



PERIPHYTON NUTRIENT LIMITATION AND MAXIMUM POTENTIAL PRODUCTIVITY IN THE BEAVER LAKE BASIN, UNITED STATES¹

Andrea Ludwig, Marty Matlock, Brian Haggard, and Indrajeet Chaubey²

ABSTRACT: The objectives of this study were to measure periphytic growth responses to enrichment with nitrogen (N), phosphorus (P), and simultaneous N and P using *in situ* bioassays in streams draining Beaver Reservoir Basin, Northwest Arkansas; compare periphytic growth responses measured with *in situ* bioassays with a range of land use and point sources; and test the lotic ecosystem trophic status index (LETSEI) as a simplifying metric to compare effects of nonpoint-source pollutant-limiting variables of N, P, and sediment across the basin. P limitation was observed at sites across a transect of stream orders throughout the basin; however, at the two sites with highest ambient nitrogen concentrations, limitation was often coupled with nitrogen limitation. Nutrients were at nonlimiting levels at both of two sites below wastewater treatment plants in all seasonal deployments. A Michaelis-Menten growth equation described LETSEI as a function of ambient PO₄-P concentrations ($p < 0.05$); the midpoint (LETSEI of 0.50) corresponded with a PO₄-P concentration of approximately 3 µg/l. Change-point analysis indicated a threshold point at LETSEI of 0.80 and 15 µg/l PO₄-P. These low values show that the periphytic community has a high affinity for available P, and that the watershed as a whole is sensitive to available nutrient inputs.

(KEY TERMS: water quality; nutrient-diffusing substrata; watershed management; riparian; eutrophication.)

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INTRODUCTION

In 2004, states, tribes, territories, and interstate commissions reported that of the 700,000 miles of rivers and streams assessed in the United States (U.S.), 56% fully supported their designated uses and 44% were impaired for one or more designated uses (USEPA, 2009). This was an increase from the 2000

assessment, when 39% of rivers and streams were reported as impaired (USEPA, 2000). The leading pollutants in these assessments were found to be pathogens, habitat alterations, organic enrichment, and nutrients. Agriculture was the reported source of impairment of over 35% of streams and rivers. These assessments were reinforced by a recent analysis of wadeable streams across the U.S. that concluded that the three most critical pollutants were

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²Respectively, Assistant Professor (Ludwig), Department of Biosystems Engineering and Soil Science, University of Tennessee Institute of Agriculture, 2621 Morgan Circle, Knoxville, Tennessee 37996; Professor (Matlock) and Associate Professor (Haggard), Department of Biological and Agricultural Engineering, University of Arkansas, Fayetteville, Arkansas 72701; and Associate Professor (Chaubey), Department of Agricultural and Biological Engineering and Department of Earth and Atmospheric Sciences, Purdue University, West Lafayette, Indiana 47907 (E-Mail/Ludwig: aludwig@utk.edu).

phosphorus (P), nitrogen (N), and sediment (USEPA, 2006). In March 2011, the USEPA published a guide for states on establishing numeric nutrient criteria thresholds (USEPA, 2011). Progressive states are adopting numeric nutrient criteria to halt the eutrophication of waterways and are looking to functional relationships between nutrients and algae to assist in identifying these targets (Kiesling *et al.*, 2001). Selecting numeric nutrient criteria must take into account the designated uses of waterbodies, and many of these uses are impaired by the presence of nuisance algal growth. Scientists have provided recommendations for numeric nutrient criteria based on the likelihood of harmful algal bloom occurrence (Dodds and Welch, 2000), but adopting a single numeric threshold is difficult when so many other factors are at play.

Instream nutrient concentrations have been correlated with human activity in the corresponding basin (Gergel *et al.*, 2002). As ambient nutrient concentrations increase, stream physical characteristics such as light availability become increasingly important in governing benthic periphyton growth (Jones *et al.*, 1984; Morgan *et al.*, 2006). Many studies have linked ambient nutrient concentrations to periphyton biomass (Horner and Welch, 1981; Biggs and Close, 1989; Biggs, 1990; Lohman *et al.*, 1992; Dodds *et al.*, 1997; Tank and Dodds, 2003; Stevenson *et al.*, 2008) and shown that both N and P can colimit the growth of periphyton (Fairchild *et al.*, 1985; Biggs, 2000). Longitudinal position within a basin and basin characteristics such as geomorphology also affect productivity in periphytic assemblages in nutrient-enrichment experiments (Snyder *et al.*, 2002). Because of their sensitivity to these changes associated with anthropogenic disturbance, periphyton can be used as a bioindicator of watershed and stream ecosystem health.

Periphyton biomass accrual and the development of nuisance algae have been shown to be strongly associated with nutrient enrichment in northern Ozark streams (Lohman *et al.*, 1992). A lotic ecosystem trophic status index (LETSI) was developed from nutrient-enrichment studies in Texas, as a metric to describe the trophic state of streams (Matlock *et al.*, 1999). Measuring the variables that govern periphyton productivity *in situ* requires the consideration of ambient conditions such as light availability and nutrient concentrations (Hill and Knight, 1988). These variables have been measured and correlated individually to periphyton growth (Stevenson *et al.*, 1996), and nutrient and light availability has been documented to colimit periphyton growth in small streams (Hill and Fanta, 2008). In order to understand these relationships, it is necessary to measure levels of nutrient availability *in situ* across a gradient

of selected conditions while accounting for variations in biomass accrual due to secondary factors such as light availability, temperature, flow, substrate, anthropogenic impacts, and losses due to scour and grazers.

The objectives of this study were to measure periphytic growth responses to enrichment with N, P, and simultaneous N and P using *in situ* bioassays in streams draining Beaver Reservoir Basin, Northwest Arkansas; compare periphytic growth responses measured with *in situ* bioassays to a range of land use and point sources; and test the LETSI as a simplifying metric to compare effects of nonpoint-source (NPS) pollutant-limiting variables of N and P across the basin.

