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Associations Between Fish and Benthic Macroinvertebrate Biotic Integrity and Non-Point Source Pollution Estimates in the Nolichucky River Watershed

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Abstract—We used the Soil and Water Assessment Tool (SWAT), a GIS-based method that estimates sediment, phosphorus and nitrogen yields using remotely-sensed land cover, hydrologic, and geomorphic characteristics of watersheds, to determine if metrics of biotic integrity are associated with non-point source runoff. During 2014, SWAT model estimates were made for 19 sites in the Nolichucky River watershed that were subsequently sampled during 2014–2016 for fish and benthic macroinvertebrates using standard procedures developed by the Tennessee Valley Authority (TVA) and the Tennessee Department of Environment and Conservation (TDEC), respectively. Partial canonical correspondence analysis, after accounting for spatial proximity of sites, showed a significant association between benthic macroinvertebrate IBI metrics and SWAT model estimates, whereas partial correspondence analysis suggested a strong relationship between some fish metrics and non-point source pollution. For benthic macroinvertebrates, organic nitrogen and phosphorus yield were positively associated with % nutrient-tolerant taxa, but negatively associated with % clingers. Sediment yield was negatively associated with % clingers, taxa richness, and Ephemeroptera-Plecoptera-Trichoptera (EPT) richness. For fishes, sediment and nutrients were positively associated with % hybrids and catch per unit effort, but negatively associated with native species richness. The SWAT model is a publically-available GIS tool that managers and researchers can utilize to assess non-point source pollution impacts on freshwater ecosystems. Our findings are limited to the Nolichucky River watershed, but other researchers should use SWAT models and indices of biotic integrity to examine the link between sediment and nutrient loads and aquatic ecosystem health in other regions with different fauna.

Introduction

Since the passage of the USA Clean Water Act in 1972, regulatory agencies around the world have developed indices of biotic integrity (IBI) to assess the relative "health" of aquatic ecosystems (Karr, 1981; Couceiro et al., 2012; Jun et al., 2012; Huang et al., 2015). Fish and benthic macroinvertebrates tend to be the biological communities that are most often sampled (Karr, 1991; Klemm et al., 2003; Ruaro and Gubiani, 2013) due to their relative ease of capture, identification, and their strong response to site-specific environmental stressors like chemical pollutants, sedimentation, temperature or dissolved oxygen change (Sutherland et al., 2002; Wang et al., 2006; Utz et al., 2010). The Tennessee Valley Authority (TVA) and Tennessee Department of Environment and Conservation (TDEC) have developed IBI's for fish (modified from Karr, 1981) and benthic macroinvertebrates (Kerans and Karr, 1994; TDEC, 2011), respectively, which are used to assess the health of streams in Tennessee. As part of TDEC's stream monitoring program, reaches are sampled every five years and are scored based on a set of metrics that reflect community diversity, food web structure, behavioral and reproductive traits, productivity, and disease risk. Scores, and individual metrics that comprise the scores, are strongly associated with in-stream water quality, channel alteration, and riparian degradation (Simon and Lyons, 1995; Roth et al., 1996; Allan et al., 1997; Stoddard et al., 2008). However, landscape-level associations between non-point source stressors and IBI metrics have not been adequately assessed.

Sedimentation and nutrient runoff from non-point source pollution are the primary stressors of stream ecosystem health (Johnson et al., 1997; Zamor and Grossman, 2007; Paulsen et al., 2008; Kemp et al., 2011; Clapcott et al., 2012). However, variables describing pollutant loads derived from land use activities are rarely assessed, aside from studies that measure "snap shot" estimates of nutrients and sediment at local scales (e.g., a stream reach). The Soil and Water Assessment Tool (SWAT) is a publicly accessible watershed-scale model created by the USA Department of Agriculture that quantifies land use impacts on water, sediment, and nutrient yields (organic nitrogen and phosphorus). The main inputs of the SWAT model are rates of precipitation, surface runoff, return flow, percolation, evapotranspiration, transmission losses, pond and reservoir storage, crop growth and irrigation, groundwater flow, reach routing, nutrient and pesticide loading, and water transfer. These data are publicly available and can be incorporated in a geographic information system (GIS) and used to estimate nutrient and sediment yield at a particular point in a stream. To our knowledge, SWAT model estimates of pollutants have not been used to examine if the association exists between non-point source pollutants and stream biotic integrity. We used the Nolichucky watershed in east Tennessee as a case study for testing the hypothesis that SWAT model estimates of sediment and nutrient runoff transported from

