Development and Application of a Lotic Ecosystem Trophic Status Index

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Abstract. *We used the Matlock Periphytometer (Matlock et al., 1998) to measure in situ nutrient limitations and trophic status at five stream sites in the Bosque River Watershed in north-central Texas during July 1997. Periphytic chlorophyll a production from the Matlock Periphytometer was also used as an indicator of baseline primary productivity and of maximum primary productivity (MPP) in response to nutrient enrichment (nitrogen and phosphorus). The measured MPP was interpreted to represent the rate of periphytic growth when nutrients are not limiting. We developed a lotic ecosystem trophic status index (LETSI) using the ratio of baseline primary productivity to MPP. Nitrogen (N) and phosphorus (P) limitations were evaluated using a modified LETSI as the ratio of either N or P enriched growth to MPP. The LETSI is by definition a functional index, and may provide a classification tool for lotic ecosystem trophic status. Using the LETSI indices, we observed differences in nutrient limitations in the streams and detected co-limitations of nitrogen and phosphorus at two of the stream sites.*

Keywords. *Nutrient enrichment, Periphyton, Lotic ecosystem trophic status index, Nitrogen, Phosphorus.*

Lotic ecosystem responses to stress, including anthropogenic pollution, have been the subject of scientific inquiry for more than a century (Patrick, 1949). Patrick and others recognized as early as 1949 that there was a quantifiable relationship between the physical and chemical stress of lotic ecosystems and characteristics of the submerged attached micro-community, or periphyton (Patrick, 1949; Kolkwitz and Marsson, 1967). The periphytic assemblage (also referred to as the periphytic micro-community) is a useful bio-indicator due to its cosmopolitan distribution (Cairns, 1991) and the high complexity and diversity of species within the micro-community (Wetzel, 1975; Stanley et al., 1990). The periphytic community is also the principal community to accumulate and retain dissolved nutrients and toxicants (Rodgers et al., 1979; Ghosh and Gaur, 1994).

Historically, assessments of the degree to which lotic ecosystems are stressed have focused on evaluating the structural characteristics of communities within the ecosystem (Aloi, 1990). A relatively recent shift in the view of lotic ecosystem from structural, or descriptive relationships to functional levels of organization has resulted in the development of innovative methods for measuring functional indices of perturbation (Cummins, 1974; Rodgers et al., 1979). The objective of this project was to develop a lotic ecosystem trophic status index using periphyton, to make the system simple to use, to characterize the impact of nutrient enrichment on stream chlorophyll production over a two-week period, and to test this method with field studies at five stream sites.

Article was submitted for publication in June 1998; reviewed and approved for publication by the Soil & Water Division of ASAE in February 1999.

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Methods

Our approach in developing the lotic ecosystem trophic status index was to quantify the response of periphytic communities in five stream segments to nutrient enrichment and to develop an index for comparing these responses based on a measure of periphytic community function. Five stream sites in the Bosque River Watershed were selected for investigation. The study streams were sampled using the Matlock periphytometer in July 1997, to determine their limiting nutrient(s) and trophic status.

Site Description

The Bosque River Watershed covers about 430 000 ha in central Texas, 74% of which is represented by the drainage of the North Bosque River (fig. 1). Other major drainages in the Bosque River Watershed include Hog Creek and Middle and South Bosque Rivers. The northern two-thirds of the Bosque River Watershed is in the Central Plains Ecoregion, while the southern third is in the Blackland Prairie Ecoregion of Texas (Omernik, 1987). The Central Plains Ecoregion is characterized by irregular plains on sand and clay soils supporting mid-grass prairie species with a juniper, mesquite and oak savanna on many of the hills. The Blackland Prairie Ecoregion is characterized by fairly deep, dark, alkaline clay soils, tall-grass prairies, expansive forests and savannas, and hilly

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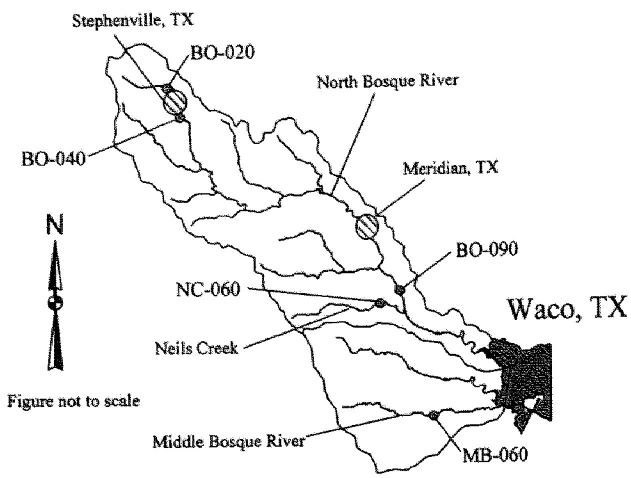

