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EFFECT OF MACROPHYTE-DRIVEN TRANSIENT STORAGE ON PHOSPHORUS UPTAKE IN SPRING COULEE CREEK

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EFFECT OF MACROPHYTE-DRIVEN TRANSIENT STORAGE ON PHOSPHORUS UPTAKE IN SPRING COULEE CREEK

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ABSTRACT

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Investigations of phosphorus cycling and transport in streams lend insight into potential mechanisms of nutrient sequestration and can help mitigate human impact. In this study, we show a relationship between transient storage and phosphorus uptake in a cold-water stream in western Wisconsin. Hydrologic characteristics, nutrient spiraling metrics, macrophyte biomass, and geomorphological properties were quantified in seven reaches of Spring Coulee Creek. In each reach a solute transport model was used to estimate the median travel-time spent in transient storage, and uptake velocity was measured using pulsed additions of PO_4^{3-} to the surface water. Stepwise linear regression was used to build models for transient storage and uptake velocity. Macrophyte biomass, stream bed slope, and riffle to pool ratio accounted for 99.6% of the variation in transient storage ($P < 0.001$). Transient storage, canopy cover, and slope accounted for 98.0% of the variation in uptake velocity ($P= 0.002$). This study shows that transient storage, primarily resulting from macrophyte beds, is positively correlated with phosphorus uptake but acknowledges the importance of other factors as well.

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INTRODUCTION

Excess nutrients from human activities often have an adverse effect water quality (Allan, 2004). In landscapes dominated by agriculture, the pollutants of concern are often nutrients (e.g., nitrogen and phosphorus) originating from practices that include fertilization and manure production (Carpenter *et al.*, 1998). Excess amounts of these nutrients generated from raising crops and livestock make their way into the waterways and can cause disruptions in water quality that affect resident aquatic organisms and terrestrial species that use the impacted resource. Lakes, reservoirs, large rivers, and marine ecosystems suffer detrimental effects from harmful algal blooms and hypoxia (Rabalais *et al.*, 2002; Dybas, 2005). These effects intensify as anthropogenic nutrient inputs to the environment continue to increase because of human population growth and land use development. Small streams account for the greatest proportion of the drainage network and the natural processes within these low order streams have the potential to reduce loading of nutrients into downstream water bodies. Headwater streams can recycle nutrients, trap sediment and pollutants, and provide the resource base which maintains biological diversity downstream (Meyer *et al.*, 2003). It is important to study and understand nutrient dynamics in streams in order to make the most of these ecosystem services, and mitigate harmful effects on the environment.

Both biogeochemical and geomorphic variables influence nutrient dynamics in a stream. The latter has an indirect effect on uptake by controlling residence time, the

amount of time water and solute are exposed to biochemically reactive substrates (Valett *et al.*, 1996). Increased exposure to these reactive substrates can facilitate biotic and abiotic uptake. Furthermore, geomorphic characteristics such as gradient, sinuosity, and cross-sectional area affect hydrologic transport processes and ultimately dictate the size of the transient storage zone (anywhere water is temporarily retained, e.g., surface pools, macrophyte beds, and the hyporheic zone). While it is intuitive that transient storage would be an important driver of nutrient uptake, a review of the nutrient spiraling literature has concluded that that the relationship between transient storage and nutrient uptake remains unclear (Ensign and Doyle, 2006). Some found no significant relationship between storage and uptake (Martí *et al.*, 1997; Webster *et al.*, 2003), and some assert the importance of surface sediments (over hyporheic storage) to nutrient retention (Hart *et al.*, 1992; Butturini and Sabater, 1999). In contrast to these findings, other studies conclude hyporheic transient storage is important to nutrient uptake. For example, transient storage was responsible for about 43% of phosphorus retention in Hugh White Creek (Mulholland *et al.*, 1997), and for similar amounts of nitrate retention in another North Carolina stream (Thomas *et al.*, 2003). Furthermore, others have found that manipulating natural and artificial structures alter surface (in-channel) transient storage and subsequently influence nutrient uptake (Ensign and Doyle, 2005).

One reason for the inconsistency in the literature is that generic measures of transient storage do not distinguish between surface and hyporheic storage zones. These zones have very different implications for nutrient uptake at different times or in different locations (Hall *et al.*, 2002; Thomas *et al.*, 2003). For example, during peak autotrophic growth, benthic uptake predominates, while low biomass conditions tend to favor

hyporheic uptake (Orr *et al.*, 2009). Some studies therefore found that the hyporheic zone has the larger potential for nutrient processing (Triska *et al.*, 1989) while others emphasize the importance of surface storage for nutrient uptake (Ensign and Doyle, 2005). Furthermore, surface storage is more important in streams where the size of the hyporheic zone can be negligible relative to surface storage (Jin and Ward, 2005; Stofleth *et al.*, 2008). Since distinguishing between these two types of storage can be difficult, particularly when considering dynamic spatial and temporal scales, it is helpful if researchers attempt to identify the key features that contribute to storage. Information regarding these geomorphological and biogeochemical features, along with the nonspecific measure of transient storage, can aid in understanding stream nutrient dynamics.

One transient storage feature that can play a key role in stream nutrient dynamics is macrophyte beds. They have a large indirect influence on nutrient dynamics by altering stream flow and sedimentation processes. Studies have found that dense macrophyte beds decrease near-bed water velocity and significantly increase residence time and the trapping of particulate sediment and organic matter, both of which contribute to phosphorus retention in streams (Sand-Jensen, 1998; Schulz et al., 2003; Gurnell et al., 2006). Macrophytes also directly alter water chemistry and nutrient dynamics via photosynthesis and by taking up nutrients through their roots (from sediment) and shoots (from water column). Furthermore, macrophytes provide surfaces for the establishment of epiphytic biofilms, which can be very efficient at removing nutrients from the water column (Hein et al., 1995; Pelton et al., 1998; Levi et al., 2015).

The dynamic relationships between biogeochemical and geomorphic stream elements are complex, and determining their collective role in nutrient dynamics is challenging. In this study we simplified these interactions by providing a longitudinal, intra-stream assessment to understand the relationships that exist between geomorphological features, macrophyte biomass, transient storage, and phosphorus uptake. Much of the previous nutrient cycling work in streams has focused on nitrogen; partly because nitrogen can be studied using stable isotopes. However, studies of phosphorus dynamics should not be neglected, particularly because phosphorus is the nutrient often linked to eutrophication of freshwater systems (Schindler *et al.*, 2008; Schindler *et al.*, 2016). In addition, previous research in our study stream shows that phosphorus is limiting (Mooney *et al.*, 2014), which allows us to quantify phosphorus uptake via nutrient addition experiments.

The objectives of this study were: (i) to quantify transient storage and phosphorus uptake in Spring Coulee Creek, and (ii) to identify the variables that have the greatest influence on transient storage and phosphorus uptake. It was hypothesized that transient storage and phosphorus uptake would be positively correlated in Spring Coulee Creek and the primary factor promoting both storage and uptake would be macrophyte biomass.