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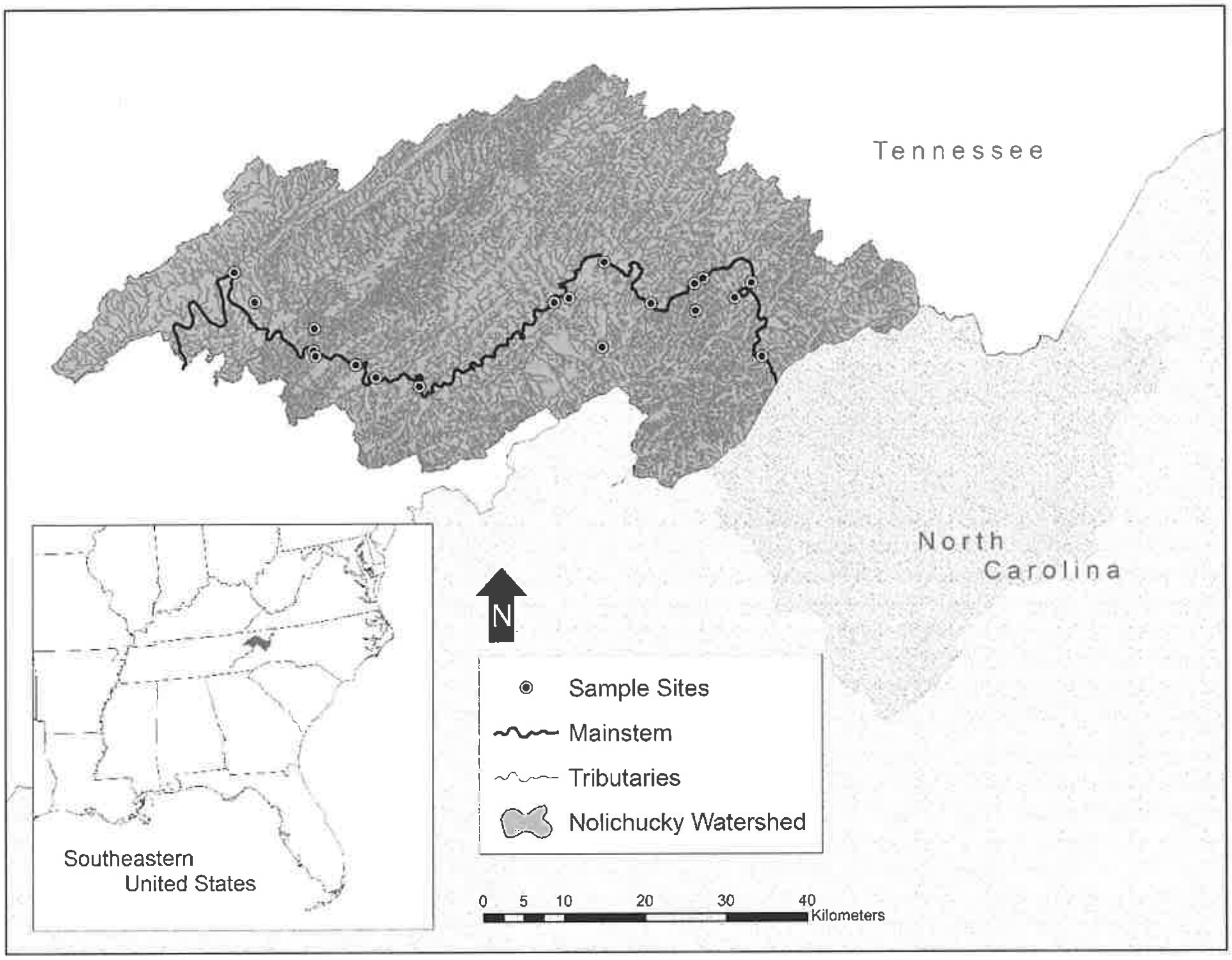