MATERIALS AND METHODS

Study Site

The Beaver Reservoir Basin is in Northwest Arkansas and drains a 300,000-hectare area (Figure 1). The White River flows north into Beaver Reservoir, which was created in the 1960s for flood protection and hydropower generation. The Arkansas Department of Environmental Quality designates the waters in the basin for the propagation of fish and

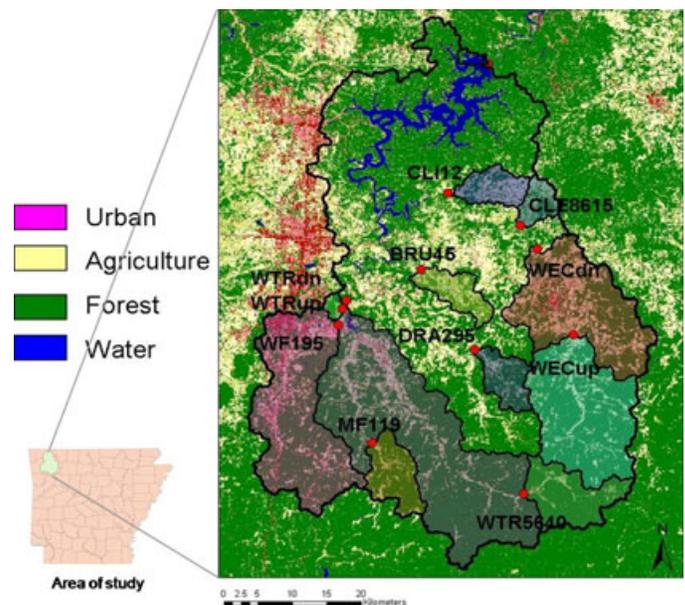


FIGURE 1. Beaver Reservoir Basin in Northwest Arkansas. Sampling sites (red dots), site subbasins, and land uses within the basin as delineated from 2004 land use data (Center for Advanced Spatial Technologies, 2004).

wildlife, primary and secondary contact recreation, and domestic, agricultural, and industrial water supplies. Beaver Reservoir is the drinking water supply for the cities of Fayetteville, Springdale, Rogers, and Bentonville as well as many small suburbs. The metropolitan area that these cities comprise was ranked as the sixth fastest growing area in the nation (U.S. Census Bureau, 2001). Level 3 ecoregions in the basin are Ozark Highlands and Boston Mountains (Omernik and Gallant, 1987). Land uses in the basin are forest (>60%), agriculture (mainly pasture, >25%), and others including urban areas (Table 1). The main tributaries of the White River in the Beaver Reservoir Basin are the Middle and West Forks of the White River, Richland Creek, and War Eagle Creek.

Trophic conditions in Beaver Reservoir were reported as eutrophic in the riverine zone and mesotrophic in the transitional zone and upper lacustrine zone (Galloway and Green, 2006). Trophic conditions are a product of nutrient loading to the reservoir from the drainage basin. Total nutrients annually exported from the basin into Beaver Reservoir were estimated to be an average of 34 Mg SRP, 75 Mg TP, 862 Mg NO₃-N, and 1,329 Mg TN (Haggard *et al.*, 2003). Alteration of riparian corridors have been identified as a likely cause of increased sediment loads and nutrient loads to the basin and the resulting eutrophication (Galloway and Green, 2006; Sen *et al.*, 2006).

Study sites for this study were selected throughout the basin to obtain a gradient of agricultural use intensity and ambient nutrient concentrations. Eleven sites were selected based on the reported ambient water chemistry by Haggard *et al.* (2003). Point-source discharges were bracketed, resulting in sampling upstream and downstream from effluent, and only perennial systems were selected. Land use

across each study site catchment was predominately forest and impacted most by NPS pollution from agriculture and point-source pollution of wastewater treatment plants (WWTP) (Table 1, Figure 1). Sites WTRup and WTRdn bracketed the Fayetteville WWTP outfall, whereas the sites WECup and WECdn bracketed the Huntsville WWTP outfall. It should be noted that basin land use percentages also changed between the upstream site and downstream site, most notably between the WEC sites.

Sample Collection

Field data collection was performed in June 2005, August 2005, and January 2006. These periods were selected to correspond to annual seasonal minimum flows in summer and increased base flows in January. However, between the summer and winter collections in 2005, there were no runoff events within the study watershed, thus low-flow conditions persisted. Seasonal extremes such as canopy cover and temperature that may affect algal growth were monitored through the seasonal deployments to document variation between site conditions. Data collections consisted of two-week deployments of a passive diffusion periphytometer (PDP) (described in detail in a following section), monitoring of ambient physical and chemical water-quality constituents, and land use characterization of each study subbasin.

Field Methods

The study reach of each study site was selected by controlling for three primary criteria, other than nutrient availability, that affect primary production: (1) canopy cover, (2) continuous flow, and (3) stream stage.

TABLE 1. Beaver Reservoir Basin in Northwest Arkansas.

Subbasin	Site Name	Land Use Classification Area (%)				Basin Area
		Pasture	Urban	Forest	Other	
White River	MF119	16.6	0.0	75.6	7.8	6.0
	WF195	17.2	10.8	63.8	8.2	26.0
	WTR5640	6.9	0.5	89.6	3.0	8.6
	WTRup	10.4	2.9	50.8	35.9	81.3
	WTRdn	6.2	1.7	50.2	41.9	82.1
Richland	DRA295	20.3	0.0	69.9	9.8	4.3
Brush	BRU45	38.4	0.3	51.0	10.3	4.2
War Eagle	CLE8615	43.8	0.0	40.0	16.2	2.6
	CLI12	51.7	0.0	36.0	12.3	4.0
	WECup	13.9	0.0	80.3	5.8	22.3
	WECdn	14.5	0.8	46.0	38.7	43.8
Beaver Reservoir Basin	BEAVER	26.5	2.2	61.5	9.8	100.0

Note: Basin divided into subbasins created by study site locations and its respective land use classifications.

