Supplementary Text, Tables, and Figures for Shuman et al. Influence of physical and chemical characteristics of sediment on macroinvertebrate communities in agricultural headwater streams.

Supplementary Text

Laser Diffraction Particle Size Analysis Details

Laser diffraction particle size analysis samples weighing between 0.5 and 3 g were combined with 100 ml of deionized water and 100 ml of 5% sodium hexametaphosphate solution. The slurries were allowed to set for 24 hours. Samples were then shaken at 100 rpm for 30 min and analyzed using the following measurement settings: 1) particle type - opaque particle-Fraunhofer approximation; 2) material name - kaolinite -refractive index; 3) dispersant - water (density 1.33 g/ml); 4) red and blue background measurement duration - 15 sec; 5) sample measurement duration - 30 sec; 6) obscuration range - 10 - 30%; 7) stir speed - 3000 rpm; 8) ultrasound mode - pre-measurement; 9) data analysis - general purpose; 10) result setting range - 0.005 - 2000 μm; and 11) result type - volume distribution. Size distribution by laser diffractometry is volume-based so we set clay-silt break at 6 μm as suggested by Miller and Schaetzl [30] to enable comparisons of the size distribution with that obtained by the traditional pipette method.

Physicochemical, Nutrient, Trace Metal, and Pesticide Analysis Details

Nutrient, trace metal, and pesticide samples remained frozen during transport to USDA National Soil Erosion Research Laboratory for analyses. Frozen samples were thawed in 4 ^{o}C storage and then the water was decanted from sediment samples. The remaining sediment in the sample was transferred to a 600 ml freeze drier flask, sealed, and rolled in a benchtop shell freezer for 30 min at -42 ^{o}C. Once frozen, sediment samples were transferred to a freeze drier and dried at -45 ^{o}C and 4 Pa vacuum pressure. Dried sediment samples were sieved through a 2 mm sieve and stored at 4 ^{o}C in an amber HDPE bottle until analyzed for physicochemical characteristics, nutrients, trace metals, and pesticides. A slurry containing a 1:1 ratio of dried sediment and nanopore water was made, shaken for 10 min at 100 rpm, and allowed to settle before measurement of electrical conductivity and pH. Electrical conductivity measurements were obtained with a conductivity meter and pH measurements were collected with a pH meter. Total carbon and total nitrogen were measured from 1 g of freeze-dried sediment with dry combustion using an elemental analyzer. Extractable ammonia-N and nitrate-N from KCl (potassium chloride) extracted samples obtained from dried sediment samples were quantified colorimetrically with a flow injection analyzer. A 1 M KCl solution (1:5 sediment weight/KCl volume) was shaken for 1 h at 100 rpm and gravity filtered through #42 filter paper. Ammonia-N and nitrate-N standards were made as a set and the standards from both analytes ranged from 0.1 to 20 mg/L. Method detection limit for ammonia-N was 0.035 mg/L and for nitrate-N was 0.005 mg/L. Ammonia-N analysis was conducted using the QuikChem Method 12-107-06-02-A [32] and the following parameters: 1) carrier - 1M KCl; 2) manifold tubing- 0.8 mm i.d; 3) sample loop - 35 cm x 0.8 mm i.d.; 4) flow cell - 10mm path length; 5) interference filter - 660 nm; 6) heating apparatus - 650 cm x 0.8 mm i.d. and heated to 60 °C; and 7) back pressure loop – 150 cm x 0.6mm i.d. Nitrate-N analysis was conducted using the QuikChem Method 12-107-04-1-B [33] with the following settings: 1) carrier - 1M KCl; 2) manifold tubing - 0.8 mm i.d; 3) sample loop - microloop; 4) flow cell - 10 mm path length; 5) interference filter - 520 nm; and 6) an inline cadmium column. Concentrations of 10 nutrients and trace metals (aluminum, calcium, copper, iron, potassium, magnesium, manganese, phosphorus, sulfur, zinc) were extracted with the Mehlich-3 solution [34] with a 1:10 solid/solution ratio, shaking for five min, and then gravity filtration through #42 filter paper. Concentrations of these 10 Mehlich-3 nutrients and trace metals were then measured from the filtered solution using an inductively coupled optical emission spectrometer.