FIG. 1. Sample locations for fish and benthic macroinvertebrates in the Nolichucky River watershed during 2014–2016.

watersheds upstream of fish and benthic macroinvertebrate sample sites are correlated with IBI metrics that reflect stream ecosystem health at the local scale.

Materials and Methods

Sample site selection—The Nolichucky River watershed straddles the border of Tennessee and North Carolina (Figure 1). It drains the Blue Ridge Mountains and is a tributary to the French Broad River. All sampling of fish and benthic macroinvertebrates for this study occurred on the Tennessee side of the watershed, where there is a total of 3,803 stream kilometers (TDEC, 2008). The watershed lies within the U.S. EPA's level 1 ecoregion, which is defined as Eastern Temperate Forest (Omernik, 1987). This ecoregion contains two nested level III ecoregions—the Blue Ridge Mountains to the east and the Ridge and Valley to the west (Commission for Environmental Cooperation, CEC, 1997). The Blue Ridge Mountain landscape is mostly comprised of an oak-pine (*Quercus-Pinus*) forest with a small amount of agriculture consisting of apple orchards and fields of tobacco and hay/pasture. The Tennessee portion of the watershed is mostly in the Ridge and Valley ecoregion, which historically was covered with oak-pine forests that were interspersed with grassland barrens. Presently, 47% of the Tennessee portion of the watershed is covered by agriculture, mostly in cattle pasture and hay production (TDEC, 2008). The Ridge and Valley ecoregion is mainly underlain by highly soluble carbonate parent rock that can make the water slightly alkaline (Lloyd and Lyke, 1995). The soil type is comprised of brown loamy soils and red clay soils. In the Blue Ridge Mountain ecoregion, mean annual precipitation ranges from 1,020 mm to 1,270 mm, with about 20% being snow fall. Mean annual temperature is approximately 10°C to 16°C. In the Ridge and Valley ecoregion, mean annual precipitation ranges from 900 mm to 1,400 mm annually, and the mean annual temperature ranges from 13°C to 16°C (McNab, 1996).

For this study, 19 sample sites in the Tennessee portion of the watershed were selected to reflect a gradient in land cover types that contribute to variation in sediment and nutrient loads. Variation in benthic invertebrate and fish assemblages due to natural geomorphic factors were accounted for by selecting sites within an elevation gradient and watershed size (Table 1). This was done because benthic invertebrate and fish assemblages are different with respect to natural changes in water temperature (e.g., higher elevation, shaded canopy sites), discharge (e.g., lower in tributary sites), and substrate size (e.g., larger substrates in tributaries) (Vannote et al., 1980). One tributary (Clarks Creek) and one main stem site on the Nolichucky River were sampled in 2014 and 2015 to check for temporal changes in fish and benthic macroinvertebrate assemblages as a function of natural changes in hydrology; however, the assemblages were highly similar with respect to

TABLE 1. Summary statistics for watershed size and position, non-point source pollutant yield from SWAT model estimates during 2014, and metrics reflecting biotic integrity of fish and benthic macroinvertebrates in the Nolichucky River watershed, Tennessee, during 2014–2016. Detailed descriptions of each metric and how they are calculated can be found in Gotwald (2016).

