measured using molybdate-blue colorimetry (APHA 2005) on an autoanalyzer (AA3; Seal Analytical Inc., Mequon, Wisconsin). For all water chemistry analyses, blanks and certified commercial standards were analyzed in each run to check for data quality.

The measured and background-corrected nutrient concentration and specific conductivity values for each TASCC release are reported in this dataset. The stream water concentrations from the steady-state nutrient additions are reported in the Griffiths and Johnson 2018 publication in Freshwater Science.

Nutrient uptake and saturation kinetic calculations:

From the TASCC pulse additions, 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) were calculated (as described in detail in Covino et al. 2010). Uptake length ($S_{w\text{-}add\text{-}dyn}$; m) 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 (74 m). 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]):

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

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. 2010). 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 stream water nutrient concentration (ambient [nutrient]), and $V_{f\text{-}amb}$ was calculated as U_{amb}/ambient [nutrient]. Stream discharge (Q) was estimated by integrating under the conductivity pulse of each release and mean stream width (w) was calculated from stream-width measurements taken every ~2 m along the 74-m study reach. Stream velocity was calculated as the time to peak specific conductivity during the TASCC release divided by the stream reach length (74 m). Stream depth was calculated as stream discharge divided by the product of stream velocity and width.

To estimate saturation kinetic metrics, areal uptake for each sample ($U_{add\text{-}dyn}$) on the falling limb was calculated by multiplying Q/w by the measured and expected geometric mean nutrient concentrations given the conservative tracer concentration divided by the uptake length of the added nutrient ($S_{w\text{-}add\text{-}dyn}$) (Covino et al.

2010). Total areal uptake (U_{total}) was then calculated for each sample by summing U_{amb} and $U_{add\text{-}dyn}$. Saturation kinetics (U_{max}, K_m) were calculated by fitting a Michaelis-Menten model to U_{total} vs total [nutrient]:

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

Uptake length of the added nutrient ($S_{w\text{-}add\text{-}dyn}$), total nutrient uptake (U_{total}), and total nutrient concentration are reported in this dataset. Further, the nutrient concentration and specific conductivity of each TASCC nutrient injectate and stream discharge, width, velocity, and depth are reported for each TASCC release in each season. 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) are reported in the Supplemental Tables associated with the Griffiths and Johnson 2018 Freshwater Science publication.

Phosphorus sorption assays:

Laboratory P sorption assays used sediments collected from Walker Branch in spring to determine P isotherms (McDaniel et al. 2009) and the phosphorus sorption index (PSI; Bache and Williams 1971). Five cores (6 cm wide × 3 cm deep) were collected from each of 6 locations along the 74-m reach in areas where gravel and fine benthic organic matter (FBOM) accumulate. These 30 cores were composited into one sample, and a subsample of the sediment was wet sieved in the laboratory to produce a <8-mm size fraction for sorption assays.

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 second set (n=21) was prepared to measure abiotic sorption by killing biota on 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 equilibrium P concentration at zero release or retention (EPC_0), where P is neither adsorbed nor desorbed from the sediments, was calculated from the P isotherms. The increase or decrease in P during the assay was scaled to g DM of sediment (µg P/g DM) for each sample against the final equilibrium P concentration in the sample. Then, the *x*-intercept of this relationship was used to estimate the EPC_0 (Froelich 1988, McDaniel et al. 2009). The PSI was calculated 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). The 50 µg P/L standard from the PSI assay with live sediments was used to estimate the capacity for the sediments to remove P at a concentration and time-scale similar to that of the pulse nutrient additions.

6. References:

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.

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

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.

Curlin, J.W., and D.J. Nelson. 1968. Walker Branch Watershed project: objectives, facilities, and ecological characteristics. ORNL-TM-2271. Oak Ridge National Laboratory, Oak Ridge, Tennessee.

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.

Genereux, D.P., H.F. Hemond, and P.J. Mulholland. 1993. Spatial and temporal variability in streamflow generation on the West Fork of Walker Branch Watershed. Journal of Hydrology 142:137-166.

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.

Griffiths, N.A., and L.T. Johnson. 2018. Influence of dual nitrogen and phosphorus additions on nutrient uptake and saturation kinetics in a forested headwater stream. Freshwater Science 37:810-825.

Johnson, D.W. 1989. Site description. Pages 6-20 *in* D.W. Johnson and R.I. Van Hook (editors). Analysis of biogeochemical cycling processes in Walker Branch Watershed. Springer-Verlag, New York, New York.

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.

Kardol P., D.E. Todd, P J. Hanson, and P.J. Mulholland. 2010. Long-term successional forest dynamics: species and community responses to climatic variability. Journal of Vegetation Science 21:627-642.

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.

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.

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

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 records for Walker Branch Watershed. Biogeochemistry 70:403-426.

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.

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.

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.

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.

7. Data Access:

For public access to ORNL TES SFA data please visit the TES SFA Web Site: https://tes-sfa.ornl.gov/home

Contact for Data Access Information: https://mnspruce.ornl.gov/contact