METHODS

Study Area

Spring Coulee Creek is a 1st-4th order cold-water stream located in southwestern Wisconsin (43.712 N, -90.990 W). The Spring Coulee Creek watershed (ca. 32 km^2) drains predominantly type B (82.9%) moderate infiltration hydrologic soils (Soil Survey Staff, 2016) and land cover within the watershed is 42.8% deciduous forest, 33.8% cultivated crops, and 15.5% pasture (Homer *et al.*, 2015). The stream is located in the US EPA Driftless Area Ecoregion (Ecoregion 52), which was bypassed by the last continental glaciation, resulting in a landscape dominated by karst topography with rolling hills, sinkholes, caves, and cold-water streams. Land use in the Driftless Area is largely agricultural (Juckem *et al.*, 2008). About 60% of the land is classified as cultivated or pasture. Driftless Area streams tend to exhibit relatively high nitrogen and low phosphorus concentrations, with summer median total dissolved N and P concentrations of 2.9 mg N/L and 15 μg P/L, respectively (Olmanson, 2014).

Seven reaches of Spring Coulee Creek were selected for investigation, herein referred to as SC-1 through SC-7 (Figure 1, Table I). Reach lengths, measured with a field tape, ranged from 215 to about 315 m. Data were collected in August 2015, during stable baseflow conditions. Despite stable flow, larger discharges in SC-5, 6, and 7 were the result of a significant tributary entering the stream below SC-4. Stream substrate generally consists of a sandy bed with overlying cobbles in areas with increased slope.

During data collection, ambient PO_4^- concentrations ranged from 17-30 µg L^{-1}, pH 8.3-8.5, conductivity 480-490 µS/cm, and temperature was generally between 16-18°C.

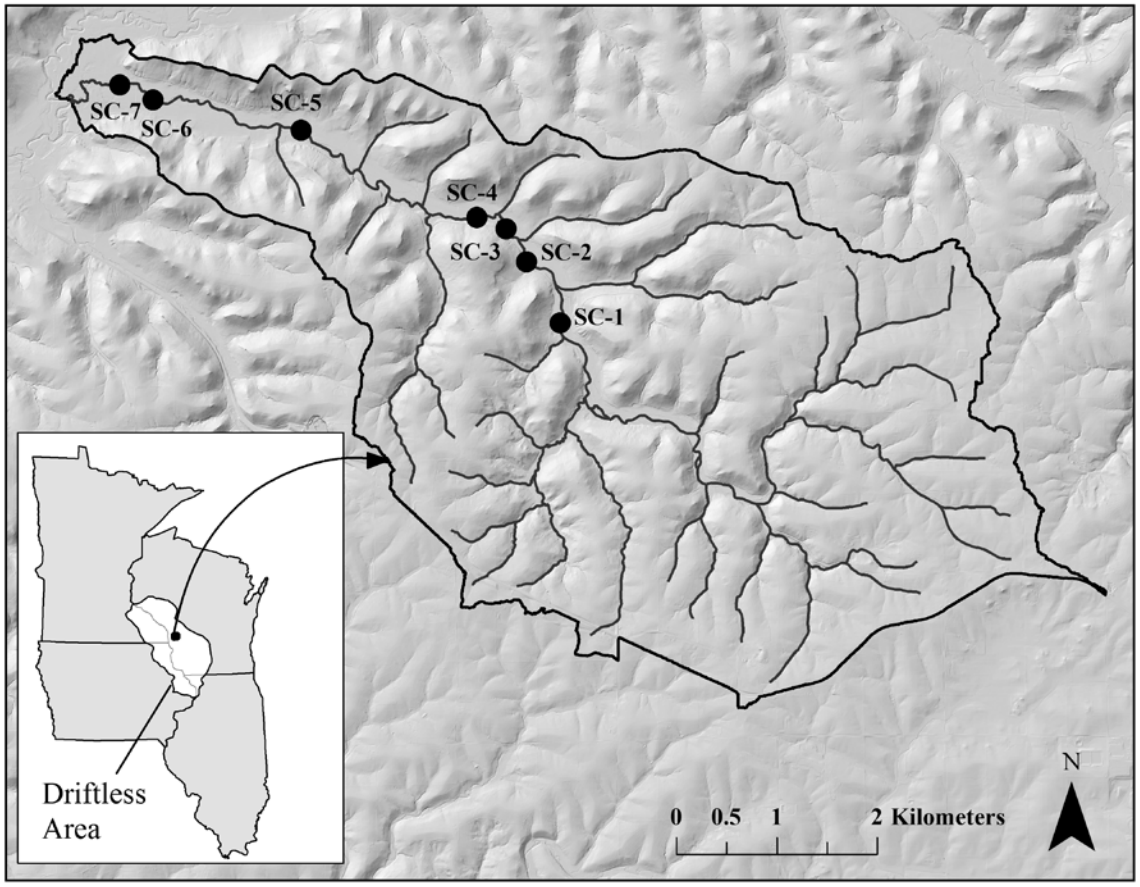

Figure 1. Location of study reaches (SC-1 to SC-7) within the Spring Coulee Creek watershed.

Table I. Channel geometry, geomorphic characteristics, and vegetation abundance of seven study reaches in Spring Coulee Creek.

	SC-1	SC-2	SC-3	SC-4	SC-5	SC-6	SC-7
Baseflow discharge (L/s)	131	131	130	134	231	225	231
Length (m)	215	298	262	280	276	300	316
Width (m)[a]	4.77 ± 1.45	4.45 ± 1.48	4.18 ± 1.69	4.08 ± 1.00	4.52 ± 2.17	3.95 ± 0.84	3.91 ± 1.37
Depth (m)[a]	0.21 ± 0.07	0.24 ± 0.07	0.39 ± 0.13	0.28 ± 0.10	0.50 ± 0.33	0.41 ± 0.12	0.40 ± 0.17
Velocity (m/s)[a]	0.34 ± 0.13	0.34 ± 0.12	0.29 ± 0.15	0.43 ± 0.21	0.41 ± 0.28	0.39 ± 0.16	0.44 ± 0.28
R:P[b]	0.67	0.33	0.00	0.33	0.40	0.00	0.40
D_{50} (mm)[c]	53	51	33	53	34	9	8
Bed slope (%)	0.91	0.75	0.53	1.03	0.80	0.25	0.46
Sinuosity	1.08	1.16	1.22	1.22	1.22	2.19	1.28
Canopy (%)[a]	63 ± 24	14 ± 18	40 ± 36	62 ± 36	3 ± 6	39 ± 29	13 ± 20
M_c (%)[ad]	26 ± 38	50 ± 44	42 ± 40	26 ± 41	36 ± 42	37 ± 42	25 ± 28
M_b (g/m^2)[ae]	62 ± 103	93 ± 91	126 ± 183	123 ± 244	147 ± 239	45 ± 59	37 ± 41

[a]Mean and standard deviation reported from 11 measurements
[b]Riffle to pool ratio
[c]Median grain size
[d]Macrophyte cover
[e]Macrophyte biomass

Physical Measurements

Each reach was divided into 11 equidistant transects where measurements of wetted width (w), thalwag depth (d), and thalwag velocity (v) were taken immediately following the series of releases. An OTT MF Pro velocity meter was used to measure velocity and determine discharge (Q) via the velocity-area method using at least 10 points measured at 0.60 depth. Froude number (Fr, equation 1) was calculated to categorize habitat type at each transect.

$$Fr = \frac{v}{\sqrt{g \times d}} \qquad \text{(Equation 1)}$$

Where g is gravitational acceleration (m/s^2), d is the thalwag depth (m), and v is the average thalwag channel velocity at 0.60 depth (m/s). The Froude numbers were used to classify each transect as a pool, riffle, or run (Jowett, 1993).

