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A Tale of Two Sylamores: Understanding Relationships Among Land Use, Nutrients, and Aquatic Communities Across a Subsidy-Stress Gradient

Danielle Braund
University of Central Arkansas

Andrew Feltmann
University of Central Arkansas

George Gavrielides
University of Central Arkansas

Katherine Lang
University of Central Arkansas

Jennifer Main
University of Central Arkansas

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Erratum

Due to a submission error, an earlier version of this manuscript was entered into the hard copy print edition of Volume 73. The manuscript on this website is the corrected and current version of this article.

Authors

Danielle Braund, Andrew Feltmann, George Gavrielides, Katherine Lang, Jennifer Main, Anastasia Mogilevski, Justin Mosbey, Rebecca Relic, Calvin Rezac, Brianna Trejo, Ginny Adams, and Steven R. Adams

A Tale of Two Sylamores: Understanding Relationships Among Land Use, Nutrients, and Aquatic Communities Across a Subsidy-Stress Gradient

D.M. Braund, A.J. Feltmann, G.B. Gavrielides, K.L. Lang, J.R. Main, A.K. Mogilevski, J.H. Mosbey, R.L. Relic, C.R. Rezac, B.K. Trejo, G.L. Adams, and S.R. Adams*

Department of Biology, University of Central Arkansas, Conway, AR 72035

*Correspondence: radams@uca.edu

Running Title: Subsidy-Stress Gradients in the Sylamore Creek Watershed

Abstract

Agricultural land use can negatively impact primary producers, macroinvertebrates, and fishes. Small-scale changes in land use can subsidize an aquatic ecosystem, where an increase in nutrients allows nutrient-limited biota to flourish, and minor increases in sedimentation may help support populations of collector-filterers. The stimulation in performance caused by small disturbances is part of the subsidy-stress gradient, where increasing perturbation subsidizes an ecosystem until a certain threshold is reached, at which a decline in performance and increased variability starts to occur. The North and South Sylamore watersheds provide a useful template to investigate the subsidy-stress gradient in relation to land use. North Sylamore flows through the Ozark National Forest and had a heavily forested catchment, while South Sylamore flows through mostly private land, some of which was pasture (23%). Physicochemical, macroinvertebrate, and fish data were collected from four sites within each watershed to determine if South Sylamore was exhibiting a response to pasture/agriculture characteristic of a subsidy-stress gradient. Sites within South Sylamore had significantly higher nitrate levels, larger macroinvertebrate populations dominated by collector-filterers, and greater abundance of algivorous fish, suggesting South Sylamore may be subsidized by the surrounding pastoral lands. However, South Sylamore also had a significantly lower proportional abundance of sensitive macroinvertebrate taxa and more unique tolerant fish taxa, suggesting South Sylamore is experiencing stress as well. Habitat quality of South Sylamore could be improved by restoration of trees within the riparian zone. Monitoring aquatic systems for subsidy-stress responses can inform restoration/management decisions and guide intervention prior to watersheds and aquatic communities becoming overly stressed.

Introduction

Civilization has long been understood to both enhance and impair natural environments depending on the magnitude and scale of impact, yet the idea of the "subsidy-stress gradient" has only recently gained prevalence in ecological fields since its conception in Odum *et al.* (1979). The subsidy-stress gradient describes a response where increasing perturbation stimulates performance until a certain threshold is surpassed, at which a decline in performance and increased variability starts to occur (Odum *et al.* 1979). Numerous studies document subsidy-stress responses within aquatic ecology, such as macroinvertebrate community biomass responses to phosphorus gradients (King and Richardson 2007), bacterial abundance responses to salinity gradients (Kefford *et al.* 2004), and periphyton responses to water velocity gradients (Biggs *et al.* 1998). Land-use gradients in catchments have also been shown to induce subsidy-stress responses in stream communities, whereby invertebrate density and algal biomass were higher in catchments with more pastoral land cover (Niyogi *et al.* 2007).

Subsidy-stress responses are crucial to understand given land-use alteration could reach a threshold level inducing substantial change to stream ecosystems. Identifying ecological thresholds is important for conservation but difficult to quantify. However, increased forest loss led to increased variance in community metrics of some Neotropical forest animal communities (Roque *et al.* 2018). Resultant losses in biodiversity could in turn affect ecosystem processes, particularly in ecosystems that experience fluctuations on seasonal time scales (Keddy and Reznicek 1982). Thus, catchment systems with relatively little agricultural land-use could provide an excellent model to study the subsidy-stress hypothesis in relation to land-use gradients. Whereas small increases in pastoral land-cover and subsequent abiotic shifts may subsidize stream biodiversity, increases in heteroscedasticity of community metrics may be indicative of an

approaching threshold (Barnosky *et al.* 2012; Roque *et al.* 2018).

Agricultural land use has been recognized as degradative to streams because of its nonpoint-source pollution and alterations to stream channel flows, riparian zones, and instream habitat (Allan 2004). Higher agricultural land use is associated with higher inputs of nutrients, sediments, and pesticides into streams. High nutrient loads support more algal growth in streams (Smith *et al.* 1999), which can lead to hypoxic conditions in localized areas of slow-moving water (Allan 2004). High sediment loads have also been shown to negatively impact primary producers, benthic macroinvertebrates, and fish through a number of mechanisms, including light obstruction, impaired respiration, and reduced suitability of substrate habitat (Wood and Armitage 1997; Piggott *et al*. 2015; Waite *et al*. 2019).

Conversely, elevated nutrient and sediment loads from increased agriculture could subsidize stream communities if they do not surpass a stress threshold. For example, nitrogen and phosphorus inputs could support biotic diversity due to their limited availability (Johnson *et al.* 2009), as shown in a study where higher numbers of macroinvertebrate taxa were found in pastoral lands with higher nutrient loads (Riley *et al.* 2003). Excessive sedimentation may interfere with feeding by collector-filterers if nets are buried by fine sediment, but minor increases in organic matter sedimentation may support their populations (Angradi 1999).

