FACTORS AFFECTING PHOSPHORUS UPTAKE IN KARSTIC RIVERS OF THE EDWARDS PLATEAU, CENTRAL TEXAS

by

Aaron Swink, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
May 2014

Committee Members:

Weston Nowlin, Co-chair

Benjamin Schwartz, Co-chair

Alan Groeger

FAIR USE AND AUTHOR'S PERMISSION STATEMENT

Fair Use

Duplication Permission

DEDICATION

I dedicate this thesis to my mother, Valerie Swink, who first instilled in me a love of science.

ACKNOWLEDGEMENTS

To say that this thesis would not have been possible without the support, inspiration, guidance, and friendship of my advisors, Weston Nowlin and Benjamin Schwartz, would be an understatement. I am eternally grateful for their patience and good since of humor throughout this project. Also thanks to Dr. Alan Groeger for serving on my committee and keeping me grounded when I was having delusions of biogeochemical grandeur. I am also so thankful for the immense help in the field and lab by our lab technician Gabrielle "Lab Angel" Timmins, my labmates Lauren Loney, Amelia Everett, Kelly Rodibaugh, Jesse Becker, and fellow grad students Dave Ruppel, Ben Hutchins and Ben Tobin. Lastly, to Kara Rhodes, for being such a kind and patient companion on this journey. Thanks to everyone here, and to all my family and friends in San Marcos for making these last two years so amazing.

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Phosphorus (P) is a limiting nutrient for microbial primary producers in many aquatic systems and an overabundance of it via urban and agricultural runoff has led to eutrophication of waterways across the globe. There are a variety of ways to study nutrient dynamics, but nutrient spiraling theory is often used as a measure of efficiency and limitation in lotic ecosystems. However, there is a relative dearth of knowledge on nutrient uptake in larger rivers (> 200 L s^{-1}) due to methodological constraints. Recent improvements in methodology (pulse-tracer addition, Tank et al. 2008) have allowed for measurements of nutrient uptake to be made in larger systems. Furthermore, the relative contribution of various biotic and abiotic factors that affect nutrient uptake is less studied in lotic systems than in other aquatic ecosystems (i.e lakes and wetlands). Therefore, the purpose of this study was to quantify P uptake and examine the factors that influence P uptake in relatively larger discharge riverine ecosystems, specifically, in karstic, spring-fed rivers of the Edwards Plateau, in central Texas. I utilized a pulsed tracer addition method to measure P uptake in 6 rivers and one large creek of varying discharges, channel morphology, and biological assemblages. These uptake estimates were calculated according to the traditional nutrient spiraling metrics as well a method put forward here to express the maximal instantaneous capacity of these systems for P uptake. The abundance and chemical makeup of benthic biofilms present in these systems were also studied to determine the degree of influence these biofilms have on nutrient uptake.

I found that levels of chlorophyll *a* and particulate P in benthic biofilms were significant predictors of uptake rates. In general, there was a high degree of correlation between benthic chlorophyll-*a*, benthic particulate P, water column SRP and dissolved mineral load indicating that biological and physicochemical factors are highly interrelated and may work in concert to affect P cycling in these systems. My results indicate that P uptake rates for the rivers in this study are rapid when compared to similarly sized non-karst rivers due to (1) the low availability of dissolved phosphate in the river, (2) abundance of algae-dominated biofilms, and (3) interaction with dissolved minerals (especially Ca^{2+}), resulting in precipitation of insoluble mineral forms of P onto benthic surfaces. We have also shown that pulsed tracer additions can be a simple and effective tool for studying nutrient dynamics in streams and rivers.

.

I. FACTORS AFFECTING PHOSPHORUS UPTAKE IN KARSTIC RIVERS OF THE EDWARDS PLATEAU, CENTRAL TEXAS

Introduction

Nutrient pollution is a major problem affecting waterways across the globe (Camargo and Alonso 2006; Elser et al. 2007) , leading to substantial economic costs (Tilman et al. 2001) and loss of biodiversity (Paerl and Pinckney 1998). Human-impacted landscapes dominated by agricultural or urban landuse often generates runoff are high in dissolved inorganic nitrogen (N) and phosphorus (P), causing increased growth of osmotrophic algae and bacteria, leading to deterioration of water quality. At the community- and ecosystem-level, the processes that control inorganic nutrient uptake and retention are complex and varied, thus not all ecosystems respond in the same fashion to nutrient loading (Smith et al. 1999). However, understanding the factors that influence nutrient uptake and retention is important to predicting which systems may be more sensitive to the potential consequences of external nutrient loading. In freshwater lotic ecosystems, nutrient uptake and retention is related to the composition and biomass of biological communities and the physicochemical properties of the streams in question (Webster et al. 2003). However, biological nutrient uptake and physicochemical conditions are often interrelated, potentially yielding complex and interactive mechanisms for nutrient uptake.

One major focus of many studies on stream nutrient cycling has been the determination of the direct and indirect effects of hydrology on nutrient uptake (Ensign and Doyle, 2006). Stream hydrologic characteristics such as discharge, channel

morphology, transient storage zones, and seasonal flow regimes play large roles in nutrient cycling by controlling the transport rates of dissolved nutrients and the degree interaction with benthic substrates and hyporheic zones (Stream Solute Workshop 1990). Despite attempts to account for hydrologic variability within and between streams, hydrologic factors such as discharge, transient storage, and lateral dispersion have been shown to not be independent from nutrient uptake metrics (Runkel 2007; Tank et al. 2008). Interrelated with these indirect factors, the processes that directly affect nutrient uptake are chemical and biological in nature. Abiotic, physicochemical factors such as dissolved mineral load (especially Ca^{2+}) and N and P stoichiometry have also been shown to be a major part of P cycling in other aquatic systems (Dodds 2003; Noe et al. 2003; Scinto and Reddy 2003) but the relative contribution of these abiotic factors is less studied than hydrologic and biologic factors. Thus, understanding how biotic, physicochemical, and hydrologic characteristics interact to affect nutrient uptake rates is critical for developing an understanding of the fates and consequences of nutrients in lotic systems.