Figure 1–Location of sample sites in central Texas.

terrain. The Blackland soils are capable of supporting intensive row crop agriculture, while the Central Plains soils are primarily used for improved pasture and less frequently peanut production in the sandier areas.

Water quality throughout the Bosque River watershed has been declining due to nutrient enrichment (TCRP, 1996). In the North Bosque River drainage, dairy waste has been identified as a major water quality problem (TWC and TSSWCB, 1991). The dairy industry represents a potential source of increased nutrient loading with more than 34,000 dairy cows in the upper portion of the North Bosque River Watershed as primarily located in Erath County (McFarland and Hauck, 1997). Manure produced by these dairies is primarily surface applied to permanent pasture at rates based on crop nitrogen demand. This practice results in excess phosphorus application and soil phosphorus build-up. High in-stream phosphorus concentrations are associated with drainages dominated by dairy waste application fields in the upper portion of the North Bosque River (McFarland and Hauck, 1997). In the Middle and South Bosque Rivers, elevated nitrogen levels associated with intensive row crop agriculture are considered a threat to maintaining water quality in these two rivers (TNRCC, 1996). These nutrients threaten water quality in Lake Waco at the outlet of the Bosque River Watershed, the primary supply of drinking water for the city of Waco, Texas (with a population of about 140,000 people).

Five stream sites with different nutrient characteristics were selected in the Bosque River Watershed to compare the lotic ecosystem trophic status under varying conditions. Water quality samples at each site were analyzed for nutrients just prior to the study period on 14-15 July 1997 and again towards the end of the study on 28-29 July 1997. Samples were collected as grab samples; no measurable rainfall fell in the watershed during the sample period. Nutrient analyses for nitrite-nitrogen (NO_2^--N), nitrate-nitrogen (NO_3^--N), total Kjeldahl nitrogen (TKN), orthophosphate-phosphorus ($PO_4^{\equiv}$-P), and total phosphorus were conducted by the water chemistry laboratory at the Texas Institute for Applied Environmental Research at Tarleton State University using EPA-approved methods (EPA, 1983). Total nitrogen was derived as the sum of NO_2^--N, NO_3^--N, and TKN. Three monitoring sites were on the North Bosque River, the main drainage feature in the watershed; the remaining sites were on Neils Creek, a sub-watershed of the North Bosque River, and on the Middle Bosque River, a major drainage to Lake Waco (fig. 1).

Progressing down-watershed, the first site (BO-020) is on the North Bosque River above Stephenville, Texas (pop. 14,000). This is a third-order stream with silt-clay substrate, steep banks, low base flow, and closed canopy. The stream is 3 to 4 m wide and 1.5 m deep at BO-020. About 12% of the land area above BO-020 is used for dairy waste application. The next site (BO-040) is on the North Bosque River below the Stephenville Waste Water Treatment Plant (WWTP), about eight river kilometers below BO-020. The North Bosque River is a fourth-order stream at this location, 5 to 6 m wide and 1.5 m deep, silt-clay bottomed with open canopy. While dairy production is an important feature in the drainage area above BO-040, the predominant source of nutrients at base flow at BO-040 is the Stephenville WWTP (McFarland and Hauck, 1997). The last site on the North Bosque River is BO-090, a fourth-order stream at Clifton, Texas (pop. 3,400), about 95 river kilometers below site BO-040 and 64 km above Lake Waco. The drainage area for BO-090 is approximately 254 000 ha, with about 4% of land area designated for dairy waste application. There are four small municipal WWTPs contributing nutrients to the river above BO-090 as represented by the cities of Stephenville, Hico (pop. 1,400), Iredell (pop. 350), and Meridian (pop. 1,400).

The Middle Bosque Sub-watershed covers approximately 31 000 ha, and the land use is predominantly cropland and rangeland. The sample site (MB-060) has episodic nutrient loading associated with runoff, but is not as nutrient-enriched as BO-040 or BO-090 (table 1). This site is located about 19 km upstream from Lake Waco and east of the city of Crawford (pop. 600). The river is third order, about 1.5 m deep and 10 m wide, with gravel, cobble, and boulder substrate. The canopy is open, and there is very little sediment in the water.