The North and South Sylamore watersheds in north Arkansas may provide a useful comparison by which to investigate the subsidy-stress gradient in relation to land use. North Sylamore Creek is located in the Springfield-Salem Plateau section of the Ozark Plateau in north-central Arkansas, and flows southeast into the White River (Mast and Turk 1999). South Sylamore Creek is located just south of North Sylamore Creek, and confluences with North Sylamore Creek just upstream of its confluence with the White River (Figure 1). North Sylamore Creek has remained mostly within a protected area (Ozark-St. Francis National Forest) since the early 1900's (Strausberg and Hough 1997); therefore, its land cover consisted primarily of forest and habitat surrounding North Sylamore Creek was relatively undisturbed (Mast and Turk 1999). Land cover in South Sylamore Creek catchment had more agricultural activity, with ~20% of the land cover consisting of pastoral land, but the catchment remained heavily forested (~ 75%). Based on these land-use patterns, it is possible to see a subsidizing effect of pastoral land on the South Sylamore stream community, as influenced by the physicochemical factors associated with agricultural land-use.

We hypothesized that in comparison to North Sylamore Creek, South Sylamore Creek would exhibit indictors of subsidy and/or stress based on responses to increased pasture land use. Increased runoff and nutrients from pasture land use may have resulted in increased basal food subsidies (*e.g.*, fine particulate organic matter and periphyton); therefore, we expected to find higher macroinvertebrate abundance, higher proportions of filter-feeding invertebrates, and higher proportions of scraping/grazing invertebrates and fish in response to food subsidies. In contrast, increased pasture may have led to harsher abiotic conditions (*e.g.*, lower dissolved oxygen, higher temperatures, higher contaminants, etc.) resulting in decreases in taxa richness and relative abundance of sensitive macroinvertebrates and fishes and increases in tolerant taxa in South Sylamore relative to North Sylamore. Through measuring overall habitat quality and community metrics in each system, we assessed whether South Sylamore Creek appears to be subsidized and/or stressed by moderate increases in pasture land use relative to the more forested North Sylamore Creek.

Methods

Study Site

Sylamore Creek is a spring-fed stream that flows east through the Springfield Plateau in the Ozark Highlands ecoregion of northern Arkansas. It is a 5th order tributary to the White River composed of two 4th order branches or subcatchments, North Sylamore Creek (NS) and South Sylamore Creek (SS). The two confluence to form Sylamore Creek approximately 0.8 river kilometers upstream of the White River. The NS flows through a highly forested, public land riverscape while SS flows through mainly private land. According to the National Land Cover Database 2011 (NLCD), land cover in NS is composed of 95% forest, 2% pasture, and 3% development. In the SS system, land cover consists of 72% forest, 23% pasture, and 5% development. We selected eight total sites on the longest perennial reach of each system: four sites on NS and four sites on SS (Figure 1). All four sites on NS were on the NS main stem: Barkshed (BD), Gunner Pool (GP), Blanchard Springs (BS), and Rosa Hole (RH). Four sites were selected along the Roasting Ear Creek/South Sylamore continuum as it represented the longest contiguous perennial segment: Roasting Ear/Mill Creek (RM), Roasting Ear Clearwater (RC), Double Bridge (DB), and

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Angler's Resort (AR). All sites were sampled once during fall of 2018 (14-15 September or 12-13 October) (Table 1).

Land Use/Land Cover (LULC)

Upstream catchments of each site were delineated using StreamStats (United States Geological Survey). Using ArcGIS Desktop 10.6 and the 30-meter resolution National Land Cover Dataset (NLCD) 2011 raster file, land cover percentages (forest, pasture, and development) were calculated for each site's upstream catchment. Wooded riparian buffer width (up to 200 m) was calculated at each site using Google Earth satellite imagery and the measuring tool. Ten measurements (five equidistant measurements per bank) were made corresponding to reach length and averaged for comparison. Measurements greater than 200 m because of contiguous forest were recorded as "200" when calculating means.
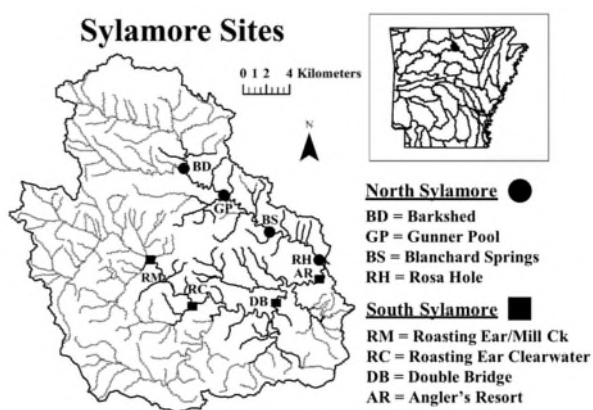

Figure 1. Sylamore Creek watershed with sites labelled corresponding to which system they are in. Dotted grey lines represent intermittency. Inset map in the top right represents the watershed shaded black.

Physicochemical

At each site we measured dissolved oxygen (DO; mg/L), specific conductivity (μS/cm), and temperature (°C) using a YSI 85 handheld water quality meter (Yellow Springs Instruments Inc., Yellow Springs, OH). Turbidity (NTU) was measured with a Hach 2100P Turbidimeter. Within the selected stream reach, we chose two representative riffles and depending on its length, created two or three transects perpendicular to the riffle, giving a total of four to six transects per site. We measured the width of each transect in meters. At each of five points distributed evenly across each transect, we measured depth and substrate (modified Wentworth Scale [Cummins 1962]: bedrock, boulder, cobble, pebble, gravel, sand/silt). At least one transect per riffle, we measured stream

velocity using a Marsh-McBirney flow meter (FloMate 2000, Marsh-McBirney Inc., Frederick, MD) at five equidistant points to calculate discharge.