Previous investigators have examined the relationship between nutrient uptake and physicochemical, hydrological, and biological characteristics of stream ecosystems; however, the bulk of these studies have taken place in relatively small, temperate zone streams within forested catchments or in highly degraded streams that suffer from agricultural pollution (Payn et al. 2005). Indeed, the overwhelming majority of research examining nutrient uptake in streams has been on relatively small discharge systems (e.g. 90% of N uptake studies in <200 L s^{-1}, Tank et al. 2008). In addition, multiple studies have assessed the factors that influence the uptake and retention of N in lotic ecosystems

(Martí et al. 1997; Webster et al. 2003; Tank et al. 2008; Dodds et al. 2008), but studies that have focused on the factors that influence the uptake and retention P in lotic systems are much less common (Ensign and Doyle 2006). Given that P-limitation in autotrophs is widespread in streams (Elser et al. 2007) and the fact that humans have greatly increased the amount of P cycling in the biosphere (Tilman et al. 2001), there is clearly a need to understand which factors contribute to P uptake and retention across lotic ecosystems so that decisions regarding P control in rivers and streams can be better informed.

The purpose of the study presented here is to quantify P uptake and examine the factors that influence P uptake in relatively larger discharge riverine ecosystems (>200 L s^{-1}); specifically in karstic spring-fed rivers of the Edwards Plateau in central Texas. These rivers present a unique context in which to study P uptake because they emanate from springs discharging from the Edwards and Edwards-Trinity limestone aquifers and are similar in hydrochemisty (e.g., high dissolved solids, especially Ca^{2+}), high water clarity, and relatively stable base flows. In addition, all of these systems tend to be nutrient poor (especially in regards to P) and algal growth has been shown to be strongly P-limited (Stanley et al. 1989; Groeger et al. 1997). In this study, I utilized a pulse addition nutrient uptake method (Tank et al. 2008) and coupled these P uptake estimates of with measurements of a diversity of in-stream and reach-level variables which are likely to exhibit influences on P uptake and retention in lotic ecosystems. I hypothesized that demand for P would be high as compared to other systems, as they are hydrologically stable, clear-water systems that support abundant populations of macrophytes, periphyton and epiphytes and have low available dissolved P in the water column. I predicted that factors such as benthic biofilm density and abundance would be a strong predictor of P

uptake rates among riverine systems. Additionally, I hypotheised that the unique hydrochemistry of these karstic systems would contribute to the uptake of P through abiotic precipitation of insoluble mineral forms of P.

Methods

Study sites – The study was conducted in six rivers and one relatively large discharge creek located throughout central Texas (Table 1). All sites are located within or along the margin of the Edwards Plateau, a region with a semi-arid subtropical climate. All sites are perennially-flowing and are spring-fed from the same regional aquifer pool; thus, there is relatively little variation among the upper reaches of these streams in water chemistry. The Edwards-Trinity Aquifers are karst systems and water emanating from springs generally has high water clarity and total dissolved solids (TDS), primarily composed of Ca^{2+}, Mg^{2+}, and bicarbonate (HCO_3^-). Study reaches in each river were selected so that they encompassed a range of conditions (e.g., discharge, channel morphology, and benthic substrate types) across rivers to facilitate the examination of roles of within-reach and within-stream factors which can affect P uptake while keeping the general geographic location and water chemistry relatively consistent. The Devils River, Dolan Creek, the Frio River, the Guadalupe River, the San Marcos River and the South Llano River each had one P uptake experiment conducted during the study period, and I conducted two P uptake experiments in the Nueces River on separate dates (June and December 2013) at two different study reaches.

The study was conducted from December of 2012 - December 2013. At each site, we conducted a pulsed-addition phosphorus uptake experiment using a modified method presented in Tank et al. (2008). Briefly, a slurry of ~100 L of river water containing

added dissolved inorganic phosphorus (as 100-1000 g KH_2PO_4 or NaH_2PO_4) and a non-biologically reactive tracer (4-11 kg NaCl) was released rapidly into the thalweg of each site and sampled at different points downstream as the solute pulse traveled downstream. The amount of phosphate and NaCl used at each site was dependent on the discharge at the site because the goal of each addition was to elevate the conservative tracer concentration above baseline concentrations to be detectable by conductivity probes at the furthest downstream station at each site.

Prior to the start of an addition experiment, digital conductivity probes (YSI Model 85 or a Hannah Multi-parameter water quality meter) were placed at sampling stations downstream from the addition point in order to track the progress of the pulse plume as it moved downstream. At each site, crews were stationed in the thalweg at three sites downstream from the pulse addition point. At the arrival of the pulse plume at a sampling station, grab samples were taken from the midpoint in the water column with acid-washed high density polyethylene (HDPE) bottles at regular intervals (every 30 sec to 2 min) until conductivity readings returned to roughly background (pre-pulse) levels. Samples were immediately put on ice in coolers and transported to the lab for analysis. Phosphorus samples were filtered with pre-ashed Pall A/E filters within 24 hours of collection and acid preserved with H_2SO_4 and stored at 4^{o}C until analysis. Phosphate concentration in samples was measured as soluble reactive phosphorus (SRP) with the molybdenum blue method (Wetzel and Likens 2000). Samples for the conservative tracer (NaCl) were filtered through pre-ashed Pall A/E filters within 24 hours of collection and stored in 60 mL HDPE bottles at 4^{o}C until analysis on a Dionex ICS 1600 ion chromatograph.

Quantification of P uptake - Traditionally, quantification of nutrient demand in lotic systems has been performed in the context of nutrient spiraling theory (Webster 1975; Newbold et al. 1981), which focuses on the downstream movement and transformation of inorganic nutrients from a dissolved state, to biological or abiotic removal from the water column and subsequent release back into the water column. The principal metrics for quantifying the rate of nutrient uptake have been uptake length (the average distance a nutrient molecule travels in its dissolved form before uptake; S_w) and uptake velocity (the mass transfer coefficient for the benthic compartment, expressed as units of length time^{-1}; v_f) (Stream Solute Workshop 1990). Cross-system comparisons of nutrient uptake have widely used v_f ($v_f = (Q/w)/S_w$) because it is assumed that the effect of discharge (Q, normalized for width, w) is removed and allows for comparison of uptake across streams (Wollheim et al. 2006; Ensign and Doyle 2006). However, use of v_f cannot account of for other hydrological factors such as lateral dispersion, advection, and transient storage, which are increasingly important as channel complexity and discharge increase (Runkel 2007). Thus, because my study focuses on relatively large discharge systems in which hydrological processes like dispersion, advection, and transient storage are likely to play an important role, I have additionally attempted to express P uptake in terms of mass of nutrient removed per distance (moles P removed m^{-1}) to supplement calculations of v_f.

The pulse-addition method utilized by this study allows for the integration of more complex hydrological processes, such as advection and dispersion and assumes that the concentration of both the nutrient (i.e., PO_4^{3-}) and the conservative tracer (i.e., NaCl) are both subject to same dilution due to hydrologic effects of advection and dispersion.