Characteristic	Mean	Max	Min
Upstream catchment area (km^2)	1,798.9	4,229.0	14.2
Elevation above mean sea level (m)	462.5	637.6	315.8
Yield estimates (kg/ha) from SWAT model simulations			
Sediment	8,164.7	46,629.3	181.4
Organic nitrogen	5.3	12.9	0.6
Organic phosphorus	0.9	2.1	0.1
Fish IBI metrics			
Native richness [NATIVE]	19.3	26.0	3.0
Darter richness [DARTER]	6.0	8.0	0.0
Sunfish richness minus *Micropterus* [SUNFISH]	2.0	6.0	0.0
Sucker richness [SUCKER]	2.0	5.0	0.0
Pollution-intolerant richness [INTOLERANT]	3.5	5.0	0.0
% Pollution-tolerant [%TOLERANT]	7.7	26.6	0.0
% specialized insectivores [%INSECTIVORE]	58.1	86.8	0.0
% omnivores [%OMNIVORE]	14.0	43.0	1.9
% piscivores [%PISCIVORE]	1.2	7.8	0.0
Catch per unit effort (fish/m^2) [CPUE]	19.5	43.5	5.0
% hybrids [%HYBRID]	8.8	58.0	0.0
% with disease, anomalies, lesions, tumors [%DALT]	0.5	2.7	0.0
Benthic invertebrate IBI metrics			
Taxa richness [TR]	28.5	41.0	11.0
Ephemeroptera-Plecoptera-Trichoptera richness [EPT]	14.1	25.0	2.0
% EPT minus *Cheumatopsyche* [%EPT-C]	55.9	74.9	21.2
% Oligochaeta and Chironomidae [%OC]	5.8	16.2	0.2
North Carolina Biotic Index [NCBI]	4.1	4.7	3.1
% clingers [%CLINGER]	50.4	78.5	14.1
% total nutrient tolerant organisms [%TNUTOL]	19.9	36.3	5.2

species composition, and no changes were detected with respect to land use or hydrology (Gotwald, 2016).

Land cover data acquisition and classification—Landsat 5 Thematic Mapper (TM) and Landsat 8 Operational Land Imager (OLI) satellite data at path 18, row 35 with minimal cloud cover were acquired for 2014 during the visible growing season. Satellite images were preprocessed using the software ENVI version 4.8 (Exelis Visual Information Solutions, 2010). Radiometric correction was performed to produce reflectance values, and atmospheric correction was completed with a dark body subtraction (e.g., Level II Normalized Difference Vegetation Index, NDVI, Rouse et al., 1974). Scenes that were derived from Landsat surface reflectance images were acquired from the USGS's Earth Resources Observation and Science Center (EROS) to aid the classification and differentiation between row crops and open spaces. Aerial photographs that were coincident with the satellite data were acquired to provide training areas for supervised classifications and ground truth sample sites for accuracy assessments of the resulting thematic maps.

To help separate row crops from open spaces (i.e., hay, pasture, and fallow fields), a mask was created for the NDVI time series using 2011 National Land Cover Dataset's (NLCD) impervious surface layers, the National Hydrography Dataset's (NHD) vector data of open water, and the forested land cover identified in the supervised classification. They were applied to the satellite images with the closest corresponding acquisition year. A maximum likelihood supervised classification approach in combination with a normalized difference vegetation index calibrated density slice (NDVI, Rouse et al., 1974, Tucker, 1979) was used to produce LULC maps from the processed Landsat time series. The maximum likelihood supervised classification produced 4 classes: forest, impervious, row crop, and open space (i.e., hay, pasture, and fallow fields). Upstream catchments for each site were delineated using the Arc Hydro toolset version 2.0 (Djokic et al., 2011). Using U.S.D.A National Agriculture Imagery Program (NAIP) photos for 2014, land use was digitized and summed for each of the 19 catchments.