Filtered (500 ml) water samples were collected upstream at each site for both nitrate and phosphate. Water samples were kept on ice until laboratory analysis. Nitrate was analyzed following the Cadmium Reduction Method (Hach 2015; Method #8192). Phosphate was analyzed using the Ascorbic Acid Method (Hach 2015; Method #8048).

Unfiltered (1 L) water samples were collected upstream at each site for total dissolved solids (TDS) and total suspended sediments (TSS). Water samples were kept on ice until laboratory analysis. To measure TSS, the unfiltered water sample was filtered through an ashed, pre-weighed Whatman® glass microfiber filter (4.7 cm diameter; 1.6 microns pore size) and then weighed again after drying in the oven at 60 °C. Difference in dry filter weight before and after filtration was TSS (mg/L). TDS was measured by collecting the filtrate from TSS in a pre-weighed container. Containers were placed in the drying oven at 60 °C for the liquid to evaporate, and the container was re-weighed to get TDS (mg/L).

Chlorophyll a

Six periphyton samples per site were collected using a divot sampler (sample area equaled 4.91 cm^2) following Lamberti and Steinman (1997) and Burgad *et al.* (2018). Periphyton samples were collected at approximately 25%, 50%, and 75% of wetted width. Specific sampling locations were determined by haphazardly tossing a 0.5 m^2 polyvinyl chloride frame and sampling the largest rock within the frame. Divot samples were primarily taken on cobble in riffles at depths of 2-55 cm. A spherical crown densiometer was used to measure canopy cover corresponding with each periphyton sample. Samples (periphyton slurries) were filtered in the field with pre-weighed filters and kept on ice until laboratory analysis.

The laboratory analysis followed Havel (2016), where filters were heated in a 95% ethanol solution (5 minute exposure at 78°C), kept in the dark (24 hrs), and centrifuged. A 0.25 M HCl acid addition was used to correct for pheophytin (degradation products) in samples. Concentrations of chlorophyll *a* (μg/L) were determined using the volumetric formula described in Havel (2016). In some instances, the entire sample extracted with the divot sampler could not be filtered; therefore, we recorded volume filtered in the field for each sample and used the volumetric formula.

Macroinvertebrates

Standard benthic macroinvertebrate collections were made using a 25.4 cm x 30.5 cm D-frame dip net having 500 micrometer mesh generally following Barbour *et al*. (1999). A composite sample for each site consisted of six kick sets stratified across riffles similar to periphyton samples. Substrate was disturbed by foot ~ 0.5 m upstream of the dip net, and any large substrates were rubbed by hand. Samples were preserved in 70% ethanol. All individuals were sorted and identified in the laboratory using Merritt and Cummins (1996), McCafferty (1998), and Voshell (2002). Identifications were to the family level with the following exceptions: order level for Amphipoda, Isopoda, and Oligochaeta, genus level for *Hexatoma* sp. and *Corbicula* sp., and Chironomidae were separated into either sub-family Tanypodinae or non-Tanypodinae.

Functional feeding group designations were made following Barbour *et al*. (1999) and Cummins *et al*. (2005). Taxa assigned to multiple feeding groups were split evenly across groups following Greathouse and Pringle (2006). Tolerance values were assigned to taxa based on multiple sources in an attempt to assign values that best fit taxa in our watershed. Preference was given to values reported from systems in proximity to Arkansas. Tolerance values were predominantly taken from Bressler *et al*. (2006) since they dealt with macroinvertebrates in Mississippi. Taxa not listed in Bressler *et al*. (2006) were taken from Lenat (1993), as those values reflected macroinvertebrates in North Carolina. Lastly, tolerance values for any remaining unassigned taxa were taken from Barbour *et al*. 1999. Tolerant taxa had values equal to or greater than 7, and intolerant taxa had values $\leq$ 3 (Hotz 2010). Hilsenhoff Biotic Index values were calculated for each site (Hilsenhoff 1987). Biotic index values ranged from 0 to 10, where 0 indicated excellent water quality and 10 indicated very poor water quality.

Fishes

Fishes were sampled in riffles, runs, and pools within a 174 to 317 m reach at each site. Fish were sampled in different riffles than macroinvertebrates and periphyton. Composite fish samples per macrohabitat were collected using a 1.2 x 4.6 m seine (pools and runs) and a 1.2 x 2.4 m seine (riffles), both having a mesh size of 0.48 cm. Macrohabitats were exhaustively sampled with kick sets and hauls as described by Matthews (1986 and 1990) and Burgad *et al* (2018). Larger individuals collected were identified, counted, and released in the field. Smaller fishes were fixed in 10% formalin and returned to the lab for identification (UCA IACUC Protocol # 17-004). All specimens were identified to species, stored in 70%

ethanol, and catalogued into the UCA Fluid Vertebrate Collection. Fishes were classified as tolerant or sensitive following Dauwalter *et al*. (2003) for Ozark fishes. Percent relative abundances of Centrarchidae and grazing fishes (*Campostoma* spp.) were calculated as common indicators of disturbance in Ozark streams (Dauwalter *et al*. 2003).

Data analysis

Most statistical tests were performed in R (version 3.4.4; R Core Team 2018). Means of abiotic and biotic variables comparing SS (n=4) versus NS (n=4) were evaluated with Student's t-test (Welch's adjustment). A Log_{10} transformation was performed with data that did not meet assumptions of normality. If normality could not be attained, means were tested with Wilcoxon Rank Sums Test. Spearman's rank correlation (rho) examined relationships between key continuous variables of interest (Chl *a* versus canopy cover, nutrients, and grazers). Macroinvertebrate FFGs and fish assemblage structures were evaluated with Nonmetric Multidimensional Scaling (NMDS) ordination performed in PCORD Version 6. Relative abundances of FFGs and fishes were arcsine square root-transformed. Distance matrices were constructed using Bray-Curtis dissimilarity. Multi-response permutation procedures (MRPP) tested null hypotheses that FFG composition and fish community structure were equal between NS and SS. Significance was determined using an alpha level of 0.05.