However, the nutrient is additionally undergoing uptake and sequestration into the benthos through biologic and abiotic processes. Thus, if there were no uptake, the ratio of the concentration of the nutrient in question to the concentration of the conservative tracer should remain unchanged from the initial point of addition to the most downstream sampling point. However, if there is active uptake and sequestration of the nutrient in question to the benthos, then the ratio of the nutrient in question to the conservative tracer should decline as it moves downstream from the point of addition (Tank et al. 2008). I utilized the five samples in the middle of each curve for each downstream point because 1st order uptake rates are assumed to be maximal when P concentrations are also highest (Stream Solute Workshop 1990; Payn et al. 2005) and the water samples collected along the tails of each time series curve tended to have P:Cl ratios that would overestimate P uptake because the loss of even a small amount of P resulted in a larger proportional change in concentration. S_w is calculated by taking the natural log of the ratio of the added nutrient to the conservative tracer at each station and plotting these ratios over distance. S_w is the inverse of the absolute value of the slope of a regression through these points.

I also calculated the amount of P uptake (in moles) per meter of distance over each study reach by first determining the amount of PO_4^{3-} that would be expected in each sample at each sampling point in the reach (background corrected), assuming that there was no uptake and retention using

$$P_E = (P{:}\,Cl_I)(Cl_O)$$

where, P_E is the expected PO_4^{3-} concentration (moles PO_4^{3-} - P L^{-1}) of each individual water sample, $P{:}Cl_I$ is the molar ratio of P and Cl^- in the initial pulsed slurry added to the

study reach, and Cl_O is the observed Cl^- concentration (moles Cl L^{-1}) of each sample (background corrected). I then estimated the P concentration "deficit" for each water sample using

$$P_D = P_E - P_O$$

where, P_D is the amount of P deficit from each sample, P_O is the observed P concentration in each sample, and P_E is the expected P concentration of each sample. One would expect that P_D values should decrease in water samples as the pulsed solute plume travels downstream and P is transferred to the benthos. I then calculated the proportion of the original added total PO_4^{3-} still remaining in each water sample at each downstream site by dividing P_D by P_E. This proportional value for each water sample can then be multiplied by the initial amount of PO_4^{3-} added in solute pulse to calculate the approximate amount of P that has been taken up at that point in time at a specific point in the river. In order to estimate P uptake over the entire study site reach, I calculated the mean P uptake of the five water samples at each downstream point that coincided with the peak of the solute plume as it passed by the point (Figure 2). Finally, the P uptake values for each downstream station were plotted as a function of the distance downstream from the solute addition point and ordinary least-squares linear regression was used to estimate the slope of this relationship; the slope parameter yields a reach-scale estimate of amount of P taken up per unit river distance (mmol P m^{-1}). Since the P concentrations during the peak of the pulse were 100-1000 times greater than background concentrations, it is assumed that available P during the pulse is in excess of the capacity of the stream to assimilate, therefore the slope of this line represents a maximal, instantaneous, assimilatory capacity for P by the stream. This estimate is hereafter

referred to as assimilatory capacity - U_d. The proposed advantage of U_d over v_f is that it measures the removal of P from the water column, but does not require measurements of discharge or channel dimension, and therefore is less susceptible to error from these measurements. Additionally, the definition of v_f as the "vertical velocity of nutrient molecules through the water column towards the benthos" (Ensign and Doyle 2006) is arguably difficult to conceptualize in a real-world system. Furthermore, this assumes that all uptake is occurring in the benthos, which may not be the case.

Site-specific environmental variables - Immediately prior to conducting an uptake experiment at each study site, I determined mean channel width and mean depth and benthic substrate size at each sampling station within the study reach. I also collected a grab sample at each sampling station for the initial background water chemistry prior to conducting an experiment. Bottles were immediately placed in a cooler on ice and were analyzed for PO_4^{3-}, Na^+, and Cl^- in the lab with the same methods as the water samples from pulse addition experiments. The concentrations of a suite of other cations and anions (Ca^{2+}, in particular) were also measured in water samples using the Dionex ICS 1600 ion chromatograph. Ammonium (NH_4^+) and nitrate (NO_3^-) were measured on filtered water samples using the phenate method (Solorzano 1969) and second-derivative spectroscopy (Crumpton et al. 1992), respectively. Instantaneous measurements of water velocity and where necessary, discharge) were determined at each site with a Marsh-McBirney Flo-Mate 2000 flow meter along three to four points in the thalweg of each study reach. Where possible, we utilized the nearest United States Geological Survey (USGS) stream gauging station to estimate discharge at the time of the pulse addition experiment. Water temperature (^oC) and specific conductance (SC: $\mu S\ cm^{-2}$) were

measured in the field using a hand-held field probes (YSI Model 85 or a Hannah Multi-parameter water quality meter). The area of each study reach was determined by using the software Google Earth Pro to draw polygons over satellite imagery of the reach. Mean percent coverage of macrophytes along each study reach was reported as the average of visual estimation at 3-4 points along the main channel for each study reach; dominant species at each point were recorded. Size of the dominant benthic substrate type was estimated along each study reach by visually estimating the dominant substrate type at 3-4 points in the thalweg using a modified Wentworth scale in which substrates were categorized from 0 to 5 as bedrock, silt, sand, gravel, cobble, or boulder; the mean substrate size of each site reach is reported as the average of these 3-4 measurements. In order to estimate the abundance of periphyton cover along each study reach, I collected four to five cobble-sized rocks, throughout the reach. In river sites with abundant macrophyte cover (i.e., the San Marcos River), I additionally collected four to five leaf samples from the dominant macrophyte species along the reach in order to determine epiphyton biomass. I measured the area of each leaf with a ruler and used the aluminum foil method (Davies and Gee 1993) to determine the area of each cobble sample. Cobbles and leaf samples were scraped to remove biofilms (cobbles were scraped with a nylon bristle brush, material was removed from leaves by gently rubbing with a gloved finger) and the scraped materials were rinsed with Milli-Q water and a portion of this slurry was then filtered onto pre-ashed and pre-weighed Pall A/E filters for determination of organic and inorganic matter content of biofilm materials. Inorganic materials were determined by drying filters for 48 h at 60ºC and reweighing to determine total biofilm dry mass (DM; mg) and filters were then ashed at 500 °C for 5 hours and reweighed to determine

the mass of inorganic material. The amount of chlorophyll-*a* in the biofilms was determined by filtering a portion of the slurry onto Pall A/E filters, followed by extraction with HPLC-grade acetone for a minimum of 8h in the dark. After extraction, chlorophyll *a* concentration was measured on a Turner Designs fluorometer. The P content of biofilm samples was determined by filtering a portion of the slurry onto Pall A/E glass fiber filters, digestion with HCl, and analysis for PO_4^{3-} with the molybdenum blue method (Wetzel and Likens 2000).