The location of the deployed PDP dictated the location of field data collection. PDPs were deployed in the main flow of the channel, directly above or below a riffle. Locations were selected within the reach that had maximum light availability by considering the effects of bank height, canopy cover, and bank angles. Simultaneous deployments in each season ensured that each PDP experienced similar weather conditions and cloud cover. The stream stage at each selected location was a minimum of approximately 25 cm, the depth required for the PDP to float in the channel. After the location was selected, grab water samples and physicochemical data were taken upstream of the PDP and in the main flow. Care was given to not sample or record measurements in the water column influenced by suspended silt disturbed by sampling.

Ambient physical water-quality constituents were measured using hand-held probes during each visit to the study sites, four to five times during the periphytometer deployments. A YSI 550a probe was used to measure dissolved oxygen (DO) (Water Solutions, Austin, Texas), and a YSI63 probe was used to measure the temperature, conductivity, and pH (Water Solutions). Probe readings were taken in the main flow of the channel.

Water Chemistry Analyses

Ambient nutrient concentrations in the water column were monitored with grab samples taken from the most concentrated flow upstream from the PDP four to five times throughout the two-week periphytometer deployment. When analyses called for sample preparation, water samples were filtered with single-use 0.45 μm filters and preserved with hydrochloric or sulfuric acid in the field. Ambient chemical constituents measured from grab samples were total phosphorus (TP), total nitrogen (TN), nitrate-nitrogen ($\text{NO}_3\text{-N}$), phosphate-phosphorus ($\text{PO}_4\text{-P}$), and ammonia-nitrogen ($\text{NH}_3\text{-N}$) (Table 2). All reported values are averages of measured concentrations during each deployment. Concentrations

below the practical quantitative limits (PQLs) were assigned a value of half of the PQL for averaging purposes. N:P ratios were calculated from the TN and TP averages.

Passive Diffusion Periphytometers

Limiting nutrients (N and/or P) of periphyton were determined for each stream site using PDPs with modification (Matlock *et al.*, 1998). This method measured *in situ* periphytic responses to nutrient enrichment using a passive diffusion nutrient-enrichment periphytometer system to enrich the artificial periphyton growth media with growth-limiting nutrients (N and P). The PDPs were chosen as the nutrient-enrichment method because they were relatively inexpensive and easy to assemble, of adequate size for study sites, and minimally susceptible to bias introduced by the method (Matlock *et al.*, 1998).

PDPs were constructed of a 0.45- μm nylon membrane filter and glass fiber filter, attached over the top of a 250-ml low-density polyethylene container with a 2.5-cm diameter hole cut in the lid (Figure 2). The nylon membrane acted as a biofilter, allowing only nutrients to diffuse into and out of the bottle (Figure 3). The glass fiber filter was the growth media for the periphyton and was the point of nutrient saturation. The bottles were filled with the treatment solutions, and attached to a floating apparatus using plastic zip ties. The four treatment solutions were constituted in reverse osmosis (RO) water with bottle nutrient concentrations of 4 to 10 times ambient water quality previously reported at study sites (Table 3) (Haggard *et al.*, 2003). These concentrations represent excess based upon ambient conditions. The concentrations were varied to insure that seasonal *in situ* variability did not interfere with enrichment.

PDP treatments were arranged in randomized blocks consisting of a treatment array of four treatments per block, and 10 replicates of each block per site. Each treatment array of 40 PDPs was supported on a steel utility panel of approximately 1 m \times 1.5 m,

TABLE 2. Water-Quality Methods.

Constituent	Method	MDL (mg/l)	PQL (mg/l)
TN	Method 5310B (APHA, 1998)	0.1	0.1
$\text{NO}_3\text{-N}$	EPA Method 300.1: Detection of Inorganic Anions in Drinking Water by Ion Chromatography (EPA, 1997)	0.008	0.002-0.005
$\text{NH}_3\text{-N}$	Method 4500NH3 F (APHA, 1998)	0.02	0.002
TP	Method 4500P E (APHA, 1998), Method 4500 E (APHA, 1998)	0.019	0.0032-0.032
$\text{PO}_4\text{-P}$	EPA Method 300.1: Detection of Inorganic Anions in Drinking Water by Ion Chromatography (EPA, 1997)	0.01	0.002-0.005

Note: Procedures used for chemical analysis of grab samples with respective method detection limits (MDLs) and practical quantitative limits (PQLs).

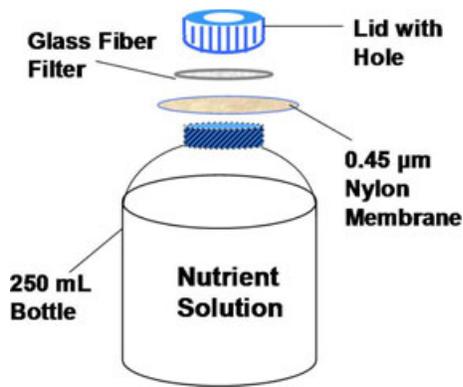


FIGURE 2. Modified Passive Diffusion Periphytometer (Matlock *et al.*, 1998).

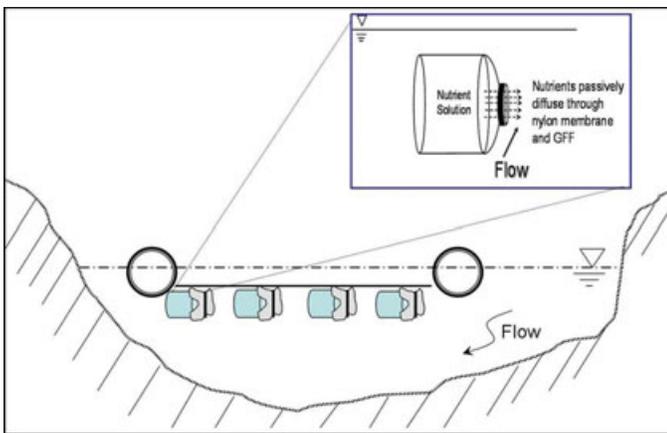


FIGURE 3. Deployed Passive Diffusion Periphytometer in Stream Channel. Stream flow is parallel to glass fiber filter surface and perpendicular to nutrient diffusion (Matlock *et al.*, 1998).

attached to PVC pontoons, and anchored in the main flow of the channel at the selected site location (Figure 3). The treatments were attached to the panel with growth surfaces perpendicular to the water surface and parallel to stream flow (Matlock *et al.*, 1998).