Results

Land use/land cover

Upstream catchments of sites in NS ranged from 83.9 to 197.9 km^2 and those in SS 119.2 to 367.8 km^2 (Table 1). Larger catchment sizes in SS were confounded by much of the upstream portions of the watershed being intermittent (Figure 1). All sites in NS had nearly 95% forest cover within upstream catchments. All sites in SS had at least 20% less forest cover in their upstream catchments. Conversely, SS catchments had 19-25% pasture while NS had less than 3%. There was a small portion of development in every upstream catchment, but most of the SS sites had slightly more development from sprawl of Mountain View in the southeast corner of the watershed.

Mean wooded riparian buffer was highest at NS sites (146.4 m) relative to SS sites (37.1 m) (p = 0.03). Mean riparian buffer across NS sites ranged from

200.0 m at Barkshed to 103.0 m at Rosa Hole. Mean wooded riparian buffer across SS sites ranged from 67.0 m at Roasting Ear/Clearwater to 21.9 m at Angler's Resort.

Physicochemical

Pebble was the dominant substrate at all sites except Blanchard Springs (NS), where bedrock was dominant (Table 1). Riffle depth (0.09-0.23 m), riffle width (7.8-16.9 m), and discharge (0.1-0.4 m^3/s) were relatively similar across sites; however, values were higher at Angler's Resort (SS) (discharge was 0.7 m^3/s). Temperature and dissolved oxygen were mostly typical for the region, but DO level at Angler's Resort (6.12 mg/L) was lower than expected and was somewhat lower at Double Bridge (7.09 mg/L) relative to measurements at other sites. Mean canopy cover tended to be greater at NS (54.4%) compared to SS (37.1%), but the difference was not significant (p = 0.11).

Average total suspended sediment (TSS) tended to be greater in SS (71.3 mg/L) compared to NS (57.5 mg/L), but the difference was not significant. (p = 0.34) (Table 1). Roasting Ear at Clearwater had highest TSS (88.8 mg/L), and Blanchard Springs had lowest TSS (42 mg/L). Mean turbidity was not different at SS (2.19 NTU) and NS (1.54 NTU) sites (p = 0.80).

Mean conductivity was significantly greater in SS (317.9 µS/cm) compared to NS (272.3 µS/cm) (p = 0.01). Roasting Ear at Mill Creek had highest conductivity (340.1 µS/cm), and Rosa Hole had lowest conductivity (252.7 µS/cm) (Table 1). Mean total dissolved solids (TDS) tended to be higher in SS (99.6 mg/L) compared to NS (76.5 mg/L), but the difference was not significant (p = 0.24). Blanchard Springs had highest TDS (120.8 mg/L), and Barkshed had lowest TDS (48.4 mg/L).

Mean nitrate concentration was significantly higher at SS (1.375 mg/L) compared to NS (0.75 mg/L) sites (p = 0.03). Roasting Ear at Mill Creek had highest nitrate concentration (1.7 mg/L) and Gunner Pool had lowest (0.6 mg/L) (Table 1). Mean phosphate concentration tended to be higher at SS (0.22 mg/L) compared to NS (0.07 mg/L) sites, but this difference was not significant (p = 0.30).

Chlorophyll a

Mean chlorophyll *a* concentrations were highly variable and not significantly different at SS (771.0 ± 232.70 SD µg/L) and NS (1862.5 ± 431.34 SD µg/L) (p = 0.20) sites (Table 1). Rosa Hole had highest average chlorophyll *a* concentration (3592.0 ± 1043.1 SD µg/L), and Double Bridge Loop had lowest (175.5 ± 74.3 SD µg/L). Chlorophyll *a* concentrations were not associated with canopy cover (r_s = 0.14), nitrate concentrations (r_s = -0.33), phosphate concentrations (r_s = 0.03) or sampling date (r_s = 0.34, p = 0.40). However, chlorophyll *a* was positively associated with macroinvertebrate grazer relative abundance (r_s = 0.71, p = 0.04). Interestingly, there was a trend for a negative association between chlorophyll *a* and relative abundance of the algivorous fishes *Campostoma* spp. (r_s = - 0.42, p = 0.29).

Macroinvertebrates

We collected and identified 9,750 individuals that encompassed 47 taxa. In total, SS sites contained 6,192 individuals representing 39 taxa, and total abundance at NS sites was 3,558 individuals and 40 taxa (Table 2). Mean number of individuals tended to be higher at SS (1,548.0 ± 497.3 SD) sites relative to NS sites (889.5 ± 252.3 SD), but this difference was not significant (p = 0.07). Most abundant taxa were Psephenidae, Elmidae, Amphipoda, Heptageniidae, and Hydropsychidae. These taxa were mostly ubiquitous across sites; however, Amphipoda was predominately collected at Roasting Ear/Mill Creek. Both catchments had 7 unique taxa, and taxa richness did not vary between catchments (p = 0.94). The nonnative Asian clam (*Corbicula fluminea*) was only collected in SS.

Both NS and SS each had 11 sensitive taxa. The most abundant sensitive taxa were Perlidae, Corydalidae, and Helicopsychidae (Table 2). NS (0.61) had a higher mean proportional abundance of sensitive taxa relative to SS (0.40) (p = 0.02). Both NS and SS each had 7 tolerant taxa. The most abundant tolerant taxa were Caenidae, Coenagrionidae, and predatory Chironomidae. Both NS (0.06) and SS (0.08) had similar mean relative abundances of tolerant taxa (p = 0.65). All sites in both systems had biotic index values that ranged from 3.2 - 4.3 (Table 1) and were therefore classified as "Excellent" water quality (HBI = 0.00 - 3.50) to "Very Good" water quality (HBI = 3.51 - 4.50) (Hilsenhoff 1988). Mean HBI of NS sites (3.37) was within the "Excellent" range while mean HBI of SS sites (3.85) was within the "Very Good" range.