Pesticides and their metabolites were extracted using an accelerated solvent extractor following the method of Vonberg et al. Vonberg et al. [35]. Concentrations of nine pesticides and four pesticide metabolites (2,4-D (2,4-Dichlorophenoxyacetic acid), acetochlor, alachlor, atrazine, desethylatrazine, desisopropylatrazine, 2-hydroxyatrazine, clothianidin, methyl nitroguanidine, imidacloprid, malathion, s-metolachlor, simazine) with UPLC (ultra performance liquid chromatography) interfaced with a computer running MassLynx version 4.1 chromatography manager software (Waters Corp., Milford, Massachusetts). The separation of compounds was performed on a 100 mm × 2.1 mm i.d., 1.7 μm UPLC analytical column at 45 °C using 0.5 % formic acid in water and 0.5 % formic acid in acetonitrile as the mobile phases. The flow rate was set at 0.45 ml/min and the mobile phase gradient for the 0.5% formic acid in water mobile phase was set as follows: 0 min - 70%; 0.70 min - 70%; 5.50 min - 40%; 6.00 min - 25%; 6.30 min - 25%; 7.00 min - 70%, and 7.30 min - 70%. All solvents used in the UPLC system were optima grade and the autosampler was set at 10 °C during the analysis to minimize degradation. Pesticide detection and confirmation were performed using a tandem quadrupole mass detector in the multiple reaction monitoring mode, and quantification was performed using the parent and daughter ions for each compound. Calibration curves (0.3 to 40 μg/L) for all compounds were generated by using external standards, and an internal standard (5 μg/L trietizine) was added to all samples and external standards to minimize the matrix composition of the water samples. A 50 μL injection volume was used for external and internal standards and samples. Retention times in min of the compounds were: 0.61 - methyl nitroguanidine; 0.79 - 2-hydroxyatrazine; 0.90 - desisopropylatrazine; 1.04 - clothianidin; 1.12 - imidacloprid; 1.16 – desethylatrazine; 1.97 – simazine; 2.85 – atrazine; 5.14 – malathion; 5.24 – s-metolachlor; 5.27 – alachlor; and 5.29 - 2,4-D and acetochlor. The limit of detection in μg/L for each pesticide and metabolite measured were: 2,4-D - 0.465; acetochlor - 0.235; alachlor - 0.065; atrazine - 0.004; 2-hydroxyatrazine

- 0.004; desisopropylatrazine - 0.004; desethylatrazine 0.004; clothianidin - 0.056; methyl nitroguanidine - 0.019; imidacloprid - 0.038; malathion - 0.062; s-metolachlor - 0.029; and simazine - 0.004.

Table S1. Loadings from principal components analysis (PCA) of eight physical characteristics of sediment from eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. Bolded loadings are those that best characterized the underlying habitat gradients of each PCA axis.

Physical Characteristics	**PCA Axis 1**	**PCA Axis 2**
Percent large gravel	0.369	0.222
Percent small gravel	**0.412**	0.134
Percent sand	-0.055	**-0.638**
Percent silt	**-0.468**	0.207
Percent clay	-0.372	0.392
Grain size richness	0.397	0.102
Grain size diversity	0.219	**0.516**
Loss-on-ignition percent organic content	-0.359	0.231
Percentage of variance explained by axis	*49*	*27*

Table S2. Loadings from principal components analysis (PCA) of 20 chemical characteristics of sediment from eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. Bolded loadings are those that best characterized the underlying habitat gradients of each PCA axis.

Chemical Characteristics	**PCA Axis 1**	**PCA Axis 2**	**PCA Axis 3**
Conductivity	-0.292	-0.255	0.057
pH	0.178	0.071	-0.222
Percent Total Carbon	-0.226	-0.279	-0.010
Percent Total Nitrogen	**-0.320**	-0.098	-0.036
Ammonia-N	-0.221	-0.234	-0.022
Nitrate-N	-0.063	0.141	**-0.408**
Calcium	-0.019	**-0.364**	-0.248
Potassium	**-0.364**	0.068	-0.114
Phosphorus	0.040	-0.269	0.259
Sulfur	-0.246	-0.283	-0.096
Aluminum	-0.297	0.241	0.040
Copper	0.034	0.208	**-0.408**
Iron	-0.221	-0.161	0.287
Magnesium	**-0.327**	0.159	-0.184
Manganese	0.033	-0.114	-0.351
Zinc	-0.256	-0.055	-0.361
Atrazine	-0.264	0.225	0.165
2-hydroxyatrazine	-0.233	0.256	0.223
Simazine	-0.041	**0.330**	-0.004
Metolachlor	-0.212	0.293	0.120
Percentage of variance explained by axis	*31*	*21*	*10*

Table S3. Best identified random effect and *p* values from linear mixed effect model analysis of the relationships of site scores from the first three principal component axes of chemical characteristics (C-PCA axis 1, C-PCA axis 2, and C-PCA axis 3) and the first two principal components axes of physical characteristics (P-PCA axis 1, P-PCA axis 2) of sediment within eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. Bolded *p* values are < 0.05.