Macrophyte cover (M_c) and macrophyte biomass (M_b) were estimated using a grid technique modified from Bowden *et al.* (2006). A randomly placed quadrat at each of the 11 transects was used to estimate percent M_c. Additionally, the macrophytes within each quadrat were harvested to determine macrophyte biomass (M_b) in the lab via ash free dry mass (AFDM). Canopy cover was measured using a Model-A spherical densitometer during September 2015, before leaf-fall. Measurements were taken facing four directions (90° intervals from one another) at each transect. To determine reach median grain size, D_{50}, grain size distributions were constructed from Wolman pebble count data that was collected along the entire reach. Reach length was determined manually using a field tape, while elevation change and valley length were determined from point measurements taken at the upstream and downstream end of each reach with a Trimble GEOXH 6000 GPS with centimeter accuracy following differential correction. From these

measurements, bed slope (change in elevation ÷ channel length) and sinuosity (channel length ÷ valley length) were calculated.

Tracer Releases

A series of tracer releases were conducted in August 2015, during baseflow and peak biomass conditions. In each reach, a pulse (slug) release was conducted to measure phosphorus uptake, and a sustained injection release was done to quantify transient storage and other hydrologic parameters. With the exception of SC-6, where five days separate the two releases both releases were completed in either one day, or in two consecutive days. In order to ensure adequate solute mixing, an area of turbulence was selected for at the upstream end and an approximate 15-minute travel time was targeted in each reach.

Rhodamine WT dye (RWT) was used as the conservative tracer during the pulse and injection releases. We acknowledge that RWT dye can be affected by sorption processes, so it should not be used as a conservative tracer to investigate long flow paths or the hyporheic zone (Runkel, 2015). We warranted its use as a conservative tracer in this study based on limited hyporheic transient storage in Spring Coulee Creek. Further justification was based on high (97-100%) mass recovery of RWT during slug releases, low benthic organic matter in study stream, short reach lengths (215 to 370 m), and short duration injections (<1.5 hours). Target concentrations of RWT were 30 ppb. These concentrations were easily observed in the study reach and rapidly diluted to non-detectable levels downstream.

Pulse additions of RWT plus PO_4^{3-} to the surface water were used to determine dissolved phosphorus uptake. Target concentrations of PO_4^{3-} were 200 µg/L above

background. Slugs containing known amounts of phosphorus and RWT and were added at the upstream end of the stream reaches. Over the course of the pulse period, downstream concentrations of RWT were measured with a Hydrolab DS5 multiparameter probe equipped with a Turner Designs® Rhodamine WT sensor. The probe was calibrated daily and temperature corrections were applied to the RWT data (Wilson *et al.*, 1986). Additionally, solute samples were collected in 20 mL scintillation vials at the downstream end approximately every 30 seconds, beginning 10 minutes before the pulse arrived, and continuing until RWT concentrations returned to ambient levels. Samples were taken less frequently if change in dye concentration between time points was small. Samples were acidified (pH < 4) and kept refrigerated until dissolved phosphorus concentrations were measured on a Lachat QuikChem 8500 Series 2 Flow Injection Analysis System (QuikChem® Method 10-115-01-1-A).

Sustained injections of RWT were accomplished with a battery-powered FMI Lab Pump (Model QB) and voltage regulator to ensure constant injection rate. Pump rates during each release were between 55 and 95 mL/min. Injections persisted until plateau was achieved at the downstream end (about one hour) and the pump was turned off. Concentrations of RWT were monitored throughout the injection period and until background levels returned.

Uptake Calculations

The nutrient spiraling approach (Webster, 1975) uses interrelated metrics to describe uptake dynamics in streams. Uptake length (S_w), the average distance a nutrient atom traveled in dissolved inorganic form before it is removed from solution, is a function of biotic demand and hydrology (Newbold *et al.*, 1981). To standardize S_w by

taking into account velocity and depth, it is converted to a mass transfer coefficient (Stream Solute Workshop 1990) referred to as the uptake velocity, v_f (Peterson *et al.*, 2001; Valett *et al.*, 2002). This value describes how quickly a solute in transport will become taken up and immobilized, and is good for comparing uptake in reaches of differing sizes. These uptake metrics were calculated from the pulse releases using a mass balance approach (Tank *et al.*, 2008). Simply put, this method compares the recovery of both the conservative and non-conservative tracers over the duration of the pulse period to determine uptake. We compared the total mass recovery PO_4^{3-} to RWT at the downstream station ($P{:}RWT_{ds}$) to the mass ratio of the injectate ($P{:}RWT_{inj}$), and assumed any mass reduction of phosphorus relative to RWT was the result of uptake. Uptake length (S_w) was a function of this difference and reach length (L) (equation 2).

$$S_w = \frac{-1(L)}{\ln(P{:}RWT_{ds}) - \ln(P{:}RWT_{inj})} \qquad \text{(Equation 2)}$$

To calculate v_f in order to compare channels of different sizes discharge (Q) was divided by the product of channel width (W) and S_w (Covino *et al.*, 2010) (Stream Solute Workshop, 1990) (equation 3).

$$v_f = \frac{Q}{s_w W} \qquad \text{(Equation 3)}$$

Studies agree that this approach to calculating uptake is generally suitable and provides practical values (Powers *et al.*, 2009; Trentman *et al.*, 2015).

Storage Calculations

We used the field data from the sustained injections in conjunction with One-Dimensional Transport with Inflow and Storage (OTIS and OTIS-P) models to characterize the channel and determine hydrologic parameters of each reach (Runkel, 1998). This model uses the advection dispersion equation (equations 4 and 5), to

determine hydrologic channel characteristics including channel cross-sectional area (A), transient storage cross-sectional area (A_s), dispersion (D), and storage exchange rate (α).

$$\frac{\partial C}{\partial t} = -\frac{Q}{A}\frac{\partial C}{\partial x} + \frac{1}{A}\frac{\partial}{\partial x}\left(AD\frac{\partial C}{\partial x}\right) + \frac{q_{LIN}}{A}(C_L - C) + \alpha(C_S - C) \qquad \text{(Equation 4)}$$

$$\frac{dC_S}{dt} = \alpha\frac{A}{A_S}(C - C_S) \qquad \text{(Equation 5)}$$

A– main channel cross-sectional area
A_s– storage zone cross-sectional area
C– main channel solute concentration
C_L– lateral inflow solute concentration
Cs– storage zone solute concentration
D– dispersion coefficient
Q– volumetric flow rate
q_{LIN}– lateral inflow rate
t– time
x– distance
α– storage zone exchange coefficient

Preliminary fit of the RWT field data to the advection dispersion equation was done using a Mathematica visualization tool. To obtain values for A, A_s, D, and α. The preliminary parameter estimates were then optimized with non-linear regression using OTIS-P computer software (Runkel, 1998). The ratio of A_s/A is the size of the transient storage zone relative to the size of the channel, but the term does not consider the interaction between flow velocity and the exchange rate (i.e., how much water is actually entering the storage zone). Thus, we also calculated a more robust metric of transient storage, F_{med}^{200} (equation 6), the fraction of median travel time spent in storage if the reach were 200 m (Runkel, 2002).

$$F_{med}^{200} = \left(1 - e^{-200\frac{\alpha}{(Q/A)}}\right)\frac{A_S}{(A+A_S)} \qquad \text{(Equation 6)}$$

Damkohler (*DaI*) values were calculated to evaluate if the length (L) of the experimental reach allowed for reasonable parameter estimates to be obtained (Wagner and Harvey, 1997) (equation 7).

$$DaI = \frac{L\alpha\left(\frac{A+A_S}{A_S}\right)}{Q/A} \qquad \text{(Equation 7)}$$

Parameter estimates are the most reliable when *DaI* is on the order of 1.0. Calculated *DaI* values that are much less than one indicate there was not enough interaction between the tracer and the storage zone to obtain reasonable parameter estimates. This could occur when stream velocity is very high, timescales of exchange are too long, and/or the reach length is too short. In contrast, calculated *DaI* values much greater than one indicate that too much (or even all) of the tracer was able to interact with the storage zone during the release (i.e., solute exchange with the storage zone was fast relative to water velocity).