Statistics – All statistics were calculated using the Data Analysis add-on package for Microsoft Excel. To determine the relationships between the environmental variables present in this study, I calculated the Pearson r value for each combination of variable in a correlation matrix. I used ordinary least-squares linear regression to determine the influence of these environmental variables on the calculated uptake metrics.

Results

Environmental variables - Although the lotic systems included in this study were located in the same general geographic region and were fed from the same larger aquifer source, sites represented a range of discharges, channel morphologies, water temperatures, and water chemistries (Table 2). Despite these differences, all of the sites were characterized by clear water, high SC, and relatively low PO_4^{3-} concentrations. Instantaneous discharge across sites ranged from 3895 L s^{-1} (the Devils River) to 133 L s^{-1} (the Nueces in December 2013). The mean SC across all sites was 371 µS cm^{-1} and ranged from 275 µS cm^{-1} (the Devils River) to 569 µS cm^{-1} (the San Marcos River). At study sites, the SC was dominated by Ca^{2+} because all sites were fed from limestone-dominated groundwater sources; across 5 of the 8 sites we determined that SC was a

significant function of Ca^{2+} concentration ($y = 5.51[Ca^{2+}] + 41.31$, $R^2 = 0.98$, $p = 0.002$), thus SC acts as an excellent surrogate for Ca^{2+} concentration across the sites examined by this study. Ambient PO_4^{3-} concentrations ranged from 1 µg PO_4^{3-} -P L^{-1} (the Frio River) to 9.7 µg PO_4^{3-} -P L^{-1} (the San Marcos River). In contrast, NO_3^--N concentrations were relatively high in all systems: (153 µg L^{-1} in the Frio River to 2911 µg L^{-1} in Dolan Creek) so that the ratio of dissolved inorganic N (DIN = NO_3^--N + NH_4^+-N) to PO_4^{3-} ratios (molar) ranged from 304:1 (South Llano River) to 2642:1 (Guadalupe River).

The concentration of biofilm material on rocks and macrophytes ranged from 0.67 mg cm^{-2} (San Marcos River) to 52 mg cm^{-2} (Nueces River in December 2013). The proportion of organic matter (OM) in these biofilms (mg OM mg DM^{-1}) ranged from 0.13 (Frio River) to 0.45 (Dolan Creek). The areal concentrations of chlorophyll-*a* (µg chl *a* cm^{-2}) ranged from 0.28 (San Marcos) to 4.21 (Nueces, Dec 2013). Chlorophyll-*a* (Chl *a*) by mass (µg Chl *a* mg DM^{-1}) ranged from 0.08 (Devils River) to 0.38 in (San Marcos River). Biofilm areal P (µg P cm^{-2}) ranged from 0.60 (San Marcos) to 7.85 (Nueces, Dec 2013) and P content of biofilms as µg P DM^{-1} ranged from 0.13 (Nueces, Dec 2013) to 0.85 (San Marcos).

When I examined the relationships between these environmental variables (Table 3), I found that across sites, discharge was not strongly correlated with any of the other environmental variables (Pearson $r < 0.57$). Water temperature was only correlated (negatively) with the proportion of OM in the biofilms. Channel width to depth ratios varied widely (4.8 in the Devils River to 117 in Dolan Creek) and were not correlated with any other variables. SC was correlated with a number of environmental variables: SC was positively correlated with DIN:SRP, biofilm P (by mass) and biofilm Chl *a* (by

mass). All of these variables were also negatively correlated with OM proportion. Correlations were much weaker for areal OM, P, and Chl *a* than for mass-mass expressions therefore I excluded them from further analysis.

Phosphorus uptake and cycling estimates - At one site (the Guadalupe River), the channel was relatively shallow, dominated by scoured bedrock, and had many small islands and travertine dams along the reach, leading to a great deal of hydrologic complexity. Unfortunately, this complexity made it difficult for the downstream sampling crews to collect samples when the mass of the solute plume passed by sampling sites, which led to most of the sampling stations missing the center of the plume as it moved downstream. Due to this issue, there was a lack of solute peak data from two of the three sampling stations on the Guadalupe and I therefore excluded it from further analysis. Data from station 1 at the Frio River and station 3 at Dolan Creek were excluded for the same reason, however the regressions through the 3 remaining points were significant ($p \leq 0.05$) and had strong R^2 values (0.99 for both) so I did not exclude those sites. The initial amount of P added to the slurry at the Devils River experiment was not measured precisely due to equipment failure and so U_d, S_w, and v_f were calculated by only including data from the three sampling stations, and not the initial pulse amounts. The initial amount of P added does not affect the calculations of U_d, because slope of the line does not change with the initial amount added, only the intercept. Calculations for S_w nor v_f do not require the initial amount of P added, only the ratios of P to the conservative tracer. U_d at each site ranged from 11.1 mmol/m (San Marcos) to 0.5 mmol/m (Devils) (Table 4). The relationships between moles P removed and distance were all linear and significant ($p \leq 0.05$) (Figure 2). When I calculated the traditional

spiraling metrics I found that S_w ranged from 120 m (Dolan) to 1898 m (Frio) and v_f ranged from 0.05 m s^{-1} (Frio) to 0.28 m s^{-1} (Devils).

Relationships between P uptake and Environmental Variables - Across the reaches examined by this study, the calculated P assimilatory capacity, U_d (mmol P m^{-1}), and uptake velocity, v_f (mm min^{-1}), were a function of several interrelated environmental factors. Both the chlorophyll *a* content and the P content (by mass) of the biofilms were significant predictors of both U_d and v_f (Figure 3), although the R^2 values for the regressions with v_f were somewhat higher than for U_d In contrast, SC, DIN:SRP, water temperature, instantaneous discharge, and biofilm OM did not show correlation with uptake rates. S_w was not correlated with any environmental variables ($|r| < 0.53$).