The final site is on Neils Creek (NC-060); this is the least nutrient enriched stream in the watershed and is considered the reference water body for this study. Neils Creek is very similar in geomorphology to MB-060. The primary land uses in the drainage above NC-060 are forest and native rangeland.

Table 1. Water quality data from five stream sample sites in the Bosque River Watershed, composed of grab samples from 14 and 29 July 1997 (McFarland and Hauck, 1997)

		Water Quality Constituent			
Site	Date	Nitrate-Nitrogen (mg L^{-1})	Total Nitrogen (mg L^{-1})	Ortho-Phos-phorus (mg L^{-1})	Total Phos-phorus (mg L^{-1})
BO020	14 Jul 97	1.89	4.25	0.38	0.42
	28 Jul 97	2.64	3.98	0.18	0.26
BO040	14 Jul 97	1.80	3.37	0.58	0.39
	28 Jul 97	1.53	3.00	0.58	0.79
BO090	15 Jul 97	0.01	1.26	0.03	0.22
	29 Jul 97	0.07	0.92	0.02	0.10
MB060	15 Jul 97	0.66	0.96	0.02	0.08
	29 Jul 97	0.02	0.32	0.01	0.04
NC060	15 Jul 97	0.42	0.77	0.03	0.04
	29 Jul 97	0.34	0.44	0.01	0.04

Measuring Limiting Nutrients

Limiting nutrients (N and/or P) were determined for each stream site using Matlock Periphytometers (Matlock et al., 1998, with modification). Matlock Periphytometers (MPs) were constructed of a 0.45 μm nylon membrane filter (Cole Parmer CN 2916-44) as a biofilter and Whatman 934-AH glass fiber filter as the growth substrate, attached to the top of a 1-L low density polyethylene container with a 2.5-cm-diameter hole cut in the lid (fig. 2). The bottles were filled with the appropriate nutrient solution, and attached to a floating rack. The four nutrient enrichment treatments were:

1. Control (C), consisting of deionized water, with a nominal conductivity of 30 µS cm.
2. Nitrate (N), consisting of a solution of 0.35 mM (30 ppm) $NaNO_3$ in deionized water.
3. Phosphate (P), consisting of a solution of 0.11 mM (30 ppm) of Na_2HPO_4-$7H_2O$ in deionized water.
4. Nitrate and Phosphate (N+P), consisting of a solution of 30 ppm $NaNO_3$ and 30 ppm of Na_2HPO_4-$7H_2O$ in deionized water.

The periphytometer treatments were arranged in a randomized block design consisting of a treatment array of four treatments per block, and ten replicates of each block per site (fig. 3). Each treatment array of 40 MPs was supported on an iron wire frame, attached to PVC pontoons, and anchored in the middle of the river at the sample site. The MPs were attached to the wire frame with growth surfaces perpendicular to the water surface and parallel to stream flow. The treatment arrays were anchored to the middle of the stream in a run approximately 1 m deep. The algal growth surfaces were protected from fish and macro-invertebrate grazing by placing an aluminum screen (8 mesh, or approximately 3 wires/cm, 0.7-mm-diameter wire) over the face of the racks, approximately 5 cm from the glass fiber filter growth surfaces.

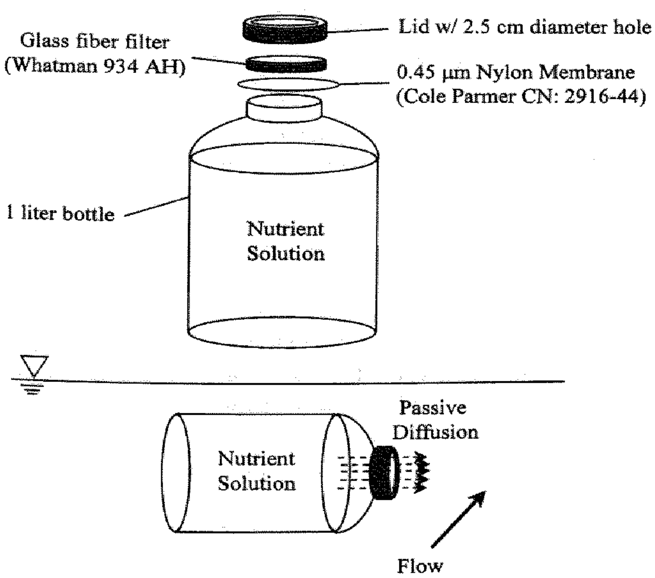

Figure 2–Diagram of Matlock Periphytometer.