Statistics

A Pearson correlation matrix was constructed using the following variables: F_{med}^{200}, v_f, M_c, M_b, log of sinuosity, canopy, D_{50}, slope, and R:P. This allowed for selection of variables for model building. Log transformations were applied to variables to satisfy the regression assumption of linearity. Stepwise linear regression was used for model building to predict storage (F_{med}^{200}) and uptake (v_f). Models were checked to ensure that added variables increased the adjusted R^2 and decreased Akaike information criterion (AIC). Statistical analyses were performed using SPSS (version 24) and RStudio (version 0.99.902).

RESULTS

There were two overarching patterns in channel characteristics within the study reaches. One pattern was the variation in channel geomorphology that was expected longitudinally due to increased channel size and discharge. Discharge was greater downstream, and in general, downstream reaches had narrower widths, greater depths and velocities, less canopy cover, finer substrates, more gradual slopes, and were more sinuous (Table I). Despite this overall trend, features of SC-4 were very similar to SC-1. Both reaches had steep slopes (>0.90%), large median grain sizes (53 mm), and high canopy cover (60-65%). This is likely because SC-4 was confined by a bluff wall on one side of the channel and by a bed embankment protecting an agricultural field on the other. The meander-preventing obstacles resulted in channel incision, and caused SC-4 to bear resemblance to SC-1. As a consequence of this "geomorphological reset," channel characteristics from SC-1 to SC-3 are comparable to the changes observed from SC-4 to SC-7. Regardless of location along the longitudinal profile, reaches with greater slopes had greater canopy cover, median grain sizes, R:P, and were less sinuous. Macrophyte cover and biomass followed less identifiable patterns. Macrophyte cover was greatest in SC-2 (50%) and lowest (ca. 25%) in SC-1 and SC-7. Macrophyte biomass was greatest in the mid-reaches (SC-3, SC-4, and SC-5) where it ranged from 123 to 147 g/m^2, and lowest in SC-7 and SC-8 (45 and 37 g/m^2 respectively). Aquatic vegetation consisted primarily of *Elodea*, but *Potamogeton* and *Ranunculus* were also present.

Elevation data from GPS measurements, used to calculate slope, were more precise in reaches with less canopy cover (SC-2, SC-5, SC-6, and SC-7). The 95% vertical precision at the upstream and downstream measurement locations (transects) for these four reaches was within 9 or 10 cm. In SC-3, 95% vertical precisions were 12 and 13 cm, and in SC-1 and SC-4 they were the greatest (16 and 29 cm, and 40 and 37 cm respectively).

Hydrological parameters estimates from study reaches in Spring Coulee Creek are presented in Table II. Mean size of the storage zone relative to size of the channel (A_s/A) was 0.15 ± 0.07. One reach, SC-5, stood out with the greatest values for A, A_s, and A_s/A (1.54, 0.39, and 0.25 respectively). These high values are attributed to two very long (>30 m), wide (>5 m), densely-vegetated pools observed in the reach. The OTIS-P model used to obtain parameter estimates fit the observed data well (e.g., Figure 2 a-c), and all seven plots of predicted (modeled) vs. observed (field data) RWT values had $R^2 > 0.99$. Further evidence for reasonable parameter estimates were Damkohler values (*DaI*) on the order of one (2.5 - 7.7), (Wagner and Harvey, 1997), and high ratios of the parameter estimate to the standard deviation of parameter estimate (Table II) (Wagner and Gorelick, 1986).

The median travel time spent in storage if the reach were 200 m (F_{med}^{200}) was calculated from the OTIS-P parameter estimates, and ranged from 1.01 to 6.16 %. This storage metric was positively correlated with both macrophyte biomass (M_b) ($r = 0.794$, $p = 0.033$) (Figure 3) and with uptake velocity (v_f) ($r = 0.756$, $p = 0.049$) (Figure 4a). However, M_b and v_f were not correlated ($r = 0.606$, $p = 0.149$) (Figure 4b). Uptake velocities, calculated from breakthrough curves of RWT and PO_4^{3-} (e.g., Figure 2 d-f), averaged 2.33 ± 1.46 mm/min in Spring Coulee Creek.

Development of regression models for F_{med}^{200} and v_f is shown in Table III. The strongest predictors of F_{med}^{200} were macrophyte biomass, slope, and R:P ($P < 0.001$, Adjusted $R^2 > 0.99$). The strongest predictors of v_f were F_{med}^{200}, canopy, and slope ($P = 0.002$, Adjusted $R^2 = 0.98$). Observed vs. predicted plots for each of these models show a close fit to a 1:1 line (Figure 5).

Table II. OTIS-P modeling output from seven study reaches in Spring Coulee Creek.

Reach	Parameter estimates Ratio[a] (95% CI)				A_s/A	*DaI*
	A (m^2)[b]	A_s (m^2)[c]	D (m^2/s)[d]	α (/s)[e]		
SC-1	1.00 426 (1.00-1.01)	0.07 34 (0.07-0.08)	0.50 59 (0.48-0.51)	1.04×10^{-4} 13 (8.87×10^{-5}-1.20×10^{-4})	0.07	2.5
SC-2	0.63 76 (0.62-0.65)	0.08 9 (0.06-0.09)	0.83 13 (0.70-0.95)	3.02×10^{-4} 4 (1.69×10^{-4}-4.34×10^{-4})	0.12	4.6
SC-3	0.80 241 (0.79-0.80)	0.19 60 (0.18-0.19)	0.78 33 (0.73-0.82)	2.94×10^{-4} 24 (2.70×10^{-4}-3.19×10^{-4})	0.23	2.5
SC-4	0.71 129 (0.70-0.72)	0.10 19 (0.09-0.11)	0.83 17 (0.74-0.93)	2.40×10^{-4} 8 (1.83×10^{-4}-2.98×10^{-4})	0.14	2.8
SC-5	1.54 110 (1.51-1.56)	0.39 31 (0.36-0.41)	0.87 19 (0.78-0.96)	2.74×10^{-4} 12 (2.29×10^{-4}-3.19×10^{-4})	0.25	2.5
SC-6	0.89 100 (0.87-0.91)	0.09 10 (0.07-0.11)	0.65 13 (0.55-0.75)	4.82×10^{-4} 5 (3.04×10^{-4}-6.60×10^{-4})	0.10	7.7
SC-7	0.97 193 (0.96-0.98)	0.09 21 (0.08-0.10)	0.73 24 (0.67-0.79)	2.69×10^{-4} 8 (2.05×10^{-4}-3.33×10^{-4})	0.09	4.2

[a]Ratio = parameter estimate ÷ standard deviation
[b]Main channel cross-sectional area
[c]Storage zone cross-sectional area
[d]Dispersion coefficient
[e]Storage zone exchange coefficient