Discussion

In the study presented here, I hypothesized that variation in the uptake and sequestration of P in karst river systems in central Texas would be related to several hydrologic, biological, and physicochemical factors. Among hydrologic factors explored by this study, I predicted that the magnitude of river discharge would be the most important variable affecting P uptake because larger rivers have more area for P uptake to take place, and channel morphology – wide and shallow rivers have a larger width to depth ratio and thus have more contact with reactive benthic biofilms. However, my nutrient uptake metric did not show any correlation with discharge, channel area, or width-depth ratios. Benthic substrate size showed some correlation with uptake rates, however this is probably because sandy and silty benthos are more likely to support macrophytes. The system with the highest U_d and v_f was the San Marcos which also had the most abundant macrophytes. Chl *a* concentrations were highest on the biofilm

samples scraped from the macrophyte leaves suggesting that epiphytic algae have a large contribution to uptake relative to their mass per area of leaf.

In the present study, the strongest predictor of P uptake using both the U_d metric and v_f was the proportion of chlorophyll in biofilms present in the river (expressed in units of μg Chl *a* mg DM^{-1}). In many clear water systems, periphyton uptake of P can be important (Gaiser et al. 2004, 2006). The significant relationship between chlorophyll-*a* in benthic biofilms and uptake rates highlights the role that algal communities are playing in P uptake in these systems. The relationship between P enrichment and chlorophyll levels is well studied in aquatic systems (Dodds et al. 1997) so it is no surprise that algae are exerting demand for P in these systems. However, the fact that U_d was as high as 11.1 mmol m^{-1} shows the magnitude of the assimilative capacity of these systems relative to the baseline P concentrations. Indeed, values for v_f in this study were high compared to what has been reported in the literature. The mean v_f for this study was 10.0 compared a mean of 4.0 from 194 studies in a 2006 meta-analysis by Ensign and Doyle.

Although the results from this study suggest that periphyton and epiphytes can have an important role in P uptake and sequestration in clear water systems, there is also an indication that the physicochemical conditions of these karstic rivers also plays a critical role in P uptake. SC was dominated by dissolved calcium and is positively correlated with benthic chlorophyll-*a*, benthic P, and DIN:SRP, and negatively correlated the amount of organic matter in the biofilms. Interestingly, water column DIN:SRP is positively correlated with biofilm chlorophyll (i.e. in the systems with less available P relative to N in the water column, there is increased algal growth), and biofilm P. One possible explanation for this is that high load of Ca and Mg (San Marcos Ca^{2+} = 96 ppm,

Mg^{2+} = 19 ppm) is reacting with free PO_4^{3-} to form insoluble Ca-P and Mg-P compounds precipitate on to the benthic surfaces (Scinto and Reddy 2003). My results show that as mineral load increases, both the inorganic/mineral content of biofilms and P content increase.

However, boundary layer chemistry on the surface of these biofilms (i.e. the release of CO_2 from respiration) may lower pH enough to cause localized liberation of these mineral forms of P, thus making them available to the biofilms (Dodds, 2003). This process is unlikely to be a major contributor to direct uptake of the pulsed P at the scale at which we were measuring, but rather in systems with higher concentrations of dissolved minerals, these minerals my make more P available to benthic communities and thus stimulate algal growth. All of these systems had dissolved P levels of less than 10 μg L^{-1} and yet benthic and epiphytic algae are abundant. These observations highlight the complexity and high degree of interrelatedness of the biological communities of these systems with their environment and how geochemical factors (e.g. karst-influenced hydrology) can play a large role in the uptake of nutrients, even if that role is predominantly indirect.

Comparison of nutrient uptake metrics – When compared to one another, v_f and U_d correlate with one another (Figure 4, Table 4). The utility of one metric over the other may depend on the application; where discharge is difficult to measure accurately or channel morphology is complex, U_d can serve as an alternative metric to study nutrient uptake. It is critical to note that the nutrient spiraling metrics are theoretically derived and may not fully account for the complexity of a real-world riverine ecosystem (Runkel 2007). In contrast, a more empirical framework for understanding nutrient dynamics is

advantageous in that it does not attempt to separate out all the components of nutrient uptake, but rather gives an estimate of actual nutrient removal from the water column.

This study has shown the pulse-addition method and subsequent uptake calculations to be a cost-effective and useful metric for gauging demand of nutrients in lotic ecosystems across a range of hydrologic conditions. Because I have shown that these uptake demands are highly correlated with other measures of stream health (i.e. chlorophyll), conducting these experiments on a regular basis could give useful information about seasonal changes in stream-level algal nutrient demand. This could be useful for managers concerned with understanding and mitigating the impacts of P loading in streams, especially when these loads are concentrated in both space and time, for example, nutrient-rich runoff from a polluted creek during rain events. This research could also be used to further understand the feedback loops between P loading and algal biomass in streams (Dodds et al. 1997) and making decisions about critical levels of P in those systems.

Table 1. Location, nearest USGS Gaging Station ID number, long-term median discharge (from the USGS gauging station), general description of stream morphology, and length of the study reach for each site.

Site	Lat, Long (°N, °W)	Nearest USGS Station ID	Median discharge (L s^{-1})	Description of channel morphology	Study reach length (m)
Devils River	29°54'12"N 101°0'7"W	08449400	8700	Highly braided channel, boulder and bedrock bottom	155[1]
Dolan Creek	29°53'9"N 100°59'35"W	08449100	368	Rocky bottom, alternating pools and gravel runs	210
Frio River	29°43'22"N 99°45'13"W	08194755	2095	Alternating gravel-dominated riffle- run habitats	192
Guadalupe River	29°58'49"N 99°26'34"W	08165500	1217	Wide and shallow, bedrock bottom	86
Nueces River	29°48'41"N 100°1'3"W	0818999010	991	Braided channel, cobble bottom	84[2], 174[3]
San Marcos River	29°53'10"N 97°56'8"W	08170500	5068	Somewhat channelized, silt and cobble bottom with abundant macrophytes	240
South Llano River	30°23'36"N 99°52'54"W	08149900	2718	Braided channel, cobble bottom	88

[1]Experiment was performed in side channel [2]June 2013 date [3]December 2013 date

Table 2. Environmental variables collected for each uptake experiment.

Site	Date	Water temp (ºC)	Discharge (L s^{-1})	SC (µS cm^{-2})	DIN:SRP (molar)	Biofilm OM (mg mg DM^{-1})	Biofilm Chl *a* (µg mg DM^{-1})	Biofilm P (µg mg DM^{-1})	Benthic Substrate Size
Devils	Dec 8, 2013	8.4	762	275	671	0.45	0.07	0.22	5
Dolan Creek	Dec 7, 2013	16.2	311	340	726	0.46	0.29	0.59	3
Frio	Jun 6, 2013	24.9	991	399	520	0.14	0.13	0.19	3.5
Guadalupe	Jun 25, 2013	27.1	282	425	2642	0.33	0.42	0.35	0
Nueces	Jun 6, 2013	24.1	991	368	445	0.19	0.21	0.22	4
Nueces	Dec 8, 2013	19.5	133	333	397	0.44	0.09	0.13	4
San Marcos	Jul 10, 2013	22.4	3115	548	1467	0.16	0.38	0.86	1.5
South Llano	Jun 25, 2013	27.8	1130	337	304	0.18	0.25	0.32	4.5

Table 3. Pearson product moment correlation r values for each combination of environmental variables.