Response Variable	Random Effect	Independent Variable	*p* value
C-PCA axis 1	Site	P-PCA axis 1	**< 0.001**
C-PCA axis 1	Site	P-PCA axis 2	0.265
C-PCA axis 2	Watershed + Site	P-PCA axis 1	0.777
C-PCA axis 2	Watershed + Site	P-PCA axis 2	0.319
C-PCA axis 3	Site	P-PCA axis 1	0.634
C-PCA axis 3	Site	P-PCA axis 2	0.483

Table S4. Number of individuals and relative abundances (percent) of aquatic macroinvertebrates captured from eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018.

Taxonomic Level	Taxa	Number	Percent
Family	Chironomidae	8603	42.7
Class	Gastropoda	1825	9.1
Family	Elmidae	1504	7.5
Order	Amphipoda	1156	5.7
Class	Turbellaria	1075	5.3
Order	Isopoda	953	4.7
Subclass	Oligochaeta	928	4.6
Subclass	Hirudinea	457	2.3
Family	Hydroptilidae	452	2.2
Family	Hydropsychidae	437	2.2
Family	Coenagrionidae	391	1.9
Family	Leptophlebiidae	358	1.8
Family	Psychodidae	357	1.8
Class	Bivalvia	335	1.7
Family	Heptageniidae	324	1.6
Family	Caenidae	148	0.7
Family	Empididae	125	0.6
Family	Hydrachnidae	113	0.6
Phylum	Nematomorpha	108	0.5
Family	Leptoceridae	101	0.5
Subclass	Collembola	57	0.3
Family	Ceratopogonidae	42	0.2
Family	Calopterygidae	36	0.2
Family	Hydrophilidae	32	0.2
Family	Simuliidae	23	0.1
Order	Decapoda	21	0.1
Family	Limnephilidae	20	0.1
Family	Culicidae	19	0.1
Family	Aeshnidae	17	0.1
Family	Veliidae	15	0.1
Class	Diplopoda	13	0.1
Family	Haliplidae	11	0.1
Family	Helicopsychidae	11	0.1
Family	Leptohyphidae	11	0.1
Family	Tipulidae	9	< 0.1
Family	Belostomatidae	7	< 0.1
Family	Dytiscidae	7	< 0.1
Family	Ephydridae	6	< 0.1
Phylum	Nematoda	6	< 0.1
Phylum	Nemertea	6	< 0.1
Family	Corixidae	5	< 0.1

Table S4 continued

Family	Dryopidae	5	< 0.1
Family	Tabanidae	5	< 0.1
Family	Capniidae	4	< 0.1
Family	Baetidae	3	< 0.1
Family	Corydalidae	3	< 0.1
Family	Polycentropodidae	3	< 0.1
Family	Corduliidae	2	< 0.1
Family	Gyrinidae	2	< 0.1
Family	Psychomyiidae	2	< 0.1
Family	Scirtidae	2	< 0.1
Family	Lepidostomatidae	1	< 0.1
Family	Lestidae	1	< 0.1
Family	Leuctridae	1	< 0.1
Family	Perlodidae	1	< 0.1
Family	Pyralidae	1	< 0.1
Family	Staphylinidae	1	< 0.1

Table S5. Best identified random effect and *p* values from linear mixed effect model analysis of the relationships of macroinvertebrate community response variables with site scores from the first three principal component axes of chemical characteristics(C-PCA1, C-PCA 2, and C-PCA axis 3) and the first two principal components axes of physical characteristics (P-PCA axis 1, P-PCA axis 2) of sediment within eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. **These results are influenced by moderate amounts of multicollinearity between C-PCA axis 1 and P-PCA axis 1 and C-PCA axis 2 and P-PCA axis 2 and are presented only so interested readers can compare the full model results with the reduced model results in Table S6 and Table S7.**