The algal growth surfaces were protected from fish and macroinvertebrate grazing by placing an aluminum screen (0.7 mm diameter wire) over the top of each PDP, approximately 3 cm from the glass fiber filter growth surfaces. The submerged growth media was suspended 5-7 cm below the water surface, under the panel. The lip of the lid over the glass fiber filter created by the cut of the hole with the aluminum screen cover establishes a quiescent zone at the growth surface, lessening the effects of scour on the periphyton assemblage.

At the end of the 14-day PDP deployment, the colonized glass fiber filters were placed in 5 ml of 90% acetone solution saturated with magnesium carbonate, wrapped in an aluminum foil, and transported

on ice to the laboratory for analysis. Samples were labeled using a blind treatment identification system to avoid field or laboratory analytical bias. Chlorophyll *a* (chl *a*) results would be decoded by treatment after the analyses were complete. This prevents inadvertent bias by the analyst. Each column represents a randomized treatment block, with a Control (C), N, P, and N + P (NP) treatment (Figure 4).

Chlorophyll was extracted from the filters for direct measurement in the laboratory using the spectrophotometric determination of chlorophyll *a*, *b*, and *c*, with the trichromatic method (Method 10200H 2c; APHA, 1998). Chl *a* from each filter sample was expressed as mass (μg) per unit of exposed surface area of the filter (5.06 cm^2) and used in comparisons as an estimation of algal biomass (Method 10200 I 1; APHA, 1998).

Passive Diffusion Periphytometer Randomized Block Design Assessment

When deployed in a stream, the PDP apparatus produces spatial bias from flow and light gradients across the panel. One row or column of bottles may receive more scouring flow than others, whereas others may be in direct sunlight for longer periods of time. These spatial biases are mitigated using randomized block design across the panel and averaging replicate treatment responses. Spatial bias was evaluated using Tukey's honest significant difference (HSD) to compare responses across row and column locations ($p = 0.05$).

Lotic Ecosystem Trophic Status Index

The LETSI was established by Matlock *et al.* (1999) as an index of algal growth potential for streams. They calculated LETSI values as the proportion of the maximum potential productivity (average NP chl *a* growth response) at ambient conditions (average Control chl *a* growth response). This calculation was altered in this study by treating each randomized block within a PDP rack as a single LETSI value, and averaging the 10 calculated LETSI values per rack. The alteration provided a means to show variance in the LETSI at each site and a means to statistically compare the LETSI across sites.

$$V = V_{\max} \frac{[S]}{[K_s + S]} \quad (1)$$

A Michaelis-Menten growth equation (Equation 1) (Shuler and Kargi, 2002) was fit to the LETSI (V) as a function of $\text{PO}_4\text{-P}$ (S , mg/l) and a Lineweaver-Burk parameter estimator was used to fit the relationship to the data (Lineweaver and Burk, 1934). This

TABLE 3. Nutrient Solutions of Passive Diffusion Periphytometers (Matlock *et al.*, 1998).

Date	Treatment	Concentration (mg/l)	Compound	Molarity (mM)
June 05	N	4.51 mg/l NO ₃ -N	NaNO ₃	0.3224
	P	0.65 mg/l PO ₄ -P	NaHPO ₄ 7H ₂ O	0.0210
	NP	4.51 mg/l NO ₃ -N, 0.65 mg/l PO ₄ -P	NaNO ₃ , NaHPO ₄ 7H ₂ O	0.3224, 0.0210
	C	RO water with nominal conductivity of 30 μS/cm		
Aug 05	N	4.51 mg/l NO ₃ -N	NaNO ₃	0.3224
	P	1.47 mg/l PO ₄ -P	NaHPO ₄	0.0473
	NP	4.51 mg/l NO ₃ -N, 1.47 mg/l PO ₄ -P	NaNO ₃ , NaHPO ₄	0.3224, 0.0437
	C	RO water with nominal conductivity of 30 μS/cm		
Jan 06	N	20 mg/l NO ₃ -N	NaNO ₃	1.4240
	P	2 mg/l PO ₄ -P	NaHPO ₄ 7H ₂ O	0.0645
	NP	20 mg/l NO ₃ -N, 2 mg/l PO ₄ -P	NaNO ₃ , NaHPO ₄ 7H ₂ O	1.4240, 0.0645
	C	RO water with nominal conductivity of 30 μS/cm		

Notes: Solution concentrations targeted to be approximately 4 to 10 times ambient nutrient concentrations reported at study sites in Beaver Reservoir Basin, Northwest Arkansas (Haggard *et al.*, 2003). C, control; N, nitrogen; P, phosphorus; NP, nitrogen + phosphorus.

	1	2	3	4	5	6	7	8	9	10
A	A1 N	A2 C	A3 NP	A4	A5	A6	A7	A8	A9	A10
B	B1 P	B2 N	B3 P	B4	B5	B6	B7	B8	B9	B10
C	C1 C	C2 NP	C3 N	C4	C5	C6	C7	C8	C9	C10
D	D1 NP	D2 P	D3 C	D4	D5	D6	D7	D8	D9	D10

FIGURE 4. Randomized Block Experimental Design. The blind treatment identification matrix used to label passive diffusion periphytometers on rack upon harvest. C, control treatment; N, nitrogen treatment; P, phosphorus treatment; NP, nitrogen + phosphorus treatment.

method was used so that the results could be compared directly with those of McFarland *et al.* (2000) in the seasonal deployments in the Bosque River, Texas. The maximum rate of uptake (V_{max}) represents a LETSI asymptote toward 1. The least-square means difference was used to optimize the half saturation constant. The inflection point of the Michaelis-Menten model (K_s) is the concentration of PO₄-P where increasing concentrations produce less algal growth response than lower concentrations. This is also the concentration that produces a LETSI of 0.5; the ambient conditions of the stream are producing 50% of the maximum potential productivity of the site. McFarland *et al.* (2000) estimated K_s of 37 μg/l for the Bosque River system in Texas.