Variable	Temp (ºC)	Discharge (L s^{-1})	SC (µS cm^{-1})	DIN:SRP (molar)	Biofilm OM (mg mg DM^{-1})	Biofilm Chl *a* (µg mg DM^{-1})	Biofilm P (µg mg DM^{-1})
Discharge	-0.46	1	--	--	--	--	--
SC	0.45	0.20	1	--	--	--	--
DIN:SRP	-0.17	0.57	0.78	1	--	--	--
Biofilm OM	-0.82	-0.05	-0.62	-0.14	1	--	--
Biofilm Chl *a*	0.38	0.04	0.71	0.64	-0.39	1	--
Biofilm P	0.03	0.30	0.72	0.87	-0.15	0.90	1
Benthic Substrate Size	-0.25	-0.04	-0.90	-0.82	0.32	-0.78	-0.84

Table 4. Calculated uptake metrics for each experiment.

Site	U_d (mmol m^{-1})	S_w (m)	v_f (mm min^{-1})
Devils	0.5	1072	3.3
Dolan Creek	8.8	120	10.7
Frio	1.8	1898	3.0
Nueces (Jun)	7.6	434	9.6
Nueces (Dec)	5.1	512	2.3
San Marcos	11.1	501	16.6
South Llano	6.5	505	10.8
Mean	5.9	720	10.0

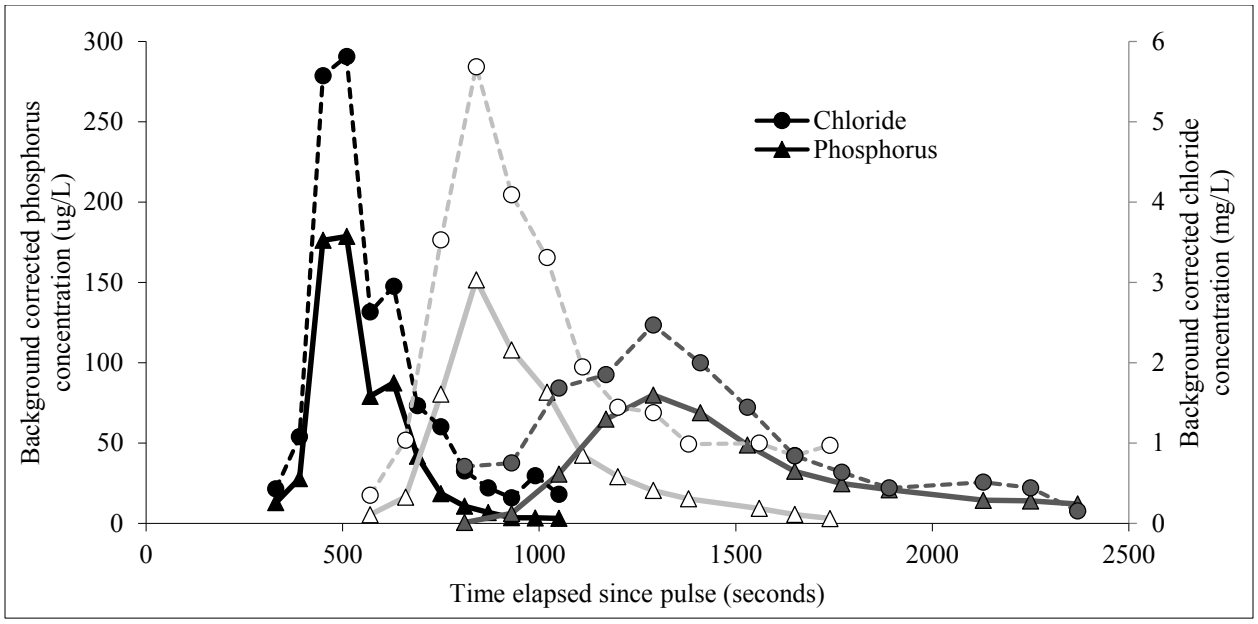

Figure 1. Chloride and Phosphorus concentrations as measured at each sampling station during pulse addition in the San Marcos River. Sampling stations were located 123, 206, and 293 meters downstream from pulse release site.