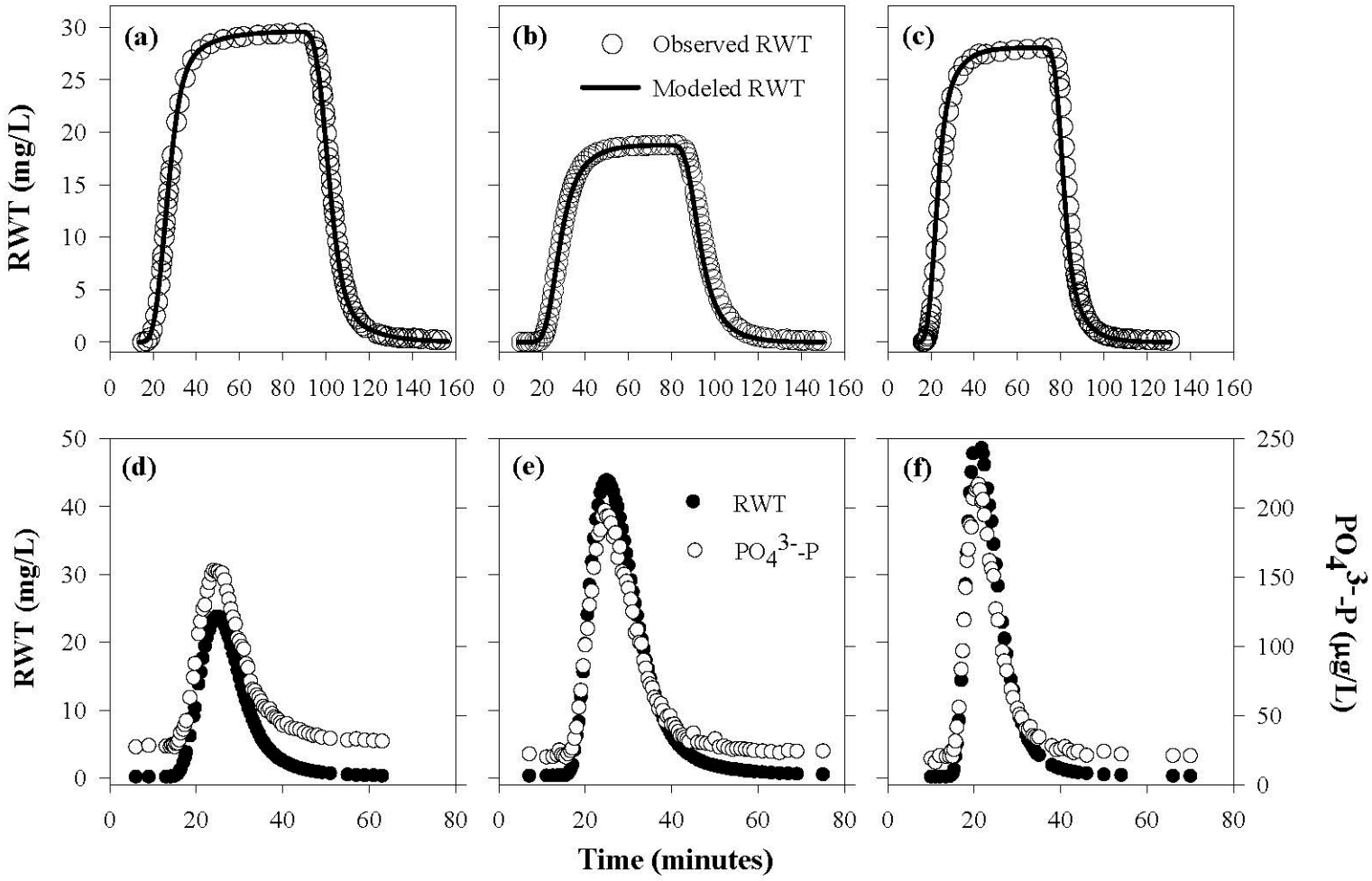

Figure 2. Representative breakthrough curves from sustained (a-c) and instantaneous (d-f) tracer releases performed in SC-1 (a & d), SC-2 (b & e), and SC-7 (c & f).

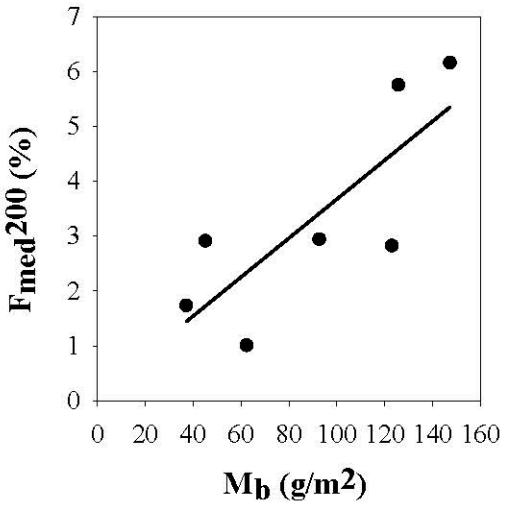

Figure 3. F_{med}^{200} as a function of M_b for seven reaches of Spring Coulee Creek (r=0.794, p=0.033).

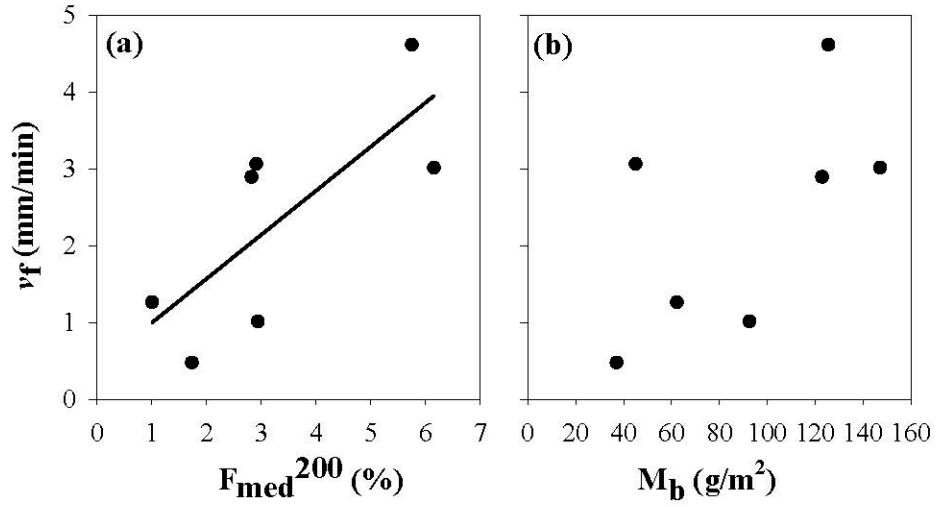

Figure 4. v_f as a function of F_{med}^{200} (a, r=0.756, p=0.049) and M_b (b, r=0.606, p=0.149)

Table III. Regression models predicting F_{med}^{200} and v_f in Spring Coulee Creek.