Response Variable	Random Effect	Independent Variables	*p* value
Abundance	Season	C-PCA axis 1	0.834
[log (x+1)]		C-PCA axis 2	0.287
		C-PCA axis 3	0.367
		P-PCA axis 1	0.400
		P-PCA axis 2	0.835
Shannon Diversity Index	Year	C-PCA axis 1	0.720
[log(x+1)]		C-PCA axis 2	0.005
		C-PCA axis 3	0.526
		P-PCA axis 1	0.341
		P-PCA axis 2	0.013
Reciprocal Berger-Parker Dominance	Year/Season	C-PCA axis 1	0.847
Index		C-PCA axis 2	0.065
		C-PCA axis 3	0.931
		P-PCA axis 1	0.440
		P-PCA axis 2	0.302
Percent Collector-Filters	Season/Watershed	C-PCA axis 1	0.768
(arcsine square root)		C-PCA axis 2	0.786
		C-PCA axis 3	0.613
		P-PCA axis 1	0.812
		P-PCA axis 2	0.612
Percent Scrapers	Site	C-PCA axis 1	0.160
(arcsine square root)		C-PCA axis 2	0.113
		C-PCA axis 3	0.347
		P-PCA axis 1	0.192
		P-PCA axis 2	0.231
Percent Chironomidae	Site	C-PCA axis 1	0.421
		C-PCA axis 2	0.730
		C-PCA axis 3	0.395
		P-PCA axis 1	0.743
		P-PCA axis 2	0.335
Hilsenhoff Biotic Index	Site	C-PCA axis 1	0.582
[log(x+1)]		C-PCA axis 2	0.304
		C-PCA axis 3	0.593
		P-PCA axis 1	0.939
		P-PCA axis 2	0.166
Invertebrate Community Index	Year/Season	C-PCA axis 1	0.176
		C-PCA axis 2	0.173
		C-PCA axis 3	0.538
		P-PCA axis 1	0.278
		P-PCA axis 2	0.854

Table S6. Best identified random effect and *p* values from linear mixed effect model analysis of the relationships of macroinvertebrate community response variables with site scores from the first three principal component axes of chemical characteristics (C-PCA1, C-PCA 2, and C-PCA axis 3) of sediment within eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. Bolded *p* values are < 0.05.

Response Variable	Random Effect	Independent Variables	*p* value
Abundance	Season	C-PCA axis 1	0.463
[log (x+1)]		C-PCA axis 2	0.121
		C-PCA axis 3	0.313
Shannon Diversity Index	Year	C-PCA axis 1	0.050
[log(x+1)]		C-PCA axis 2	0.149
		C-PCA axis 3	0.376
Reciprocal Berger-Parker Dominance	Year/Season	C-PCA axis 1	0.163
Index		C-PCA axis 2	0.073
		C-PCA axis 3	0.931
Percent Collector-Filters	Season/Watershed	C-PCA axis 1	0.918
(arcsine square root)		C-PCA axis 2	0.862
		C-PCA axis 3	0.512
Percent Scrapers	Site	C-PCA axis 1	0.607
(arcsine square root)		C-PCA axis 2	0.211
		C-PCA axis 3	0.222
Percent Chironomidae	Site	C-PCA axis 1	0.605
		C-PCA axis 2	0.560
		C-PCA axis 3	0.296
Hilsenhoff Biotic Index	Site	C-PCA axis 1	0.828
[log(x+1)]		C-PCA axis 2	0.056
		C-PCA axis 3	0.756
Invertebrate Community Index	Year/Season	C-PCA axis 1	0.109
		C-PCA axis 2	**0.014**
		C-PCA axis 3	0.652

Table S7. Best identified random effect and *p* values from linear mixed effect model analysis of the relationships of macroinvertebrate community response variables with site scores from the first two principal components axes of physical characteristics (P-PCA axis 1, P-PCA axis 2) of sediment within eight agricultural headwater streams in Indiana, Michigan, and Ohio, 2017 to 2018. Bolded *p* values are < 0.05.

Response Variable	Random Effect	Independent Variables	*p* value
Abundance	Season	P-PCA axis 1	0.196
[log (x+1)]		P-PCA axis 2	0.264
Shannon Diversity Index	Year	P-PCA axis 1	0.314
[log(x+1)]		P-PCA axis 2	0.187
Reciprocal Berger-Parker Dominance	Year/Season	P-PCA axis 1	0.241
Index		P-PCA axis 2	0.783
Percent Collector-Filters	Season/Watershed	P-PCA axis 1	0.979
(arcsine square root)		P-PCA axis 2	0.537
Percent Scrapers	Site	P-PCA axis 1	0.421
(arcsine square root)		P-PCA axis 2	0.591
Percent Chironomidae	Site	P-PCA axis 1	0.560
		P-PCA axis 2	0.340
Hilsenhoff Biotic Index	Site	P-PCA axis 1	0.668
[log(x+1)]		P-PCA axis 2	0.056
Invertebrate Community Index	Year/Season	P-PCA axis 1	0.987
		P-PCA axis 2	**0.007**

Figure S1. Map of the Saint Joseph River watershed in northeast Indiana and southern Michigan (United States) depicting the locations of three sampling sites in the southern portion of the watershed and one site in the northern portion.