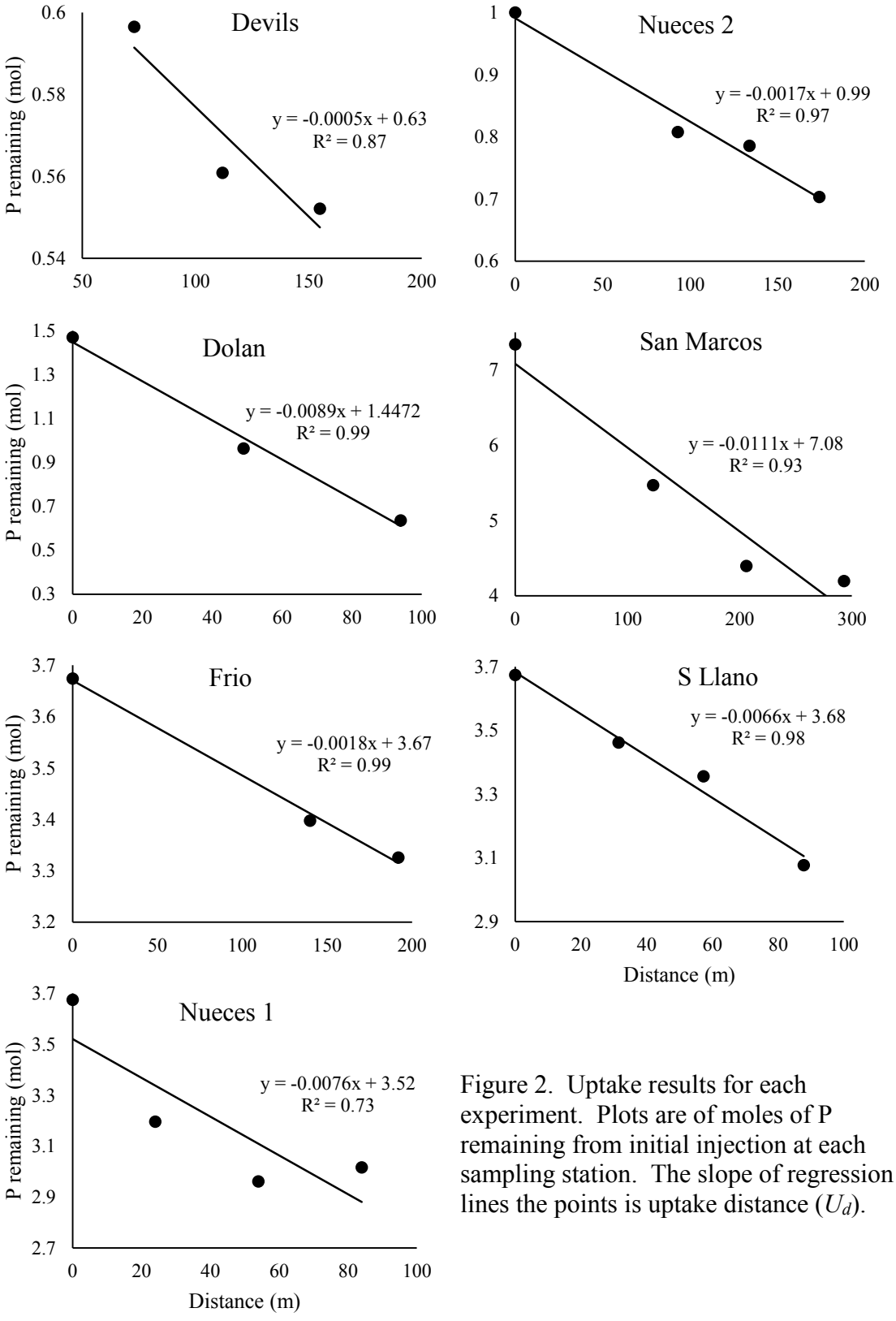

Figure 2. Uptake results for each experiment. Plots are of moles of P remaining from initial injection at each sampling station. The slope of regression lines the points is uptake distance (U_d).

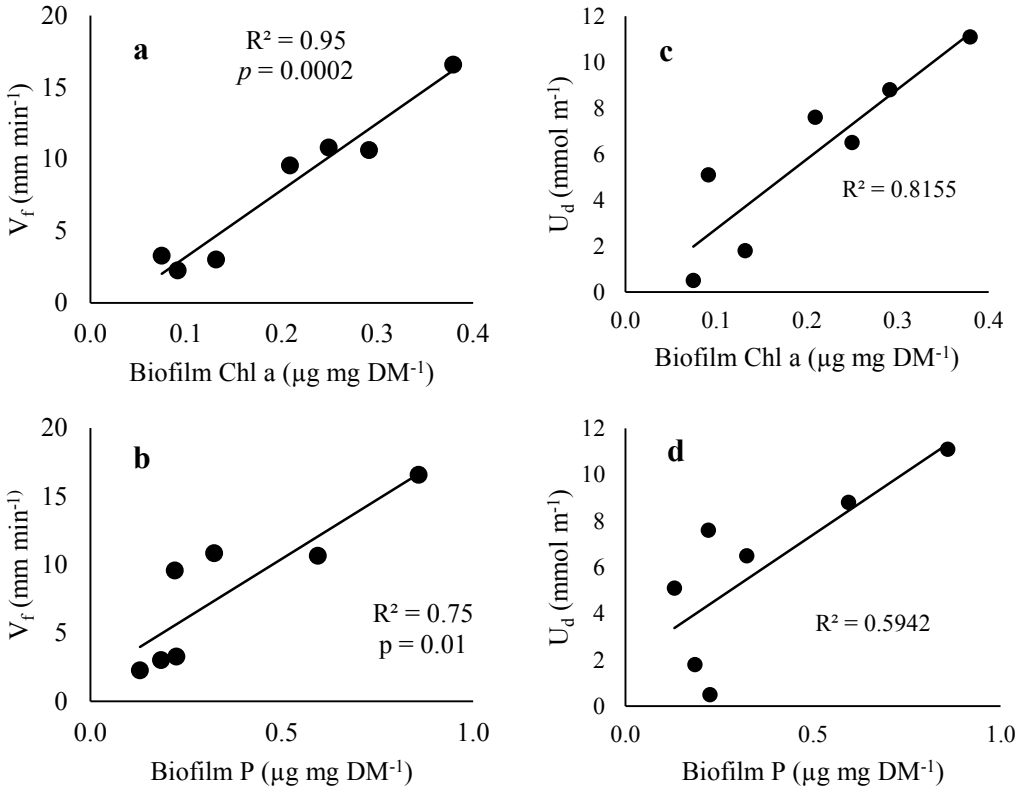

Figure 3. Relationships between biofilm Chl *a* (by mass) and biofilm P content (by mass) and the nutrient uptake metrics of v_f (a,b) and U_d (c,d) across all sites.

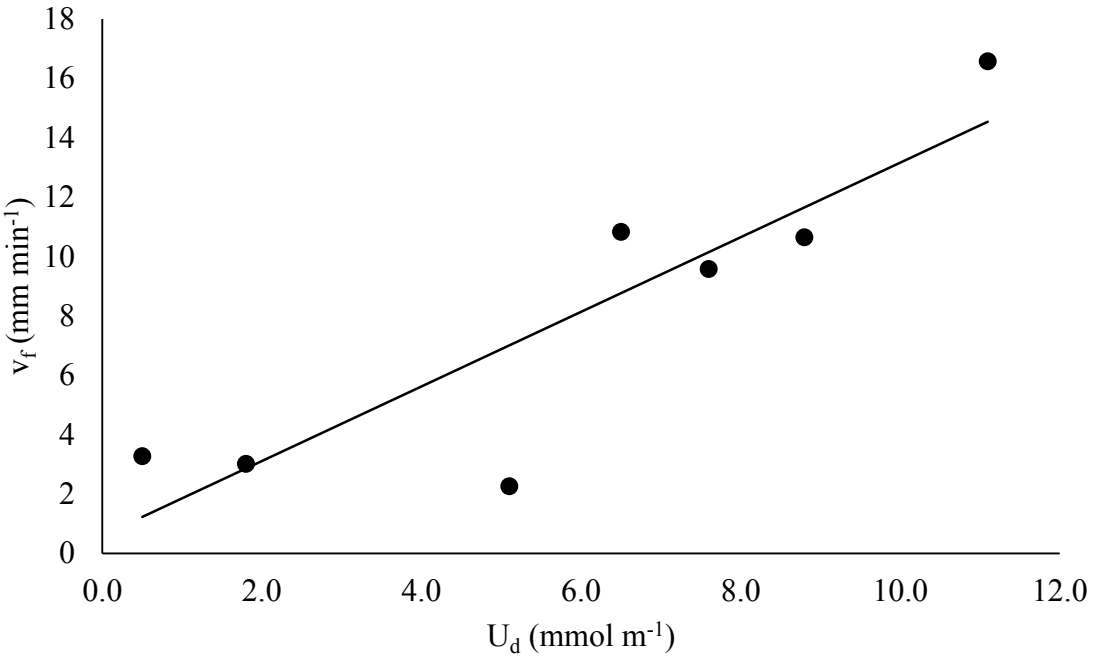

Figure 4. Correlation of uptake metrics U_d and v_f.