SWAT model estimates—To assess the amount of non-point source pollutant runoff in each catchment, the ArcSWAT 2012 model was used to simulate the amount of sediment yield for

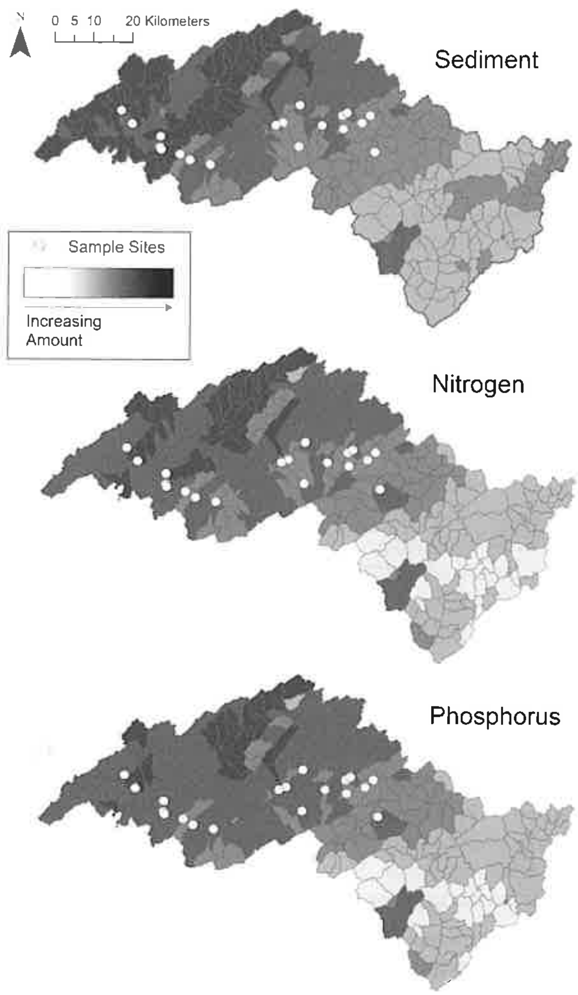

FIG. 2. Estimates of SWAT model estimates for HUC-12 catchments within the Nolichucky River watershed of Tennessee and North Carolina during 2014. Darker shades represent greater values of sediment, nitrogen, phosphorus yield for the entire year.

each sample site for 2014. Mandatory spatial input files needed for the model included the input digital elevation model (DEM), land use map, and soil layer. Using the DEM, a catchment was created for each sample site. The catchments were divided into hydrologic response units (HRUs) by land use/land cover (LULC), slope levels, and soil percentage. The HRUs are areas in the model that are calculated to have the same manner in which they conduct water through the system to the sample site. Land use and slope were reclassified in the SWAT model. Land use was classified as agriculture row crops, mixed forest, water, residential medium/low density, and pasture. The slope was classified into five classes based on natural breaks in the percent rise value in the raster.

The SWAT output file was a measurement of sediment yield, organic nitrogen yield, and organic phosphorus yield for all HRU's in the Nolichucky River watershed (Figure 2), as well as each of the 19 catchments that drained to a fish and benthic macroinvertebrate sample site. The model was run on a monthly time step during 2014. Sediment yield (SYLD) was reported as metric ton/ha and is the sediment from the catchment that is transported to the sample site during the time step. Organic nitrogen yield (ORGN) was reported as the ORGN transported out of the catchment and into the sample site during the time step. Organic phosphorus yield (ORGP) was reported as the amount of ORGP transported (Arnold et al., 2012). The measurements for all months were totaled and the amounts of SYLD, ORGN, and ORGP transported with sediment into the sample site during 2014 are reported in Table 1.

Benthic macroinvertebrate sampling—To sample the benthic macroinvertebrate community, a 500-μm mesh kick net was used to conduct two "semi-quantitative" samples in a "fast" and "slow" portion of the riffle to collect individuals. The procedures for collection, sorting, preserving, identification, and Tennessee Macroinvertebrate Index (TMI) metric calculation followed standard TDEC (2011) procedures for wadeable streams in the Blue Ridge Mountain and Ridge and Valley bioregions. Metrics from the TMI are designed to assess the overall health of wadeable streams with regard to supporting a natural benthic macroinvertebrate community. All invertebrates were identified to species, if practical, and the whole sample for each site was counted (i.e., no subsampling occurred prior to identification). Chironomid genera/species and oligochaetes were mounted in CMCP-9 low viscosity mountant and identified under a compound light microscope. All other invertebrates were identified under a dissecting light microscope. The most recent available dichotomous keys were used for identification. Quality assurance of identifications occurred with a second person of equal skill re-identifying a portion of the invertebrates.