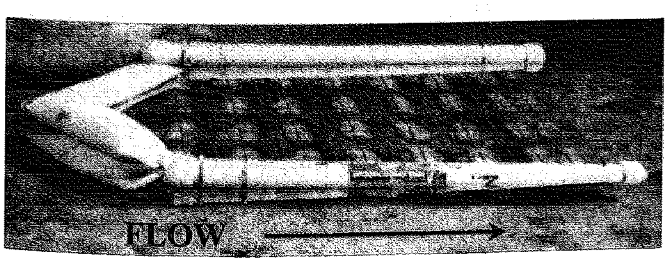

Figure 3–Matlock Periphytometer treatment arrays.

At the end of the growth period (from 17-30 July 1997) the colonized glass fiber filters were placed in 5 mL of 90% acetone solution saturated with magnesium carbonate at 5°C, wrapped in aluminum foil, and transported to the laboratory for analysis. Chlorophyll was extracted from the filters for direct measurement in the laboratory using EPA Standard Method 10200H.3 (APHA, 1989). Chlorophyll *a* from each sample site were expressed as mass (µg) per unit of exposed surface area of the filter (6.6 cm^2) for comparison. Mean chlorophyll *a* concentrations for all treatments across sites were compared using Student-Newman-Kuels' (SNK) Test (α = 0.05) in SAS/STAT© (SAS Institute Inc., 1990). Unequal replicates due to sample loss were corrected using the second approximation method as described by Steel and Torrie (1980).

Lotic Ecosystem Trophic Status Index

We developed the lotic ecosystem trophic status index (LETSI) as a tool for making comparisons of stream biotic response to nutrients. The underlying assumption of this index is that the Matlock Periphytometer N+P treatment provides a measurement of maximum potential productivity (MPP) of a stream at a given site over the sampling period. The MPP represents the level of periphytic primary productivity (measured as chlorophyll *a* production) that should occur when nutrients are not limiting. The LETSI is then defined as the ratio of the baseline primary productivity (Matlock Periphytometer control treatment) to the MPP.

The LETSI represents the proportion of maximum potential productivity manifested in the stream during the sample period. The theoretical LETSI ranges from 0 to 1, increasing as the periphytic response to nutrient enrichment declines. A stream with a LETSI of 0.50 would be at 50% of its MPP, or potential growth response to nutrients. A LETSI of 1.0 would indicate a stream is at its maximum potential productivity, and adding nutrients will not increase chlorophyll *a* production in the periphytic community. We evaluated periphytic response to phosphorus, nitrogen, and phosphorus plus nitrogen enrichment using the LETSI concept. The phosphorus LETSI (P-LETSI) is the ratio of chlorophyll *a* from phosphorus enriched treatment to the N+P treatment (MPP). Likewise, the nitrogen LETSI (N-LETSI) is the ratio of chlorophyll *a* from nitrogen enriched treatment to MPP. We calculated these indices for each data set.

Results

Nutrient concentrations monitored in the streams before and during periphytometer deployment indicated that of the five sites, site BO-040 had the highest concentration of ortho-phosphorus and BO-020 had the highest concentration of nitrate (table 1). Periphytic biomass production in Neils Creek (NC-060), the reference sub-watershed, is phosphorus limited, MB-060 and BO-090 were co-limited by N and P, and BO-020 and BO-040 were limited by something other than nutrients (table 2).

Table 2. Student-Newman-Keuls' (SNK) test comparison of chlorophyll *a* concentrations for control, Nitrogen (N), Phosphorus (P), and Nitrogen plus Phosphorus (N + P) treatments using the Matlock Periphytometer in the Bosque River Watershed during the period of 17-30 July 1997