Relative abundance patterns of functional feeding groups differed somewhat between NS and SS. SS tended to have higher proportional abundance than NS of filtering collectors (23.3, 8.8) and shredders (6.9, 0.4). NS tended to have higher proportional abundance than SS of predators (11.6, 7.8) and scrapers (59.5, 41.8). Proportion of collector gatherers was about 20% of total for both systems. Further, NMDS indicated NS and SS sites segregated in multivariate space based on functional feeding

Subsidy-Stress Gradients in the Sylamore Creek Watershed

Table 1. Land use and physicochemical characteristics of all sites sampled in the Sylamore watershed during 2018.

	Barkshed 14 Sept	Gunner Pool 13 Oct	Blanchard Springs 15 Sept	Rosa Hole 15 Sept	REC/Mill Creek 12 Oct	REC/ Clearwater 12 Oct	Double Bridge 16 Sept	Angler's Resort 15 Sept
Catchment Size (km²)	83.9	130.0	182.3	197.9	119.2	297.8	328.9	367.8
Reach Length (m)	317.0	287.0	143.0	142.2	148.0	201.0	160.0	191.6
GPS Coordinates	36.0195 -92.2495	35.9955 -92.2126	35.9681 -92.1689	35.9433 -92.1236	35.9488 -92.2866	35.9135 -92.2466	35.9119 -92.1662	35.9354 -92.1219
Mean Riffle Depth (m) ($\pm$ 1 SD)	0.09 (0.07)	0.12 (0.04)	0.11 (0.06)	0.10 (0.05)	0.23 (0.9)	0.14 (0.05)	0.14 (0.10)	0.14 (0.07)
Dominant Riffle Substrate	Pebble (40%)	Pebble (35%) & Cobble (30%)	Bedrock (40%)	Pebble (67%) & Gravel (37%)	Pebble (73%)	Pebble (67%) & Cobble (30%)	Pebble (67%)	Pebble (64%)
Water Temperature (°C)	28.5	16.1	25.8	27.2	15.6	17.8	24.6	24.4
Specific Conductivity (µS/cm)	277.9	293.8	264.9	252.7	340.1	318.6	309.1	303.9
Dissolved Oxygen (mg/L)	7.5	8.4	7.9	9.2	8.3	8.2	7.1	6.1
Mean Canopy Cover (%) ($\pm$ 1 SD)	53.3 (14.1)	37.9 (6.3)	45.8 (8.2)	80.4 (12.1)	39.1 (14.7)	43.4 (16.6)	11.0 (8.6)	34.8 (9.4)
Nitrate Concentration (mg/L)	0.80	0.60	0.70	0.90	1.7	1.5	1.2	1.1
Phosphate Concentration (mg/L)	BDL	0.12	BDL	0.15	0.21	0.02	0.10	0.56
Total Dissolved Solids (mg/L)	48.4	74.0	120.8	62.8	94.0	96.0	106.8	101.6
Total Suspended Sediment (mg/L)	50.8	73.2	42.0	64.0	84.9	88.8	66.2	45.2
Mean Chlorophyll *a* (µg/L) ($\pm$ 1 SD)	2223.4 (2134.7)	1405.0 (1483.8)	229.5 (216.4)	3592.0 (2557.0)	261.8 (271.4)	1386.0 (1654.3)	175.5 (182.2)	1263.8 (1285.3)
% Forest	96.5	95.9	94.7	94.8	76.9	78.0	70.6	72.2
% Pasture	1.0	1.4	2.0	2.0	19.2	18.4	24.9	22.7
% Developed	2.5	2.7	3.3	3.2	3.9	3.6	4.5	5.1
Mean Wooded Buffer (m) ($\pm$ 1 SD)	>200.0 (0.0)	128.7 (72.6)	153.9 (57.2)	103.0 (60.1)	23.8 (6.4)	67.0 (46.7)	35.7 (35.5)	21.9 (21.7)
Hilsenhoff Biotic Index (HBI)	3.6	3.2	3.3	3.4	3.6	3.3	4.2	4.2

Table 2. Aquatic macroinvertebrate taxa counts in North and South Sylamore. Amphipoda and Elmidae were classified under multiple FFGs (Greathouse and Pringle 2005). Chironomidae taxa were separated into Tanypodinae (T) or Non-Tanypodinae (NT).