REFERENCES

Camargo, J. a, and A. Alonso. 2006. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environ. Int. **32**: 831–49.

Crumpton, W., T. Isenhart, and P. Mitchell. 1992. Nitrate and organic N analyses with second-derivative spectroscopy. Limnol. Oceanogr. **37**: 907–913.

Davies, A. L., and J. H. R. Gee. 1993. A simple periphyton sampler for algal biomass estimates in streams. Freshw. Biol. **30**: 47–51.

Dodds, W. 2003. The role of periphyton in phosphorus retention in shallow freshwater aquatic systems. J. Phycol. **39**: 840–849.

Dodds, W. K., J. J. Beaulieu, J. J. Eichmiller, J. R. Fischer, N. R. Franssen, D. a. Gudder, a. S. Makinster, M. J. McCarthy, J. N. Murdock, J. M. O'Brien, J. L. Tank, and R. W. Sheibley. 2008. Nitrogen cycling and metabolism in the thalweg of a prairie river. J. Geophys. Res. **113**: G04029.

Dodds, W. K., K. Gido, M. R. Whiles, K. M. Fritz, and W. J. Matthews. 2004. Life on the Edge: The Ecology of Great Plains Prairie Streams. Bioscience **54**: 205.

Dodds, W., V. Smith, and B. Zander. 1997. Developing nutrient targets to control benthic chlorophyll levels in streams: a case study of the Clark Fork River. Water Res. **31**: 1738–1750.

Elser, J. J., M. E. S. Bracken, E. E. Cleland, D. S. Gruner, W. S. Harpole, H. Hillebrand, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10**: 1135–42.

Ensign, S. H., and M. W. Doyle. 2006. Nutrient spiraling in streams and river networks. J. Geophys. Res. **111**, doi:10.1029/2005JG000114

Gaiser, E. E., D. L. Childers, R. D. Jones, J. H. Richards, L. J. Scinto, and J. C. Trexler. 2006. Periphyton responses to eutrophication in the Florida Everglades: Cross-system patterns of structural and compositional change. Limnol. Oceanogr. **51**: 617–630.

Gaiser, E. E., L. J. Scinto, J. H. Richards, K. Jayachandran, D. L. Childers, J. C. Trexler, and R. D. Jones. 2004. Phosphorus in periphyton mats provides the best metric for detecting low-level P enrichment in an oligotrophic wetland. Water Res. **38**: 507–16.

Groeger, A. W., P. F. Brown, T. E. Tietjen, and T. C. Kelsey. 1997. Water quality of the San Marcos River . Texas J. Sci. **49**.

Martí, E., N. Grimm, and S. Fisher. 1997. Pre-and post-flood retention efficiency of nitrogen in a Sonoran Desert stream. J. North Am. Benthol. … **21**: 544.

Newbold, J. D., J. W. Elwood, R. V O'Neill, and W. Van Winkle. 1981. Measuring nutrient spiralling in streams. Can. J. Fish. Aquat. Sci. **38**: 860–863.

Noe, G., L. Scinto, and J. Taylor. 2003. Phosphorus cycling and partitioning in an oligotrophic Everglades wetland ecosystem: a radioisotope tracing study. Freshw. … **48**: 1993–2008.

Paerl, H., and J. Pinckney. 1998. Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA. Mar. Ecol. … **166**: 17–25.

Payn, R., J. Webster, P. Mulholland, H. Valett, and W. Dodds. 2005. Estimation of stream nutrient uptake from nutrient addition experiments. Limnol. Oceanogr. Methods **3**: 174–182.

Runkel, R. 2007. Toward a transport-based analysis of nutrient spiraling and uptake in streams. Limnol. Oceanogr. Methods **5**: 50–62.

Scinto, L. ., and K. . Reddy. 2003. Biotic and abiotic uptake of phosphorus by periphyton in a subtropical freshwater wetland. Aquat. Bot. **77**: 203–222.

Smith, V. H., G. D. Tilman, and J. C. Nekola. 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. **100**: 179–96.

Solorzano, L. 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr. **14**: 799–801.

Stanley, E. H., R. A. Short, J. W. Harrison, R. Hall, and R. C. Wiedenfeld. 1989. Variation in nutrient limitation in lotic and lentic algal communities in a Texas (USA) river. Hydrobiologia **206**: 61–71.

Stream Solute Workshop. 1990. Concepts and Methods for Assessing Solute Dynamics in Stream Ecosystems. J. North Am. Benthol. Soc. **9**: 95–119.

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

Tilman, D., J. Fargione, B. Wolff, C. D'Antonio, a Dobson, R. Howarth, D. Schindler, W. H. Schlesinger, D. Simberloff, and D. Swackhamer. 2001. Forecasting agriculturally driven global environmental change. Science **292**: 281–4.

Webster, J. 1975. Analysis of potassium and calcium dynamics in stream ecosystems on three southern Appalachian watersheds of contrasting vegetation. University of Georgia.

Webster, J., P. Mulholland, J. Tank, H. Valett, W. Dodds, B. Peterson, W. Bowden, C. Dahm, S. Findlay, S. Gregory, N. Grimm, S. Hamilton, S. Johnson, E. Marti, W. McDowell, J. Meyer, D. Morrall, S. Thomas, and W. Wollheim. 2003. Factors affecting ammonium uptake in streams–an inter-biome perspective. Freshw. Biol. **48**: 1329–1352.

Wetzel, R. G., and G. E. Likens. 2000. Limnological Methods, 3rd ed. Springer-Verlag.

Wollheim, W. M., C. J. Vörösmarty, B. J. Peterson, S. P. Seitzinger, and C. S. Hopkinson. 2006. Relationship between river size and nutrient removal. Geophys. Res. Lett. **33**: 2–5.