Fish sampling—During summers from 2014 to 2016, we followed the Tennessee Valley Authority's (TVA) standard sampling protocols for their stream fish IBI, which is a region-specific modification of the original fish IBI published by Karr (1981). Fish were sampled in riffle-run habitats by simultaneously kicking the stream bottom and back-pack electrofishing (60 Hz, AC) into a 3 m x 6 m seine net (untreated nylon, 6-mm mesh). Seine hauls were used to sample fish from pool habitats. Sample areas for electrofishing sets and seine hauls were standardized to 28 m^2, and sampling continued in a habitat type until it was "depleted" of species. That is, sampling occurred until three consecutive runs yielded no new species for riffle and run habitats. At sites with stream widths >7 m, a 51-m shoreline backpack electrofishing sample was conducted to collect fishes inhabiting bank associated habitats. All fish were identified after each electrofishing sample run, counted, and released alive immediately into the stream. Fish sampling methods were approved under IACUC protocol #2257-0414 by the University of Tennessee-Knoxville.

Statistical analysis—Partial canonical correspondence analysis (pCCA) was conducted with a main matrix of the seven benthic macroinvertebrate IBI metrics. Proportion data were arcsine-square root transformed, and continuous data were ln-transformed to improve multivariate normality. All transformed fish metric data were further standardized by watershed area, because the IBI scoring developed by the TVA takes into account the natural variation in stream fish assemblages as a function of drainage area and channel size (Vannote et al., 1980). The SWAT model estimate for each catchment was used as an explanatory variable in the second matrix of the pCCA. To account for effects of spatial proximity of sample sites within the watershed, geographic coordinates were used as a covariable matrix in the pCCA

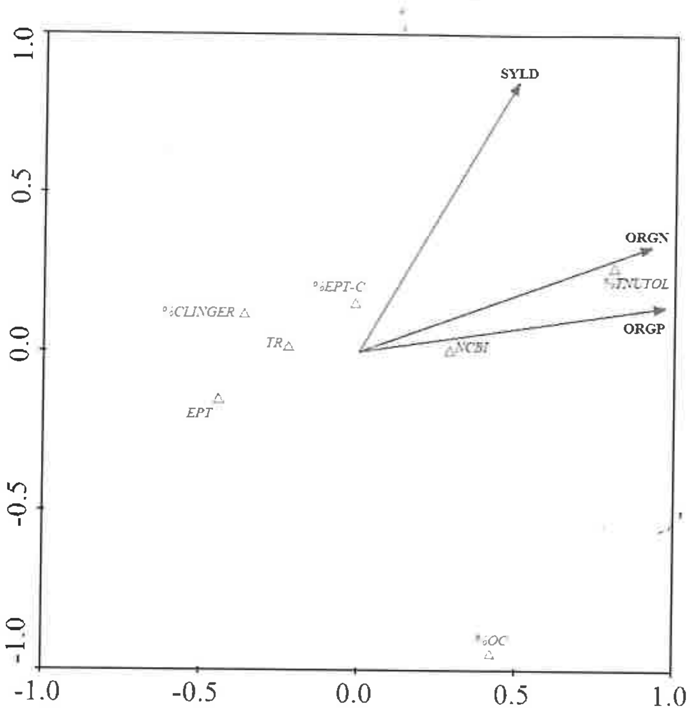

FIG. 3. Partial canonical correspondence (pCCA) of SWAT model estimates of non-point source pollutant runoff and benthic macroinvertebrate metrics of biotic integrity in the Nolichucky River watershed, Tennessee. Geographical coordinates were used as covariables to account for variation in the ordination due to spatial proximity of sample sites. Abbreviation definitions can be found in Table 1.