Dependent variable	Predictor variables in model[a]	Model	Model P value	R^2	Adjusted R^2	AIC
F_{med}^{200}	M_b*	$F_{med}^{200} = 0.036\ (M_b) + 0.121$	0.033	0.631	0.557	27.03
	M_b*** Slope**	$F_{med}^{200} = 0.052\ (M_b) - 487.50\ (slope) + 1.924$	<0.001	0.973	0.960	10.57
	M_b*** Slope*** R:P**	$F_{med}^{200} = 0.061\ (M_b) - 705.10\ (slope) + 2.493\ (R:P) + 1.850$	<0.001	0.998	0.996	-5.59
v_f	F_{med}^{200}*	$v_f = 0.572\ (F_{med}^{200}) + 0.425$	0.049	0.571	0.486	24.18
	F_{med}^{200}** Canopy*	$v_f = 0.770\ (F_{med}^{200}) + 0.039\ (canopy) - 1.533$	0.008	0.911	0.867	15.15
	F_{med}^{200}*** Canopy** Slope*	$v_f = 0.786\ (F_{med}^{200}) + 0.045\ (canopy) - 158.90\ (slope) - 0.732$	0.002	0.990	0.980	1.90

[a]Significance values of predictor variables in model: $P < 0.0001$***, $P < 0.001$**, $P < 0.01$*

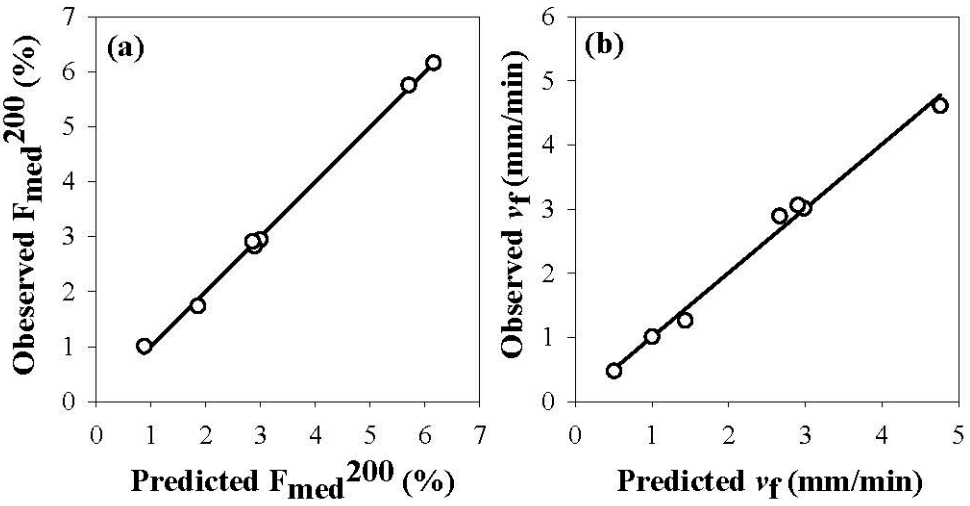

Figure 5. Predicted and observed F_{med}^{200} (a, $y = 0.999x + 0.002$, $R^2 = 0.998$) and v_f (b, $y = 1.001x + 0.005$, $R^2 = 0.990$).

DISCUSSION

Understanding the dynamics of phosphorus retention and cycling in streams are important to the development of effective methods aimed at water quality improvement. By learning which variables are key players in phosphorus removal from streams we can focus restoration efforts around them. Because of the potential to facilitate phosphorus uptake, we were interested in which factors affected transient storage. Our results suppoorted the hypothesis that macrophyte biomass was positively correlated with transient storage. Of all the variables tested, M_b was the most important variable affecting transient storage; it accounted for 63% of the variation in F_{med}^{200}. An additional 34% of the variation in the model was explained by slope, which was negatively correlated with transient storage. Greater slopes increase advective velocity which would decrease residence time of water and solutes. Stofleth *et al.* (2008) also demonstrated the inverse relationship between velocity and F_{med}^{200} in sand bed streams. The third predictor variable, R:P, was positively correlated with storage. The inclusion of R:P in the model only explained an additional 2.5% of the variation in F_{med}^{200}. This storage was likely caused by eddies in the shallow, turbulent, riffle habitats.

Based on the variables in the final predictive storage model, our results suggest that the primary mechanism of transient storage in Spring Coulee Creek was surface storage. Others have likewise concluded that sand bed streams have very little hyporheic storage (Harvey and Wagner, 2000; Jin and Ward, 2005; Stofleth *et al.*, 2008). Values of A_s/A in Spring Coulee Creek (0.15 ± 0.07) were similar to what Powers *et al.* (2009)

found in other Wisconsin streams, and were within range of the mean for sand bed streams (0.36 ± 0.22, as reviewed by Stofleth *et al.* 2008) . We observed low F_{med}^{200} values (1.01 to 6.16%), compared to Jin and Ward's (2005) observation in their sand bed stream (9.5 to 34.5%). Interestingly, values of F_{med}^{200} in Spring Coulee Creek are more akin to what is found in bedrock streams, like Walker Branch, Tennessee (Mulholland *et al.*, 1997; Hendricks, 1999) - this further indicates the negligible effect of the hyporheic zone. In fact, the domination of surface storage in Spring Coulee Creek was likely key to the positive correlation between transient storage and phosphorus uptake velocity. This would be consistent with the findings of others who have shown the greater influence of surface storage (over hyporheic storage) on uptake (Gücker and Boëchat, 2004; Ensign and Doyle, 2005; O'Connor *et al.*, 2010).

Phosphorus uptake metrics in Spring Coulee Creek were similar to those observed in other streams (Valett *et al.*, 2002; Niyogi *et al.*, 2004; Powers *et al.*, 2009). Although uptake can be affected by ambient nutrient concentration (Reddy *et al.*, 1999; Mulholland *et al.*, 2008) there was no relationship between ambient PO_4^{3-} and uptake. In addition, there was no relationship between the peak PO_4^{3-} concentration above ambient and uptake metrics, suggesting that our short-term additions did not stimulate uptake. While our hypothesis that transient storage is the main driver of phosphorus uptake was supported, other variables also helped explain variation in v_f. The addition of canopy to the model for v_f explained about 34% more of the variation. Another 8% was accounted for by slope. These additional variables demonstrate that the channel structures and materials that develop storage zones, as well as environmental factors, can be significant influences on nutrient uptake (Argerich *et al.*, 2011; Drummond *et al.*, 2016). Canopy

and slope can affect a wide array of features including substrate size, flow velocity, light availability, temperature, and organic material inputs, which in turn can have an effect on phosphorus uptake. Perhaps these factors altered algal biomass, or the nutrient composition of algal biofilms, both of which have been correlated with phosphorus uptake velocity. (Niyogi *et al.*, 2004; McMillan *et al.*, 2014; Price and Carrick, 2014; Newcomer Johnson *et al.*, 2016).

In Spring Coulee Creek, the main driver of uptake velocity was transient storage, and the main driver of transient storage was M_b. Despite the capability of macrophyte stems and leaves to take up large quantities of SRP from the water column following periods of availability (Baldy *et al.*, 2015), there was no correlation between M_b and v_f. The lack of significant relationship between these variables could be a result of: (1) differing timescales with which storage and uptake were measured and (2) ambiguity of the metric M_b. In regards to the first artifact, transient storage in each reach was determined using a 1 to 1.5 hour-long injection while uptake was measured with an instantaneous release. Based on these timescales, its reasonable to assume that some of the storage quantified by the sustained release had little to no effect on phosphorus uptake measured by the pulse release. In addition, we know that longer releases increase the measure of A_s. An unpublished study in SC-2 demonstrated that a longer injection period (six hours vs one hour) increased A_s/A by 16.7% (0.12 to 0.14) and decreased alpha (α) by 25.5% (3.02×10^{-4} to 2.25×10^{-4}). The longer release allowed RWT to travel deeper into long-term storage (presumably deeper into the macrophyte beds), and resulted in a greater measure of A_s. However, this additional storage area likely isn't important for explaining our rates of phosphorus uptake because of the lower exchange rate (α). During

our pulse releases, phosphorus was not in the channel long enough to enter these storage areas. Instead, most of the uptake that was measured occurred in storage zones where the exchange rate was high, e.g., the peripheral region of the macrophyte beds.