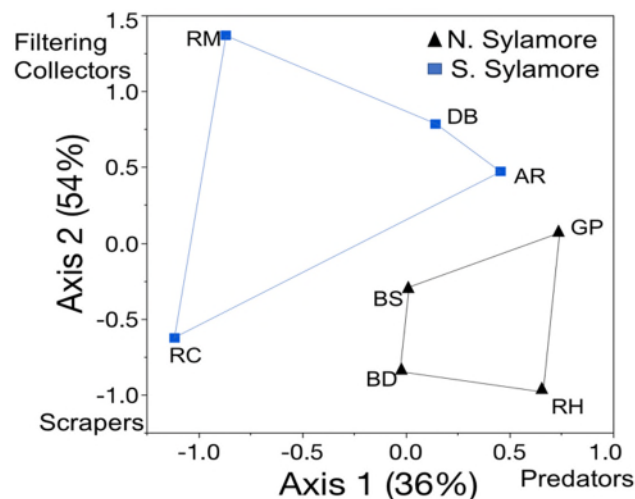| Taxon	North Sylamore	South Sylamore	Tolerance Value
Shredders			
Amphipoda (1/3)	0.33	505.67	
Capniidae	7	-	Sensitive
Haliplidae	2	-	Tolerant
Leuctridae	4	4	Sensitive
Tanyderidae	2	1	
Tipulidae	2	20	Sensitive
Filtering Collectors			
Corbicula	-	3	
Hydropsychidae	143	806	
Isonychiidae	135	474	
Philopotamidae	5	6	Sensitive
Polycentropodidae	3	-	
Simulidae	27	7	
Sphaeridae	-	5	Tolerant
Gathering Collectors			
Amphipoda (1/3)	0.33	505.67	
Baetidae	17	63	
Caenidae	39	270	Tolerant
Chironomidae - NT	60	27	
Elmidae (1/2)	594	210.5	
Ephemeridae	3	-	
Ephemerellidae	-	1	Sensitive
Hydrophilidae	2	-	
Hydroptilidae	1	3	
Isopoda	4	44	Tolerant
Leptophlebiidae	4	1	Sensitive
Limnichidae	-	1	
Oligochaeta	37	13	
Siphlonuridae	1	-	Tolerant
Tricorythidae	7	47	
Scrapers			
Amphipoda (1/3)	0.33	505.67	
Elmidae (1/2)	594	210.5	
Helicopsychidae	1	92	Sensitive
Heptageniidae	515	675	
Petrophila	4	-	
Physidae	-	1	Tolerant
Planorbidae	1	1	Tolerant
Pleuroceridae	191	257	
Psephenidae	727	1,029	
Predators			
Aeshnidae	7	2	Sensitive
Calopterygidae	3	6	
Chironomidae - T	46	35	Tolerant
Coenagrionidae	54	29	Tolerant
Corydalidae	42	113	Sensitive
Gerridae	-	1	
Gomphidae	2	6	Sensitive
Gyrinidae	-	1	
Hexatoma	9	-	
Perlidae	235	196	Sensitive
Perlodidae	1	1	Sensitive
Tabanidae	4	12	
Veliidae	22	1	

Figure 2. NMDS plot of aquatic macroinvertebrate relative abundance with sites in Functional Feeding Group space for North Sylamore (triangles) and South Sylamore (squares). For Site ID see Figure 1. Correlated functional feeding groups are labeled on each axis.

group relative abundances (Figure 2). NMDS Axis 1 (r_s = -0.04, p = 0.93) and Axis 2 (r_s = 0.38, p = 0.35) were not associated with sampling date. MRPP indicated there was not a significant grouping of sites based on functional feeding group composition, but there was a strong trend (p = 0.07). Filtering collectors were more associated with SS sites, and scrapers and predators were more associated with NS sites. Functional feeding group composition seemed to vary more among SS sites, particularly REC/Mill Creek and REC/Clearwater (Figure 2).

Fishes

Seining of riffle, run, and pool habitats within NS system yielded 1,333 individuals (23 species) and 2,364 individuals (31 species) in SS (Table 3). *Luxilus pilsbryi*, *Notropis nubilus*, *Lepomis megalotis*, and *Etheostoma caeruleum* were abundant in both systems. In total, SS had more tolerant taxa (12) than NS (7). Tolerant fishes *Luxilus chrysocephalus*, *Ameiurus natalis*, *Labidesthes sicculus*, *Lepomis cyanellus*, *Lepomis macrochirus*, and *Percina caprodes* were not detected in NS. *Semotilus atromaculatus* was the only tolerant species unique to NS (Table 3). Mean relative abundance of tolerant fishes in NS (0.29) was similar to SS (0.33). Total number of sensitive taxa was about equal between NS (16 species) and SS (19 species). *Noturus albater* was unique to NS, and *Chrosomus erythrogaster*, *Cottus immaculatus*, *Ambloplites constellatus*, and *Etheostoma zonale* were unique to SS.

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Mean relative abundance of sensitive taxa in NS (0.71) was not significantly different from SS (0.66) (p = 0.39).

Mean relative abundance of centrarchids was not different between NS (0.09) and SS (0.08) (p = 0.48). Over five-fold more stonerollers were collected in SS (Table 3), but mean relative abundance of *Campostoma* spp. was not significantly different in NS (0.03) relative to SS (0.05) (p = 0.30).

NMDS suggested NS and SS sites had different fish assemblage compositions given the segregation in the ordination (Figure 3), but this difference was not significant (MRPP, p = 0.13). NMDS Axis 1 (r$_s$ = 0.26,

p = 0.53) and Axis 2 (r$_s$ = 0.67, p = 0.07) were not associated with sampling date. NS sites and Angler's Resort tended to be associated with *Fundulus catenatus*, *Lepomis megalotis*, and *Etheostoma caeruleum*. Tolerant taxa *Notropis nubilus* and *Gambusia affinis* were most associated with SS sites Double Bridge and Angler's Resort. Based on distances observed in the ordination, there was more variation in fish assemblages across SS sites than NS sites (Figure 3).

Table 3. Total abundances of fishes collected in North and South Sylamore. * indicates a tolerant species (Dauwalter *et al.* 2003).