Site	Treatment	Number of Replicates	Mean Chl. A (μg cm^{-2})	Standard Deviation (μg cm^{-2})	SNK Group (α = 0.05)*
BO-020	Control (baseline)	10	0.88	0.60	A B
North Bosque	N	10	0.95	0.38	A B
River above	P	9	1.06	0.53	A B
Stephenville, Tex.	N + P (MPP)	10	0.98	0.53	A B
BO-040	Control (baseline)	10	4.58	0.86	E
North Bosque	N	10	5.10	1.61	D E
River below	P	9	6.22	1.91	D E
Stephenville, Tex.	N + P (MPP)	10	5.19	1.86	D E
BO-090	Control (baseline)	7	1.73	0.37	B C
North Bosque	N	5	2.08	0.38	B C
River above	P	9	2.49	0.51	C
Clifton, Tex.	N + P (MPP)	8	5.73	1.36	D E
MB-060	Control (baseline)	10	0.47	0.20	A
Middle Bosque	N	9	0.52	0.08	A
River at	P	9	0.90	0.32	A B
SH185	N + P (MPP)	10	5.87	1.93	D E
NC-060	Control (baseline)	10	0.48	0.14	A
Neils Creek	N	10	0.59	0.17	A
at SH6 - Reference	P	9	2.70	1.16	C
Sub-watershed	N + P (MPP)	10	2.67	0.82	C

* Similar letter represents similar group, within statistical parameters (α = 0.05).

According to the SNK grouping (α = 0.05), site NC-060's base chlorophyll *a* productivity of 0.48 μg cm^{-2} was not significantly different from sites BO-020 (0.88 μg cm^{-2}), and MB-060 (0.47 μg cm^{-2}). Similarly, the MPP at site MB-060 (5.87 μg cm^{-2}) was not significantly different from sites BO-040 (5.19 μg cm^{-2}), and BO-090 (5.73 μg cm^{-2}). Site BO-040, the North Bosque River below the Stephenville WWTP, had the highest base chlorophyll *a* productivity of the five sites (4.58 μg cm^{-2}).

The Lotic Ecosystem Trophic Status Indices calculated for these five sites ranged from 0.08 to 0.90 (table 3). The lowest LETSI was measured at MB-060, while the highest was measured at BO-020. Sites BO-020 and BO-040 are at MPP, while BO-090 is only at 30% of its maximum production potential. The reference stream site, NC-060, is at approximately 20% of its MPP.

Discussion

The value of the LETSI is illustrated in comparing BO-020, BO-040, and BO-090. A cursory examination of productivity estimates (chlorophyll data) suggests that BO-020 was less enriched by nutrients than the other two sites (table 2). However, according to the LETSI, site

Table 3. Lotic Ecosystem Trophic Status Indices (LETSIs) reflecting the ratios of the control, nitrogen-, and phosphorus-enriched treatment chlorophyll *a* concentrations (μg cm^{-2}) with the total nutrient concentrations for Matlock Periphytometer samples collected from the Bosque River Watershed during the period of 17-30 July 1997

Sample Site	Base Productivity*	LETSI	N - LETSI	P - LETSI
BO-020	0.88	0.90	0.97	1.08
BO-040	4.58	0.88	0.98	1.20
BO-090	1.73	0.30	0.36	0.43
MB-060	0.47	0.08	0.09	0.15
NC-060	0.48	0.18	0.22	1.01

* Measured as chlorophyll *a* production (μg cm^{-2}) over 14 days on a control surface.

BO-020 is near its maximum nutrient assimilative capacity (table 3). Table 1 also indicated relatively high N and P concentrations at BO-020. Something other than nutrients is limiting algal growth to a very low base productivity level (less than 1 μg cm^{-2} chlorophyll *a*). An obvious potential explanation is that light is limiting algal productivity at this site. This illustrates very clearly why a comparative measure of response to nutrient enrichment is critical. A simple measure of chlorophyll *a* at this site would suggest primary productivity was low, without also suggesting that nutrients are not limiting growth at the site. A common misinterpretation of this type of data has been that the site is not nutrient enriched.

While similar MPP values were indicated at three sites BO-040, BO-090, and MB-060 (table 2), the LETSI values indicated distinct differences in nutrient availability between these three sites (table 3). For example, the MPP at BO-040 is the same as BO-090 (59 miles downstream), yet the LETSI downstream is about 30% of upstream. The maximum assimilative capacity of the stream has not changed, but the nutrients available for uptake have decreased, suggesting that the nutrients have been assimilated or otherwise rendered unavailable to the periphytic community between BO-040 and BO-090. This procedure provides one way to measure ecological nutrient assimilation processes through the periphytic community's response to nutrient enrichment. There are occasions when the LETSI ratio is greater than 1.0 (see table 3, sites BO-020 and BO-040). These results suggest that either the system being measured has high variability (noisy), or there is an opportunistic algal species present. Taxonomic characterization of samples recovered provides some insight into this process, and has since been incorporated into the standard approach for MP analysis.