A variety of factors can affect the movement of water in, and around, macrophyte beds. Another reason why we see no relationship between M_b and v_f may have been because our two-dimensional measure of M_b provided no insight into these factors, including shape and density. Sand-Jensen (1998) demonstrated that flow velocity within dense macrophyte beds can be zero. These very dense beds provide long term storage, have very small exchange rates, and are not as important for uptake. However, two vegetated areas with similar biomass or cover can have differing effects on water residence time and flow dynamics depending on their contiuity across a channel (Cotton *et al.*, 2006). Residence time and potential for uptake would decrease if flow paths through or around a macrophyte bed were present. Conversely, a contiguous macrophyte bed would increase residence time and potential for uptake by slowing the velocity of the active channel. In addition to density, macrophyte shape is important as well. On a small scale, Levi *et al.* (2015) showed that macrophyte species with a higher perimeter-to-area ratio of their leaves had greater rates of ammonium uptake. On a larger scale, shape of a macrophyte bed dictates the amount of contact individual plants have with phosphorus in the water column. While effects of macrophyte beds on stream nutrient dynamics are complex and often difficult to understand (Clarke, 2002), additional information such as macrophyte density within beds, and surface area to volume ratio of beds, may result in better understanding of the relationship between macrophytes and phosphorus uptake velocity.

Conclusions

In Spring Coulee Creek, M_b, slope, and R:P accounted for 99.6% of the variation in F_{med}^{200}, while F_{med}^{200}, canopy, and slope accounted for 98.0% of the variation in v_f. Our results indicate the importance of macrophyte-driven transient storage to phosphorus uptake. We can expect features of macrophyte beds which create greater opportunity for phosphorus to interact with reactive substrates would promote uptake. These characteristics include increased surface area and higher exchange rates between storage zones and the main channel. While storage created by macrophytes is important for phosphorus retention, we cannot draw conclusions regarding relative importance of individual components within the macrophyte-driven storage. For example, we do not know the phosphorus uptake efficiency of the macrophytes themselves, their epiphytic biofilms, or the sediment within beds. These results call for further investigation into the effects of macrophyte beds on phosphorus uptake in this stream and for better metrics to describe aquatic vegetation in future studies.

REFERENCES

Allan J. D. (2004). Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 35, 257-284.

Argerich A., Martí E., Sabater F., Haggerty R., & Ribot M. (2011). Influence of transient storage on stream nutrient uptake based on substrata manipulation. *Aquatic Sciences*, 73, 365-376.

Baldy V., Thiebaut G., Fernandez C., Sagova-Mareckova M., Korboulewsky N., Monnier Y., . . . Tremolieres M. (2015). Experimental assessment of the water quality influence on the phosphorus uptake of an invasive aquatic plant: biological responses throughout its phenological stage. *PLoS ONE*, 10, e0118844.

Bowden W. B., Glime J. M., & Riis T. (2006). Macrophytes and bryophytes. In F. R. Hauer, & G. A. Lamberti (Eds.), *Methods in stream ecology* (pp. 381-414). Boston, Massachusetts: Elsevier.

Butturini A., & Sabater F. (1999). Importance of transient storage zones for ammonium and phosphate retention in a sandy-bottom Mediterranean stream. *Freshwater Biology*, 41, 593-603.

Carpenter S. R., Caraco N. F., Correll D. L., Howarth R. W., Sharpley A. N., & Smith V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications*, 8, 559-568.

Clarke S. J. (2002). Vegetation growth in rivers: influences upon sediment and nutrient dynamics. *Progress in Physical Geography*, 26, 159-172.

Cotton J. A., Wharton G., Bass J. A. B., Heppell C. M., & Wotton R. S. (2006). The effects of seasonal changes to in-stream vegetation cover on patterns of flow and accumulation of sediment. *Geomorphology*, 77, 320-334.

Covino T. P., McGlynn B. L., & McNamara R. A. (2010). Tracer Additions for Spiraling Curve Characterization (TASCC): Quantifying stream nutrient uptake kinetics from ambient to saturation. *Limnology and Oceanography: Methods*, 8, 484-498.

Drummond J. D., Bernal S., Schiller D. v., & Martí E. (2016). Linking in-stream nutrient uptake to hydrologic retention in two headwater streams. *Freshwater Science*, 35, 1176-1188.

Dybas C. L. (2005). Dead zones spreading in world oceans. *BioScience*, 55, 552-557.

Ensign S. H., & Doyle M. W. (2005). In-channel transient storage and associated nutrient retention: Evidence from experimental manipulations. *Limnology and Oceanography*, 50, 1740-1751.

Ensign S. H., & Doyle M. W. (2006). Nutrient spiraling in streams and river networks. *Journal of Geophysical Research*, 111, G04009.

Gücker B., & Boëchat I. G. (2004). Stream morphology controls ammonium retention in tropical headwaters. *Ecology*, 85, 2818-2827.

Gurnell A. M., van Oosterhout M. P., de Vlieger B., & Goodson J. M. (2006). Reach-scale interactions between aquatic plants and physical habitat: River Frome, Dorset. *River Research and Applications*, 22, 667-680.

Hall R. O. J., Bernhardt E. S., & Likens G. E. (2002). Relating nutrient uptake with transient storage in forested mountain streams. *Limnology and Oceanography*, 47, 255-265.

Hart B. T., Freeman P., & McKelvie I. D. (1992). Whole-stream phosphorus release studies: variation in uptake length with initial phosphorus concentration. *Hydrobiologia*, 235, 573-584.

Harvey J. W., & Wagner B. J. (2000). Quantifying hydrologic interactions between streams and their subsurface hyporheic zones. In P. Jones, & P. J. Mulholland (Eds.), *Streams and ground waters* (pp. 3-43). San Diego, CA: Academic Press.

Hein, M, Pedersen, M F., Sand J., & K. (1995). Size-dependent nitrogen uptake in micro- and macroalgae. *Marine Ecology Progress Series*, 118, 247-253.

Hendricks S. (1999). Relationships between hydraulic parameters in a small stream under varying flow and seasonal conditions. *Hydrological Processes*, 13, 1497-1510.

Homer C., Dewitz J., Yang L. M., Jin S., Danielson P., Xian G., . . . Megown K. (2015). Completion of the 2011 national land cover database for the conterminous United States - representing a decade of land cover change information. *Photogrammetric Engineering and Remote Sensing*, 81, 345-354.

Jin H.-S., & Ward G. M. (2005). Hydraulic characteristics of a small Coastal Plain stream of the southeastern United States: effects of hydrology and season. *Hydrological Processes*, 19, 4147-4160.

Jowett I. G. (1993). A method for identifying pool, run, and riffle habitats from physical measurements. *New Zealand Journal of Marine and Freshwater Research*, 27, 241-248.

Juckem P. F., Hunt R. J., Anderson M. P., & Robertson D. M. (2008). Effects of climate and land management change on streamflow in the driftless area of Wisconsin. *Journal of Hydrology*, 355, 123-130.

Levi P. S., Riis T., Alnøe A. B., Peipoch M., Maetzke K., Bruus C., & Baattrup-Pedersen A. (2015). Macrophyte Complexity Controls Nutrient Uptake in Lowland Streams. *Ecosystems*, 18, 914-931.

Martí E., Grimm N. B., & Fisher S. G. (1997). Pre- and post-flood retention efficiency of nitrogen in a sonoran desert stream. *Journal of the North American Benthological Society*, 16, 805-819.