| Scientific Name	North Sylamore Total	South Sylamore Total
Campostoma. spp.*	31	163
Chrosomus erythrogaster	-	214
Cyprinella galactura	1	9
Hybopsis amblops	2	1
*Luxilus chrysocephalus**	-	6
Luxilus pilsbryi	391	828
Nocomis biguttatus	11	9
Notropis boops	1	40
*Notropis nubilus**	253	185
Notropis percobromus	2	78
Notropis telescopus	161	84
*Semotilus atromaculatus**	2	-
*Ameiurus natalis**	-	1
Noturus albater	4	-
Noturus exilis	3	1
*Labidesthes sicculus**	-	79
Fundulus catenatus	139	52
*Fundulus olivaceus**	59	64
*Gambusia affinis**	17	112
Cottus carolinae	10	7
Cottus immaculatus	-	22
Ambloplites constellatus	-	7
*Lepomis cyanellus**	-	1
*Lepomis macrochirus**	-	3
*Lepomis megalotis**	89	205
Micropterus dolomieu	24	8
Etheostoma blennioides	1	1
Etheostoma caeruleum	110	98
Etheostoma flabellare	14	43
Etheostoma juliae	4	13
*Etheostoma spectabile**	4	8
Etheostoma zonale	-	21
*Percina caprodes**	-	1
Total Abundance	1,333	2,364
Total Species	23	31
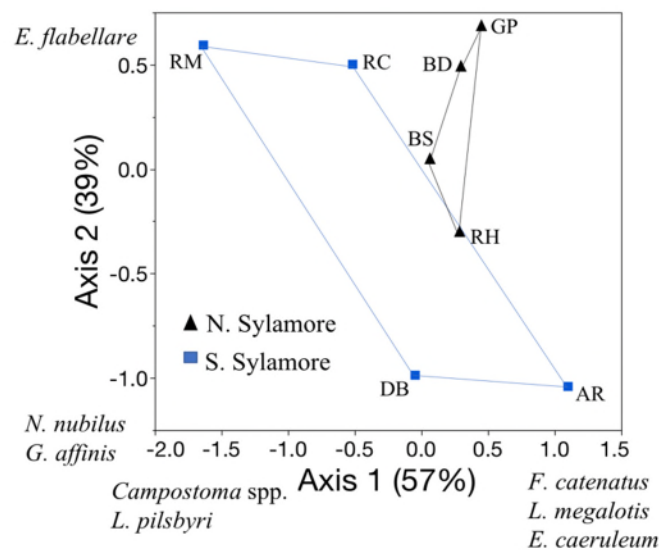
Figure 3. NMDS plot of sites in fish species space for North Sylamore (triangles) and South Sylamore (squares). For Site ID see Figure 1. Correlated species are labeled on each axis.

Discussion

Overall, physicochemical and biotic variables indicated both NS and SS are high quality Ozark streams that warrant attention from natural resource managers. For example, the macroinvertebrate-based HBI indicated both had very good to excellent water quality. Presence of 19 to 25% pasture in upstream catchments, in combination with a narrower wooded riparian buffer, created the potential for SS to have received more perturbations through time compared to NS. Indeed, some water quality parameters measured were indicative of watershed alteration in SS, providing the opportunity to study biotic responses to relatively low levels of disturbance within a subsidy-stress framework.

Physicochemical

In accordance with predicted responses to increased pasture land use within catchments, SS had higher concentrations of nitrate and tended to have

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higher concentrations of phosphate, TSS, and TDS. Similar results have been found in numerous other studies, where higher agricultural land use is associated with higher nutrient runoff into surrounding water bodies due to fertilizer inputs (Vitousek *et al.* 1997; Carpenter *et al.* 1998). Some of the nitrogen and phosphorous values measured in Sylamore, particularly SS, were similar to mean total phosphorous (~ 0.2-0.3 mg/L) and total nitrogen (~ 1.5-2.0 mg/L) measured in nutrient enriched Central Plains streams (Evans-White *et al.* 2009). Our sampling protocol was not ideal to detect alterations to dissolved oxygen; however, we did measure moderately low values for the Ozarks at two SS sites. Animal waste from domestic animals is a potential source of increased carbon and nutrients to SS. Higher rates of erosion and runoff predictably increase TSS and TDS as observed in SS (Hudson-Edwards 2003). A recent study in India found turbidity and TSS increased by 8.41% and 4.17% respectively, with every one percent decrease in forest cover (Singh and Mishra 2014). Chase *et al.* (2017) used experimental mesocosms to show that nutrient subsidy increased macroinvertebrate abundances in all taxa; however, when added with increased TSS, there was a negative stress effect in less tolerant species. Further research is needed to determine sources of increased nutrients and other solids in SS.

Chlorophyll a

Higher rates of nutrient runoff were predicted to occur in association with agriculture/pasture, and SS was therefore predicted to support higher algal growth (Smith *et al.* 1999; Dodds *et al.* 2002), potentially having a subsidizing effect on SS food webs. To our surprise, there were no differences in chlorophyll *a* values, and they even trended to be lower in SS despite elevated nutrients. Low canopy coverage and high nutrients are typically associated with higher algal abundance, as documented in previous studies (Hill and Knight 1988). However, we found no indications that chlorophyll *a* was related to canopy cover or nutrients.

Benthic algae appeared to be an important food source in both NS and SS, and biotic factors may have played a larger role than abiotic factors in its pattern of abundance during our sampling. Autotrophy can be an important carbon source in headwater streams (Minshall 1978), and chlorophyll *a* was higher than expected in headwaters of NS and SS based on predictions of the river continuum concept (Vannote *et al.* 1980) and published values. For Sylamore chlorophyll *a* divot samples that could be expressed as biomass per unit area, five were greater than 10 μg/cm^2 and seven

samples were between 5 and 9 μg/cm^2; values greater than 10 μg/cm^2 fall within the "excessive" range for temperate streams (Dodds *et al.* 2002). Sylamore stream reaches had relatively open canopies, low turbidity, and stable substrates, and nutrients did not appear to be limiting, particularly in SS. Relative abundances of scraper macroinvertebrates, particularly Psephenidae, were relatively high (40-60%) in both headwater systems. Macroinvertebrate grazers across all sites seemed to be responding positively to increased benthic algae (as indicated by chlorophyll *a*). Hillebrand and Kahlert (2001) found that grazer presence had a stronger effect on algal biomass than nutrient enrichment, and Gregory (1980) observed that high (13.3 g/m^2) and intermediate (6.7 g/m^2) densities of grazers resulted in significant decreases in chlorophyll *a* concentrations compared with low (1.1-2.2 g/m^2) grazer densities. Abundance of the algivorous fish *Campostoma* was moderately negatively associated with chlorophyll *a* across our sites and this fish tended to be more abundant in SS. This pattern further represents evidence of the potential for top-down influence during our study. Seemingly elevated benthic algal production in the headwaters of both NS and SS need further study.