Analyzing limiting nutrients using the LETSI differs from previous methods by comparing responses to nutrient enrichment to maximum potential responses, thus providing a perspective for comparison (table 3). The ratio of a nutrient enrichment response to the total maximum productivity provides a comparative analysis of the role of that nutrient in limiting primary productivity. The LETSI approach should not be used in the place of standard nutrient analyses, but should be used in concert with them to provide biological significance to the nutrient data.

Classic nutrient limitation theory was based on the response of individual organisms or monocultures of plants to nutrient limitations, and states that only one nutrient limits the growth of a plant at a time (Lawes and Gilbert, 1880). The periphytic community is not a monoculture, however, and responds to nutrient enrichment in a more complex manner. The periphytic community in an episodically nutrient-enriched stream may engender populations of algae that sequester nutrients during times of excess via luxury consumption, releasing these nutrients to the community during times of nutrient stress. The relatively low MPP in NC-060 illustrates this principle. The base productivity of NC-060 and MB-060 are similar and the base flow nutrient concentrations are fairly similar during the two week period measured, yet the MPP of MB-060 is double that of NC-060. The most obvious explanation is that the periphytic community in MB-060, subjected to episodic nutrient loading from agricultural runoff from row crops, has acclimated to take advantage of high nutrient loading. While no community taxonomy was performed (a significant deficiency), it is reasonable to expect a difference in the periphytic community structure between these sites. This is the basis for periphytic community structure as a bio-indicator: LETSI is a measure of periphytic community function rather than structure. These data also illustrate the difficulty in applying the Reference Site approach with biological indicators. The bio-indicator communities develop pollution tolerance, in some cases becoming less responsive to environmental change in unpolluted sites than polluted sites.

The response of the periphytic community to nutrient enrichment observed in MB-060 and BO-090 during the sampling period suggested that nitrogen and phosphorus were simultaneously limiting. These conclusions are best explained by considering that nutrient limitations can be induced by providing another nutrient in excess; the nutrient stimulation response of the periphytic community may be considerably different from its response to the absence of a nutrient.

Conclusion

The proposed LETSI index is analogous to the algal growth potential test (AGPT) using the *Selenastrum capricornutum* bottle assay in lakes (Raschke and Schultz, 1987). This assay measures the growth of *S. capricornutum* in bottles filled with lake water (control) and nutrient-enriched media to determine the ratio of the baseline growth to MPP. As with the index we propose, Raschke and Schultz's assay is based on the premise that the maximum yield is proportional to the amount of nutrient which is present and biologically available in minimal quantity with respect to the growth requirement of algae (APHA, 1989). A trophic status index for lentic ecosystems was developed based on the AGPT, and has been applied to lakes and reservoirs in the southeastern U.S. for over a decade (Vollenweider, 1974; Raschke and Schultz, 1987).

As with any broadly applied measure of community productivity, there are many sources of variability that must be recognized and addressed when using this method. These sources include grazing, turbulent or laminar scouring, light limitation, siltation, and temporal fluctuations in stream velocities. The growth surfaces were protected from fish and macro-invertebrate grazing by placing an aluminum screen over the surface of the treatment blocks. The screen reduced direct light by approximately 12% for all treatments (Matlock et al., 1998). Sites were selected to avoid scouring under normal (base) flow. Siltation was minimized by orienting the growth surfaces perpendicular to the stream surface. High flow events were avoided as much as possible by sampling during low rainfall seasons. The aluminum screen reduced flow across the growth surfaces, which also reduced scouring. Light and temperature were uniformly distributed across experimental blocks. The number of replicates was selected to provide an acceptable degree of confidence in the treatment response.

Acknowledgments. Research reported in this investigation was supported in part by the U.S. Environmental Protection Agency, the Oklahoma Conservation Commission, the United States Department of Agriculture—National Needs Fellowship Program, the Oklahoma Agricultural Experiment Station and Oklahoma Cooperative Extension Service. This research is a contribution to United States Department of Agriculture—Cooperative State Research Service Regional Project S-249. The authors would like to give a special note of appreciation to the Texas Institute for Applied Environmental Research for their generous assistance.

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