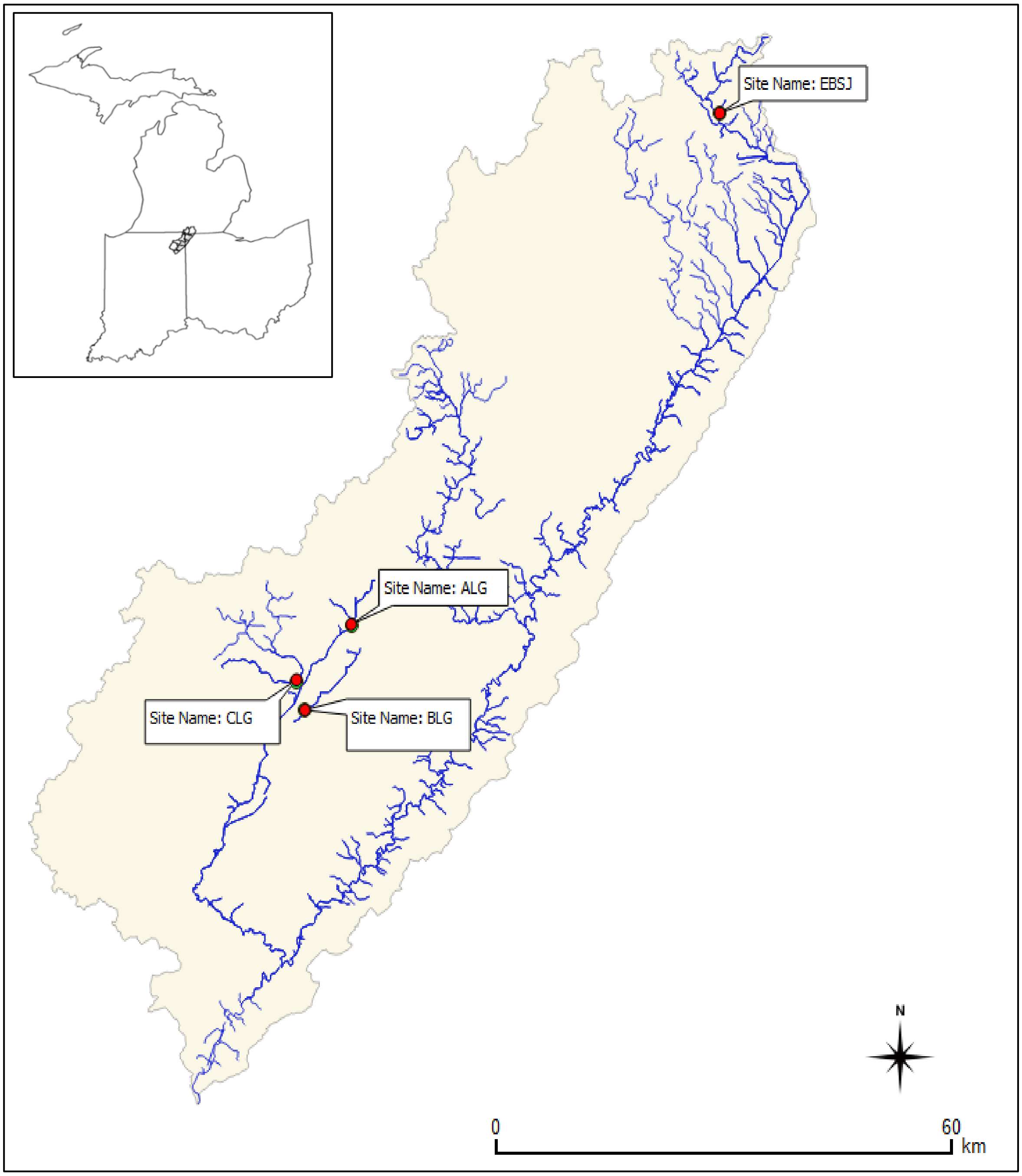

Figure S2. Map of the Upper Big Walnut Creek watershed in central Ohio (United States) depicting the locations of two sites in the southern portion of the watershed and two sites in the northern portion