Statistical Analyses

Chl *a* outliers were identified as data points falling outside 2 standard deviations from the mean of

each treatment-level response and excluded from the dataset. This represented <10% of the total data being removed. These outlying data were likely affected by something other than the ambient conditions and therefore justifies their removal from further analyses. Data were log-transformed, possibly introducing a downward bias; however, the transformation reduced the influence of extreme data and bound the dataset by zero (Newman, 1993). Log-transformed mean chl *a* concentrations for all treatments across sites were compared using Tukey’s test for HSD using JMP software (SAS Institute, Cary, North Carolina). Significant difference ($p \leq 0.05$) between treatments indicated nutrient limitation and was interpreted using specific nomenclature (Table 4).

Change-point analysis is a method of determining the statistically significant shift in trends across a series of ordered observations (Csorgo and Horvath, 1998). This analysis uses a cumulative sum method for iteratively identifying distinct regression characteristics of a curve, such that the point(s) of divergence from the common regression line are identified within a predefined confidence limit (95% for this analysis) (Pettit, 1980).

Simple analysis of variance (ANOVA) was used to determine significant differences between site conditions of ambient water chemistry and land use intensity as well as control treatment responses. An alpha of 0.05 was used to denote the significant difference, and therefore a *p*-value of <0.05 would result in the rejection of null hypotheses. Statements made in the following sections that reference significant difference between conditions were determined through this process.

TABLE 4. Nutrient Limitation Nomenclature.

Treatments and Tukey's HSD Groupings					
C	N	P	NP	Symbol	Interpretation
A	A	A	A	None	No nutrient limitation
A	A	A	B	N+P	Colimitation
A	A	B	B	P	Phosphorus limited
A	A	B	C	P*	Phosphorus limited, N secondary
A	B	B	B	N or P	Colimitation
A	B	C	C	P,2N	Colimitation, P primary, N secondary
A	B	C	D	P*,2N	Colimitation, P primary, N secondary
A	B	A	B	N	Nitrogen limited
A	B	A	C	N*	Nitrogen limited, P secondary
A	C	B	D	N*,2P	Colimited, N primary, P secondary
A	B	B	C	N or P	Colimitation

Notes: Designations used to interpret results of statistical analyses between treatment responses where treatments with the same letter would not be significantly different from each other ($p = 0.05$). C, control treatment; N, nitrogen treatment; P, phosphorus treatment; NP, nitrogen + phosphorus treatment; *, primary limiting nutrient. A, B, C, and D are bin groupings.

RESULTS AND DISCUSSION

Nutrients

Instream nutrient concentrations were found to be the greatest among sites downstream from WWTP effluent discharges and in subbasins with increased pastureland (Tables 5 to 7). War Eagle Creek below Huntsville WWTP (WECdn) and the White River below Fayetteville WWTP (WTRdn) consistently had the significantly higher TP concentrations compared with the other nine sites ($p < 0.0001$), ranging from 0.09 mg/l TP in August 2005 at WTRdn to 0.38 mg/l TP in August 2005 at WECdn. Highest TN concentrations were observed in the catchments of Clear Creek (CLE8615) and Clifty Creek (CLI12), which have relatively greater percentages of pastureland use (52 and 44%, respectively, $p < 0.05$), as well as below WWTPs ($p < 0.05$). TP generally decreased from June 2005 to January 2006 whereas TN remained constant across seasons (Tables 5 to 7). Seasonal trends were likely a result of a combination of flow regimes and biologically mediated phenomena.

Physical Parameters

There were no unexpected trends in measurements of pH, temperature, DO, and specific conductivity. All pH measurements were between pH = 6 and pH = 8 with the exception of the two sites located downstream from WWTPs, where the pH was consistently between 8 and 8.3. The mean temperature in the summer deployments was between 15°C and 16°C at the CLE8615 and CLI12 sites, whereas the tempera-

ture ranged from 26.3°C to 31.6°C at all other sites. In the winter, temperature ranged from 6.7°C to 12.2°C at all sites. Summer DO levels fluctuated between sites within the range of 2.8-13.6 mg/l DO, always above a critical 2 mg/l threshold for the onset of anoxic conditions. Winter DO levels increased, ranging from 9.9 to 13.4 mg/l DO.

Passive Diffusion Periphytometer Randomized Block Design Assessment

Statistical analyses showed spatial bias in several deployments ($p < 0.05$), particularly between row placements of PDPs (presumably a light variable impact). However, treatments were evenly distributed throughout the length and randomly across the width of the PDP apparatus, spreading the bias across all treatments. Therefore, there was no indication of bias created by the methodology used in this study.

Periphyton Growth

Control treatment growth responses were compared between sites to analyze periphyton growth conditions (grazer excluded) with *in situ* nutrient concentration. WTR5640 and CLE8615 consistently showed low amounts of periphyton accrual, whereas WTRdn, BRU45, and CLI12 showed relatively high amounts of periphyton accumulation on PDP control treatments. Differences across seasonal deployments in relative periphyton accrual were present, which were identified through simple ranking of data. In June and August 2005, a relative shift in trophic state was evident at a threshold of 0.350 $\mu\text{g}/\text{cm}^2$. In

TABLE 5. Water Chemistry Analyses Results.