Macroinvertebrates

Trends for increased total number of macroinvertebrates in SS, particularly the filtering collectors, were indicative of a subsidy-related response. Pasture runoff has likely directly and/or indirectly resulted in increased or enriched food resources in SS (mechanisms reviewed by Evans-White *et al.* 2009). For example, high abundances of Hydropsychidae and Isonychiidae indicated SS must have an abundance of fine particulate organic matter in transport. Enhanced autotrophic and detrital resources and its consumption in SS probably lead to more fine organics in transport. Pasture land use has probably subsidized basal food resources for macroinvertebrates in SS, but insight here is limited without increased spatial and temporal sampling.

Similarity in overall macroinvertebrate richness and number and abundances of tolerant and sensitive taxa between NS and SS is consistent with a subsidy-stress gradient where nutrient input is still usable by the primary producers, and not yet enough to cause a stress response in the aquatic ecosystem (Odum *et al* 1979). Abundances of tolerant and sensitive species present in a system are often used, among other metrics, as indicators of water quality (Resh and Unzicker 1975). As water quality decreases, the amount of tolerant taxa increase while the amount of sensitive taxa decrease

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(Lenat and Crawford 1994). Though proportional abundance of tolerant taxa was almost the same between the two watersheds, NS had a higher proportional abundance of sensitive taxa than SS, suggesting there could be some level of stress to sensitive taxa in SS. In support, Hilsenhoff Biotic Index (HBI) values were also slightly lower in SS relative to NS. Both study systems had good water quality, and alterations from increased pasture land use have probably had a subsidizing effect on many macroinvertebrates in SS but caused a degree of stress to some sensitive taxa.

Fishes

Similar to macroinvertebrates, fish assemblage data from NS and SS suggested responses to subsidies in SS and some evidence of stress. *Campostoma* spp., and to some extent *Notropis nubilus*, predominately feed on periphyton (Pflieger 1997) and tended to have greatest abundances in SS. These species probably benefit from increased food resources in SS. Scott and Helfman (2001) described a common phenomenon across the southeastern United States of lowland fishes expanding ranges into upland reaches of streams degraded by land use change (*e.g.*, increased pasture). These fishes tolerate aspects of the perturbation (*e.g.*, increased fine sediments) and benefit from enhanced/increased food resources, often feeding in the water column. We collected a number of fishes that are denizens of lowland habitats, often feeding in the water column (Pflieger 1997), that were unique to or most associated with SS: *Labidesthes sicculus*, *Gambusia affinis*, *Lepomis cyanellus*, and *Lepomis macrochirus*. These fishes were captured at the lowermost sites in SS, and therefore, had access to Rosa Hole in lower NS but were not detected there except for *Gambusia affinis* (in much lower abundance). Resource subsidies in SS seemed to have benefited fishes that feed directly on basal resources and probably have allowed expansion of some native lowland species.

Some sensitive fishes had interesting patterns of abundance and occurrence in NS and SS. *Chrosomus erythrogaster* and *Cottus immaculatus* were only detected in SS at either Roasting Ear/Mill Creek or Roasting Ear Clearwater. Both of these species tend to be associated with springs (Pflieger 1997), and a large spring occurs on Mill Creek that probably influenced these collections. Large springs were not present in or near our study reaches on NS, and we know from previous research that *Chrosomus erythrogaster* occurs further upstream within the intermittent reaches of NS (Walker *et al*. 2013) and in smaller tributaries (Mitchell *et al*. 2012). *Noturus albater* was detected at three out

of the four sites in NS, and was not detected at SS sites. Two individuals were collected at Blanchard Springs, one was collected at Barkshed, and one was collected at Rosa Hole. *Noturus albater* is a sensitive taxon (Dauwalter *et al*. 2003) endemic to the Ozark region of southern Missouri and northern Arkansas (Pflieger 1997). This species is typically associated with clear, high-gradient streams, in gravel riffles (Pflieger 1975), and it may be vulnerable to conditions in SS. However, our study is limited by only sampling during a single season. More extensive collecting, both temporally and spatially, is needed to better understand fish distribution patterns in Sylamore Creek.

Conclusions

The subsidy-stress framework helped to understand the current ecological status of two subcatchments within the same watershed but having different land use patterns. Relative to NS, SS showed evidence of enhanced biological response consistent with inputs of usable resources (*e.g.*, nutrients and probably organic matter) related to increased pasture land use (*sensu* Odum *et al*. 1979). Macroinvertebrate and fish data suggested the amount of perturbation in SS has become a stressor to some taxa, but the overall communities remain healthy. Amounts of pasture land use within upstream catchments of SS (19-25%) are less than thresholds reported to significantly stress macroinvertebrates (> 30%; Quinn and Hickey 1990) and fishes (> 50%; Wang *et al*. 1997) in other studies. However, land use alterations in SS are approaching these thresholds, and the watershed could be vulnerable to persistent effects of pasture. An additional future concern is higher amounts of development in SS and the subsequent potential for multiple stressors and their interactions impacting this stream. Most sites in SS, and a few specific locations in NS, had narrow or sparsely wooded riparian zones. A potential way to limit inputs from pasture into the streams is to enhance forest buffer strips, particularly along SS. These strips act like fences to effectively mitigate the movement of sediment, contaminants, and nutrients during surface runoff (NRCS 2010). Using the hypothetical performance curve predicted by the subsidy-stress hypothesis (Odum *et al*. 1979), land use change in SS may have moved this system outside the "normal operating range" and into the "subsidy effect" range based on comparisons with NS. However, this is difficult to establish without comparisons with historical conditions and more extensive sampling. Additional sampling will allow improved evaluation of the variance and stress components of the hypothesis predicted to increase with

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increased perturbation.

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