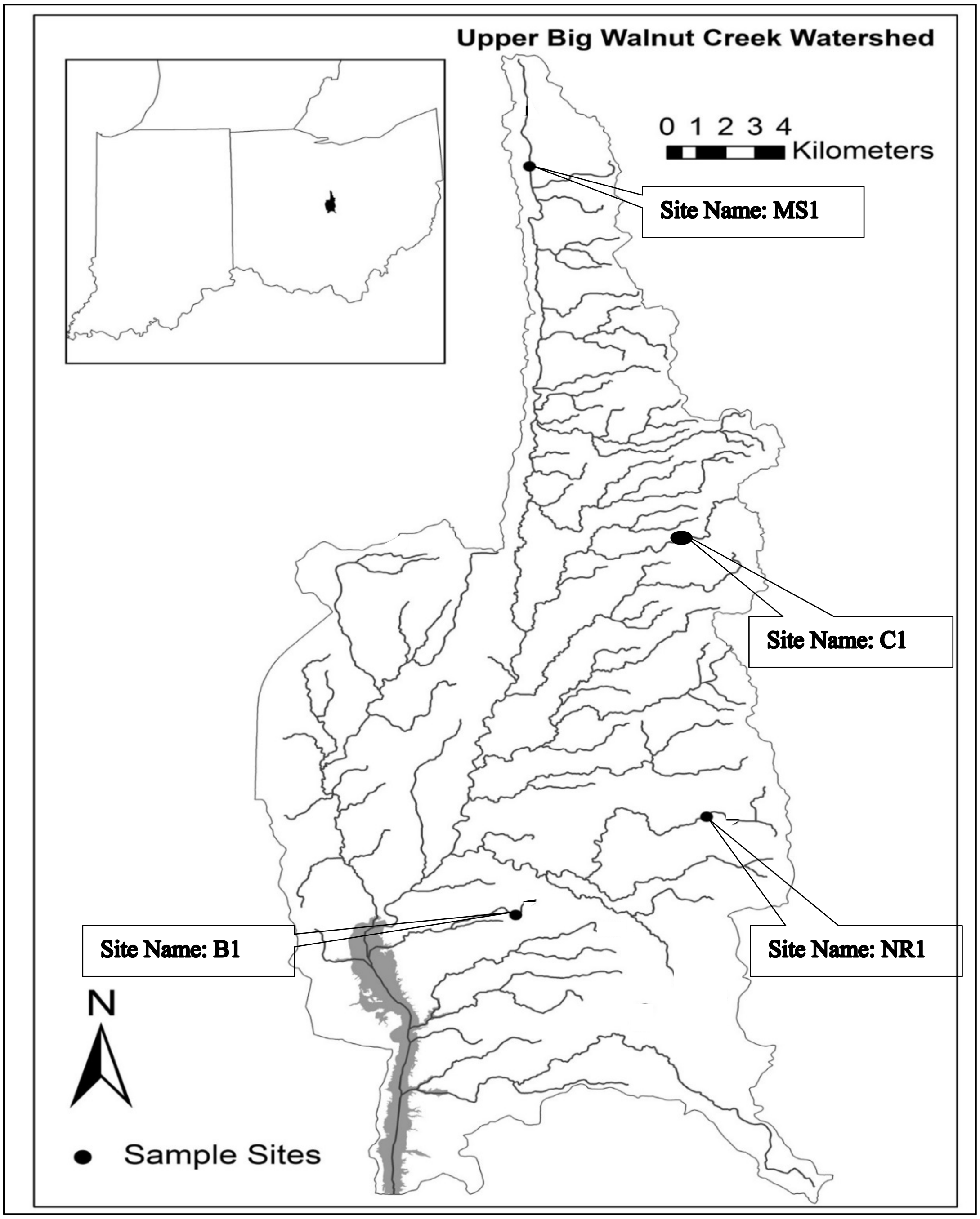

Figure S3. Each site (150 m) was delineated by six segments (25 m) and a total of 32 segments were randomly sampled among eight sites (4 per site) over two years. We used stratified random selection to sample six locations from among 72 possible locations based on 24 longitudinal positions and 3 lateral positions (left, center, right) in in each randomly selected segment during each season.