Site	Total N (mg/l)			NH ₃ -N (mg/l)			NO ₃ -N (mg/l)			Total P (mg/l)			PO ₄ -P (mg/l)		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
MF119	5	0.45	0.22	3	0.003	0.004	4	0.689	0.076	4	0.04	0.05	4	0.004	0.0001
WTR5640	5	0.35	0.02	3	0.009	0.007	4	0.351	0.239	5	0.05	0.04	4	0.002	0.003
DRA	5	0.34	0.20	2	0.001	NA	4	0.453	0.408	5	0.06	0.04	4	0.003	0.001
WECup	4	0.25	0.19	3	0.007	0.010	4	0.167	0.242	5	0.09	0.02	3	0.005	0.003
WECdn	5	0.51	0.39	3	0.004	0.006	4	1.128	0.652	5	0.16	0.09	4	0.198	0.146
WF	5	0.36	0.14	3	0.001	NA	3	0.036	0.061	4	0.06	0.02	3	0.003	0.002
CLE	5	1.37	1.14	3	0.001	0.000	4	0.964	0.183	4	0.06	0.03	4	0.012	0.002
BRU	5	0.05	0.00	3	0.020	0.033	4	2.595	1.002	3	0.07	0.04	4	0.010	0.001
WTRup	5	0.23	0.123	3	0.003	0.004	3	0.070	0.120	5	0.07	0.04	3	0.002	0.001
WTRdn	4	2.91	1.76	1	0.001	0.000	3	2.584	1.036	4	0.26	0.15	3	0.297	0.195
CLI	5	1.57	1.07	3	0.001	0.000	3	3.274	0.021	4	0.08	0.02	3	0.009	0.001

Notes: Datasets from June 2005 collected in streams in the Beaver Reservoir Basin, Northwest Arkansas, during PDP deployments. Measurements below practical quantitative limit (PQL) were assigned a value of half the PQL for averaging purposes. NA, not applicable; n, number of observations; SD, standard deviation.

January 2006, the threshold had shifted down to approximately 0.150 µg/cm². C chl *a* measurements below the two WWTPs were significantly different than those measured above the outfalls in all deployments (*p* < 0.05), except in January on the White River. High concentrations of ammonia would be a typical characteristic of WWTP effluent (Dodds, 1991); however, we measured only small amounts in the grab samples. This may be explained by the presence of large amounts of periphyton biomass that converts this readily available nutrient source quickly to obtain an accrual rate necessary to sustain the stream food web. This finding adds to the large amount of literature that documents a change in the chemical and biotic composition of streams affected by WWTP outfalls as the nutrients and other by-products are discharged from these facilities (Goudreau *et al.*, 1993; Haggard *et al.*, 2001; Gucker *et al.*, 2006; Cary and Migliaccio, 2009).

Nutrient Impacts on Periphyton Growth

During the three deployment seasons, nutrient limitation was observed at least one time at 9 of the 11 study sites (Table 8). The Middle Fork, WTR5640, DRA295, WECup, and CLE8615 always showed some form of P limitation. BRU45 was P-limited during the June 2005 deployment, but was not nutrient-limited during August 2005 or January 2006. The West Fork showed nutrient limitation in August 2005 and January 2006, but did not show nutrient limitation in June 2005. WTRup showed no nutrient limitation in June or August 2005, but showed P limitation in January 2006. CLI12 showed no limitation in August 2005, but showed P limitation in January 2006.

N:P ratios above 23:1 were measured during at least one deployment at all sites (except WECdn), indicating that low-flow concentrations of N and P from these drainages create a phosphorus-limiting

TABLE 6. Water Chemistry Analyses Results.

Site	Total N (mg/l)			NH ₃ -N (mg/l)			NO ₃ -N (mg/l)			Total P (mg/l)			PO ₄ -P (mg/l)		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
MF119	5	0.49	0.13	5	0.0392	0.0317	5	0.391	0.173	5	0.02	0.01	5	0.011	0.002
WTR5640	5	0.53	0.06	5	0.0360	0.0421	5	0.403	0.032	5	0.02	0.01	5	0.007	0.002
DRA	5	0.25	0.06	5	0.0402	0.0414	5	0.163	0.082	5	0.02	0.00	5	0.008	0.002
WECup	5	0.14	0.06	5	0.0370	0.0441	5	0.029	0.021	5	0.02	0.00	5	0.007	0.004
WECdn	5	1.16	0.41	5	0.1074	0.0310	5	0.827	0.414	5	0.38	0.25	5	0.347	0.253
WF	5	0.40	0.10	5	0.0536	0.0199	5	0.091	0.053	5	0.04	0.00	5	0.007	0.002
CLE	5	2.09	0.03	5	0.0244	0.0370	5	2.040	0.018	5	0.03	0.01	5	0.016	0.001
BRU	5	0.58	0.19	5	0.0602	0.0449	5	0.39	0.234	5	0.04	0.01	5	0.018	0.001
WTRup	5	0.47	0.14	5	0.0816	0.0409	5	0.406	0.710	5	0.05	0.01	5	0.008	0.003
WTRdn	5	1.34	0.94	5	0.0514	0.0251	5	0.399	0.470	5	0.09	0.02	5	0.018	0.014
CLI	5	2.26	0.07	5	0.0210	0.0393	5	2.222	0.0123	5	0.02	0.00	5	0.015	0.001

Notes: Datasets from August 2005 collected in Beaver Reservoir Basin, Northwest Arkansas, during PDP deployments. n, number of observations; SD, standard deviation.

TABLE 7. Water Chemistry Analyses Results.

Site	Total N (mg/l)			NH ₃ -N (mg/l)			NO ₃ -N (mg/l)			Total P (mg/l)			PO ₄ -P (mg/l)		
	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
MF119	4	0.58	0.04	4	0.009	0.013	3	0.569	0.362	5	0.03	0.04	3	0.002	0.000
WTR5640	4	0.46	0.16	4	0.011	0.013	3	0.487	0.418	4	0.03	0.03	3	0.002	0.000
DRA	4	0.26	0.03	4	0.014	0.021	3	0.201	0.089	5	0.00	0.00	3	0.002	0.000
WECup	4	0.05	0.00	4	0.022	0.029	3	0.031	0.029	5	0.01	0.03	3	0.002	0.000
WECdn	4	1.75	0.29	4	0.016	0.017	3	2.141	0.201	5	0.22	0.11	3	0.190	0.089
WF	4	0.25	0.16	4	0.017	0.032	3	0.005	0.000	5	0.02	0.02	3	0.002	0.000
CLE	4	1.85	0.05	3	0.025	0.021	3	0.797	1.373	5	0.04	0.03	3	0.002	0.000
BRU	4	1.08	0.14	4	0.028	0.021	2	1.489	0.062	5	0.01	0.00	2	0.002	0.000
WTRup	4	0.29	0.08	3	0.027	0.034	3	0.041	0.043	5	0.04	0.03	3	0.002	0.000
WTRdn	5	2.52	0.65	4	0.008	0.014	3	1.773	1.752	5	0.13	0.13	3	0.103	0.155
CLI	4	2.11	0.022	4	0.017	0.019	3	1.646	1.153	5	0.03	0.05	3	0.002	0.000