McMillan S. K., Tuttle A. K., Jennings G. D., & Gardner A. (2014). Influence of restoration age and riparian vegetation on reach-scale nutrient retention in restored urban streams. *Journal of the American Water Resources Association*, 50, 626-638.

Meyer J. L., Kaplan L. A., Newbold D., Strayer D., Woltemade C. J., Zedler J. B., . . . Zedler P. H. (2003). Where rivers are born: The scientific imperative for defending small streams and wetlands, 23 pp., Sierra Club and American Rivers.

Mooney R. J., Strauss E. A., & Haro R. J. (2014). Nutrient recycling by caddisflies alleviates phosphorus limitation in case periphyton. *Freshwater Science*, 33, 1086-1092.

Mulholland P. J., Helton A. M., Poole G. C., Hall R. O., Hamilton S. K., Peterson B. J., . . . Thomas S. M. (2008). Stream denitrification across biomes and its response to anthropogenic nitrate loading. *Nature*, 452, 202-205.

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

Newbold J. D., Elwood J. W., Neill R. V. O., & Winkle W. V. (1981). Measuring nutrient spiraling in streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 38, 860-863.

Newcomer Johnson T., Kaushal S., Mayer P., Smith R., & Sivirichi G. (2016). Nutrient retention in restored streams and rivers: A global review and synthesis. *Water*, 8, 116.

Niyogi D. K., Simon K. S., & Townsend C. R. (2004). Land use and stream ecosystem functioning: nutrient uptake in streams that contrast in agricultural development. *Archiv fur Hydrobiologie*, 160, 471-486.

O'Connor B. L., Hondzo M., & Harvey J. (2010). Predictive modeling of transient storage and nutrient uptake: Implications for stream restoration. *Journal of Hydraulic Engineering*, 136, 1018-1032.

Olmanson R. M. (2014). Effects of land use on water quality of cold-water streams in the driftless area of the midwestern U. S., University of Wisconsin - La Crosse.

Orr C. H., Clark J. J., Wilcock P. R., Finlay J. C., & Doyle M. W. (2009). Comparison of morphological and biological control of exchange with transient storage zones in a field-scale flume. *Journal of Geophysical Research*, 114, G02019.

Pelton D. K., Levine S. N., & Braner M. (1998). Measurements of phosphorus uptake by macrophytes and epiphytes from the LaPlatte River (VT) using 32P in stream microcosms. *Freshwater Biology*, 39, 285-299.

Peterson B. J., Wollheim W. M., Mulholland P. J., Webster J. R., Meyer J. L., Tank J. L., . . . Morrall D. D. (2001). Control of nitrogen export from watersheds by headwater streams. *Science*, 292, 86-90.

Powers S. M., Stanley E. H., & Lottig N. R. (2009). Quantifying phosphorus uptake using pulse and steady-state approaches in streams. *Limnology and Oceanography: Methods*, 7, 498-508.

Price K. J., & Carrick H. J. (2014). Quantitative evaluation of spatiotemporal phosphorus fluxes in stream biofilms. *Freshwater Science*, 33, 99-111.

Rabalais N. N., Turner R. E., & Wiseman W. J. (2002). Gulf of Mexico hypoxia, A.K.A. "the dead zone". *Annual Review of Ecology and Systematics*, 33, 235-263.

Reddy K. R., Kadlec R. H., Flaig E., & Gale P. M. (1999). Phosphorus retention in streams and wetlands: A review. *Critical Reviews in Environmental Science and Technology*, 29, 83-146.

Runkel R. L. (1998). One-dimensional transport with inflow and storage (OTIS): A solute transport model for streams and rivers. USGS Water-Resources Investigations Report 98 - 4018. Denver, Colorado, USA.

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

Runkel R. L. (2015). On the use of rhodamine WT for the characterization of stream hydrodynamics and transient storage. *Water Resources Research*, 51, 6125-6142.

Sand-Jensen K. (1998). Influence of submerged macrophytes on sediment composition and near-bed flow in lowland streams. *Freshwater Biology*, 39, 663-679.

Schindler D. W., Carpenter S. R., Chapra S. C., Hecky R. E., & Orihel D. M. (2016). Reducing phosphorus to curb lake eutrophication is a success. *Environmental Science & Technology*, 50, 8923-8929.

Schindler D. W., Hecky R. E., Findlay D. L., Stainton M. P., Parker B. R., Paterson M. J., . . . Kasian S. E. (2008). Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proc Natl Acad Sci U S A*, 105, 11254-11258.

Schulz M., Kozerski H.-P., Pluntke T., & Rinke K. (2003). The influence of macrophytes on sedimentation and nutrient retention in the lower River Spree (Germany). *Water Research*, 37, 569-578.

Soil Survey Staff. The gridded soil survey geographic (gSSURGO) database for Wisconsin. United States Department of Agriculture, Natural Resources Conservation Service. Available online at https://gdg.sc.egov.usda.gov/. Accessed April 20, 2016.

Stofleth J. M., Shields Jr F. D., & Fox G. A. (2008). Hyporheic and total transient storage in small, sand-bed streams. *Hydrological Processes*, 22, 1885-1894.

Stream Solute Workshop. (1990). Concepts and methods for assessing solute dynamics in stream ecosystems. *Journal of the North American Benthological Society*, 9, 95-119.

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

Thomas S. A., Maurice Valett H., Webster J. R., & Mulholland P. J. (2003). A regression approach to estimating reactive solute uptake in advective and transient storage zones of stream ecosystems. *Advances in Water Resources*, 26, 965-976.

Trentman M. T., Dodds W. K., Fencl J. S., Gerber K., Guarneri J., Hitchman S. M., . . . Rüegg J. (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.

Triska F. J., Kennedy V. C., Avanzino R. J., Zellweger G. W., & Bencala K. E. (1989). Retention and transport of nutrients in a third-order stream in northwestern California: hyporheic processes. *Ecology*, 70, 1893-1905.

Valett H. M., Crenshaw C. L., & Wagner P. F. (2002). Stream nutrient uptake, forest succession, and biogeochemical theory. *Ecology*, 83, 2888-2901.

Valett H. M., Morrice J. A., Dahm C. N., & Campana M. E. (1996). Parent lithology, surface-groundwater exchange, and nitrate retention in headwater streams. *Limnology and Oceanography*, 41, 333-345.

Wagner B. J., & Gorelick S. M. (1986). A statistical methodology for estimating transport parameters: theory and applications to one-dimensional advective-dispersive systems. *Water Resources Research*, 22, 1303-1315.

Wagner B. J., & Harvey J. W. (1997). Experimental design for estimating parameters of rate-limited mass transfer: Analysis of stream tracer studies. *Water Resources Research*, 33, 1731-1741.

Webster J. R. (1975). Analysis of potassium and calcium dynamics in stream ecosystems on three Southern Appalachian watersheds of contrasting vegetation. University of Georgia.

Webster J. R., Mulholland P. J., Tank J. L., Valett H. M., Dodds W. K., Peterson B. J., . . . Wollheim W. M. (2003). Factors affecting ammonium uptake in streams – an inter-biome perspective. *Freshwater Biology*, 48, 1329-1352.

Wilson J., J.F., Cobb E. D., & Kilpatrick F. A. (1986). Fluorometric procedures for dye tracing. In *Techniques of Water-Resources Investigations of the United States Geological Survey.* Book 3: Applications of Hydraulics.