Notes: Datasets from January 2006 collected in Beaver Reservoir Basin, Northwest Arkansas, during PDP deployments. Measurements below practical quantitative limit (PQL) were assigned a value of half the PQL for averaging purposes. *n*, number of observations; SD, standard deviation.

TABLE 8. Lotic Ecosystem Trophic Status Index (LETSI) Summary.

Site	Period	Limitation	Avg C chl <i>a</i> (µg/cm ²)	Avg NP chl <i>a</i> (µg/cm ²)	LETSI	SD (LETSI)
MF119	1	P	1.700	3.406	0.52	0.13
	2	P	1.084	2.924	0.41	0.20
	3	P	0.096	0.183	0.67	0.35
WTR5640	1	P	0.088	2.994	0.03	0.01
	2	P	0.084	3.474	0.03	0.01
	3	P	0.026	0.099	0.28	0.15
DRA295	1	P	0.984	6.710	0.15	0.07
	2	P*	0.609	4.697	0.13	0.04
	3	P	0.104	0.613	0.16	0.05
WECup	1	P	0.648	2.072	0.35	0.15
	2	N or P	0.182	1.700	0.11	0.05
	3	P	0.152	0.477	0.33	0.07
WECdn	1	None	0.198	0.208	0.98	0.27
	2	None	0.672	0.728	1.03	0.56
	3	None	0.604	0.762	0.80	0.21
WF195	1	None	0.304	0.237	1.33	0.44
	2	N or P	0.292	0.517	0.66	0.45
	3	P*	0.199	0.716	0.28	0.12
CLE8615	1	N or P	0.134	0.209	0.77	0.68
	2	P	0.363	0.756	0.88	1.06
	3	P, 2N	0.139	0.293	0.54	0.16
BRU45	1	P	1.211	4.256	0.29	0.09
	2	None	0.570	0.494	1.83	2.12
	3	P	0.223	0.762	0.32	0.11
WTRup	1	None	0.246	0.252	1.02	0.40
	2	None	0.124	0.144	1.10	0.75
	3	P	0.341	0.409	1.02	0.76
WTRdn	1	None	0.626	0.692	0.84	0.19
	2	None	1.250	1.197	1.81	2.03
	3	None	0.499	0.511	1.05	0.51
CLI12	1	Fail	Fail	Fail	Fail	Fail
	2	None	1.912	2.190	0.80	0.28
	3	P	0.346	0.464	0.78	0.26

Notes: Four sampling periods in Beaver Reservoir Basin, Northwest Arkansas: 1 – June 2005, 2 – August 2005, 3 – January 2006, and 4 – September 2005. Nutrient limitation determined using Tukey's HSD ($p < 0.05$). C, control treatment; NP, nitrogen + phosphorus treatment; LETSI, lotic ecosystem trophic status index; SD, standard deviation; *, primary limiting nutrient.

ratio (Redfield, 1958) for phytoplankton growth in the receiving Beaver Reservoir. Ratios of TN to TP (N:P supply ratios) were the least in June 2005 and

the greatest in August 2005 (Table 9). No relationship was apparent between N:P supply ratio and nutrient limitation in stream productivity. Different

TABLE 9. Nitrogen to Phosphorus Supply Ratios.

Site	June 2005		August 2005		January 2006	
	Limitation	N:P	Limitation	N:P	Limitation	N:P
MF119	P	11.0	P	43.3	P	20.6
WTR5640	P	6.6	P	77.4	P	18.1
DRA	P	5.3	P*	29.5	P	116.2
WECup	P	2.8	N or P	20.6	P	3.6
WECdn	None	3.1	None	3.3	None	8.1
WF	None	5.8	N or P	55.3	P*	13.9
CLE	N or P	22.7	P	127.4	P, 2N	52.2
BRU	P	0.7	None	32.1	N or P	173.9
WTRup	None	3.2	None	57.8	P	6.7
WTRdn	None	11.3	None	73.5	None	19.8
CLI	Fail	20.5	None	152.7	P	69.8

Notes: Ratios calculated from grab samples (nitrate to phosphate) and corresponding limiting nutrients shown in periphytometer deployments in each sampling season in Beaver Reservoir Basin, Northwest Arkansas.

species within the periphyton community may be limited by different nutrients at different times, making it difficult to detect any trend between bulk biomass accrual and N:P ratio (Allan, 1995; Stevenson *et al.*, 1996; Stelzer and Lamberti, 2001; Hall *et al.*, 2005). However, nutrients, especially P, are clearly limiting periphyton growth at many sites across the basin.

Lotic Ecosystem Trophic Status Index

The LETSI was calculated from PDP responses across all sites for three deployments. LETSI at nutrient-limited study sites ranged from 0.03 to >1.0; however, at the three sites where light limitation is suggested, responses on all treatments were consistently low, possibly resulting in inflated LETSI (Table 8). A LETSI >1 at nutrient-limited sites is a result in the variability of the data collected from the NP and C treatments. For example, during the January 2006 deployment, a LETSI of 1.02 was measured at WTRup, where there was documented P limitation (the P treatment showed a significantly different response than the C). There was also high LETSI variability, which was documented by a standard deviation of 0.76. The LETSI through all seasons were related to the ambient PO₄-P concentrations, characterized by correlated increase ($p < 0.05$), asymptoting at a LETSI = 1. This suggests the potential utility of LETSI for comparing the productivity, which varies spatially throughout an ecological system.

This relationship was similar in shape to the relationship found by McFarland *et al.* (2000); however, the response in the Beaver Reservoir Basin to low levels of the limiting P nutrient produced a much steeper lag phase than that documented in the Bosque River (Figure 5). The maximum rate of uptake (V_{\max}) was 0.999, as LETSI theoretically

asymptotes toward 1. The least square means difference was used to optimize the half saturation constant (K_s) value, estimated at 3 $\mu\text{g}/\text{l}$ PO₄-P. McFarland *et al.* (2000) estimated the K_s of 37 $\mu\text{g}/\text{l}$ for the Bosque River system, and this indicates that the periphyton community in the Beaver Reservoir system is more sensitive to increased PO₄-P concentrations, possibly due to the lack of other limiting factors (such as N or light). The low estimated K_s value supports findings of near-maximum potential productivity allowed by ambient conditions at low levels of PO₄-P (<10 $\mu\text{g}/\text{l}$ PO₄-P) when measuring low-density periphyton growth (<30 mg/m^2 chl a) (Bothwell, 1985, 1988; Ramirez *et al.*, 2003; Stevenson *et al.*, 2008). A significantly low K_s value in Beaver Reservoir Basin means that a slight increase in PO₄-P concentrations in the system would result in the possibility of reaching maximum primary production.

Change-Point Analysis

Change-point analysis using the cumulative sum method identified only one change point in the results of this study represented in the Ludwig *et al.*'s Michaelis-Menton model (Figure 5). The analysis indicated a threshold point at LETSI of 0.80 and 15 $\mu\text{g}/\text{l}$ PO₄-P. The cumulative sum method regression divergence confidence level was 95%.

CONCLUSIONS

All the study locations within the Beaver Reservoir Basin with the exception of the sites below WWTP outfalls expressed some level of annual nutrient limitation. Nutrients, mainly P, were limiting periphytic

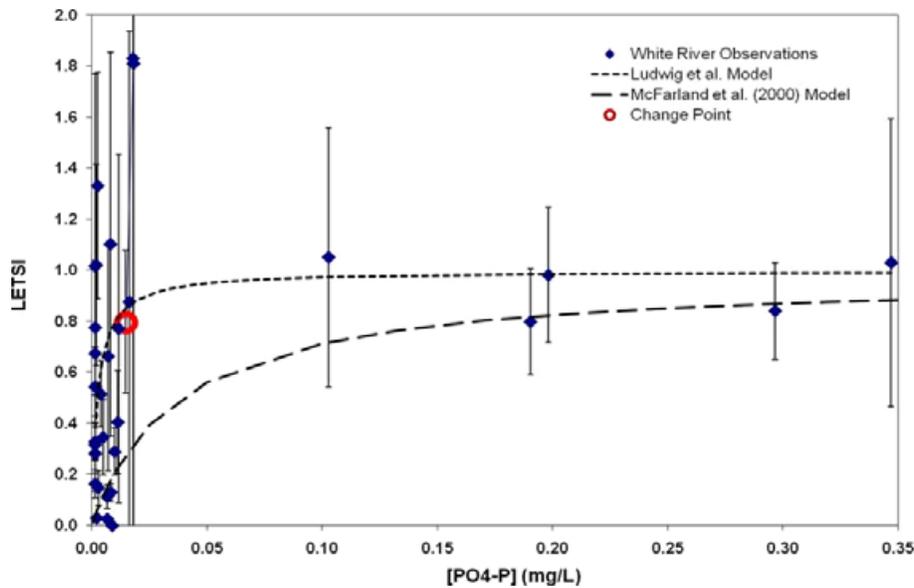


FIGURE 5. Lotic Ecosystem Trophic Status Index Determinations. Data from all periphytometer deployments in Beaver Reservoir Basin as a function of $\text{PO}_4\text{-P}$, relationship developed by McFarland *et al.* (2000) for a river system in Texas, and the relationship developed for the data collected in this study (represented as Ludwig *et al.*'s model).

algal growth at many sites in the Beaver Lake Basin. Clearly, many sites were nutrient-enriched during some periods of this study, but many of the upper reach streams measured in the Beaver Reservoir Basin had very low ambient nutrient concentrations during much of the year. As anthropogenic disturbances minimize riparian canopy cover (increasing light availability) and degrade grazer habitat (decreasing harvest), the management of waterways to keep nutrient concentrations at limiting levels to prevent nuisance growth becomes increasingly important. Periphyton biomass accrual is sensitive to a shift in any of these three dominant limiting factors (Rosemond *et al.*, 2000).

A Michaelis-Menten growth equation described LETSI as a function of ambient $\text{PO}_4\text{-P}$ concentrations ($p < 0.05$); the midpoint (LETSI of 0.50) was at a $\text{PO}_4\text{-P}$ concentration of approximately $3 \mu\text{g/l}$. Change-point analysis indicated a threshold point at LETSI of 0.80 and $15 \mu\text{g/l}$ $\text{PO}_4\text{-P}$. Periphyton in streams draining the Beaver Reservoir Basin was very sensitive to P concentrations. These results suggest that concentrations above $15 \mu\text{g/l}$ of $\text{PO}_4\text{-P}$ in the Beaver Reservoir Basin could result in significant increases in algal growth and opportunity for dissolved organic carbon inputs to the reservoir. The LETSI could serve as a management indicator for critical instream periphyton growth rates, where sites approaching their maximum potential productivity (LETSI > 0.80) would be considered impacted by NPS nutrients.

LETSI provided a quantitative relationship with available nutrients in the Beaver Reservoir Basin.

This relationship could be used to develop nutrient criteria or to prioritize streams for nutrient reduction and riparian corridor protection/restoration. In the face of an increasing amount of eutrophic waterbodies and uncertainty in the effectiveness of conservation best management practices (Boesch *et al.*, 2001), the LETSI can help planners identify stream reaches that are sensitive to nutrient inputs and predict the effects of changes in other conditions that limit the growth of harmful algae (like riparian corridor disturbance).

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