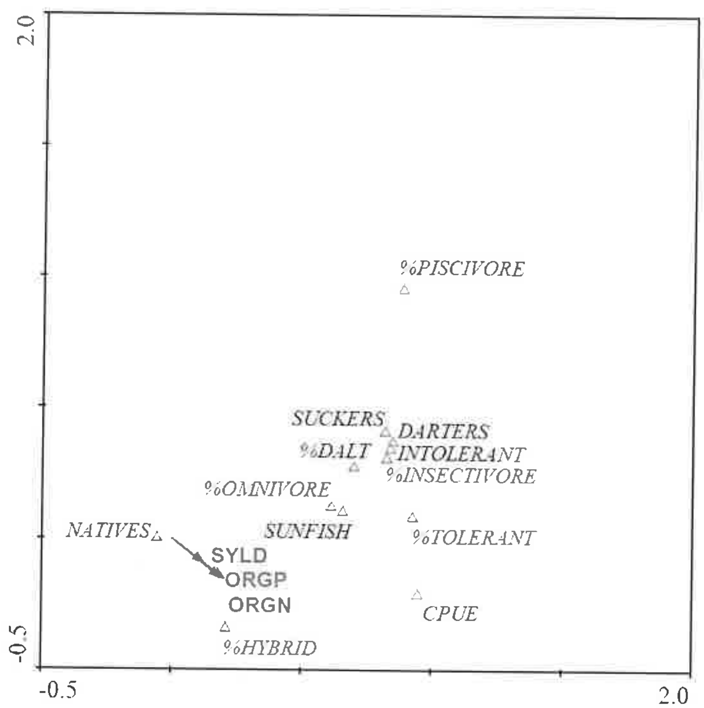

FIG. 4. Partial correspondence analysis (pCA) of SWAT model estimates of non-point source pollutant runoff and benthic macroinvertebrate metrics of biotic integrity in the Nolichucky River watershed, Tennessee. Geographical coordinates were used as covariables to account for variation in the ordination due to spatial proximity of sample sites. Abbreviation definitions can be found in Table 1.

(Borcard et al., 1992; Grand and Cushman, 2003; Alford, 2014). This procedure was done to remove any potential confounding effects of spatial proximity on the composition of fish and benthic macroinvertebrate metrics (Borcard et al., 1992). A Monte Carlo randomization procedure was run 499 times to determine if the axes and correlations between the IBI metrics and SWAT model estimates matrix were statistically significant ($P < 0.05$). A preliminary pCCA was run on the 12 fish IBI metrics versus the SWAT model estimates, however the resulting ordination of species in environmental space was not canonical ($P > 0.05$ for all canonical axes). Therefore, an indirect gradient analysis was run (partial correspondence analysis, or pCA). This procedure differs from pCCA in that in does not constrain the effects of environmental variables prior to the ordination of metrics. Both analyses were run in CANOCO version 4.5 software (ter Braak and Smilauer, 1998; Grand and Cushman, 2003; Alford 2014).

Results

A total of 10,831 benthic macroinvertebrates representing 266 taxa were collected from riffle habitats in the Nolichucky River watershed (Table 2). A combination of the caddisfly genera *Cheumatopsyche*, *Hydropsyche*, *Ceratopsyche*, the mayfly genera *Maccaffertium*, *Isonychia*, *Baetis*, the blackfly genus *Simulium*, and the riffle beetle genus *Stenelmis* comprised half of all individuals collected. A total of 11,414 fish representing 55 species were sampled from riffle, run, and pool habitats using seine hauls and backpack electrofishing (Table 3). Over half (53%) of the individuals consisted of Highland Shiner *Notropis micropteryx*, Sharphead Darter *Etheostoma acuticeps*, Greenside Darter *Etheostoma blennioides*, Telescope Shiner *Notropis telescopus*, Central Stoneroller *Campostoma anomalum*, Banded Sculpin *Cottus carolinae*, and Bluebreast Darter *Etheostoma camurum*. A summary of IBI metrics for the benthic macroinvertebrate and fish assemblages as well as estimates generated by the SWAT models for sediment, organic nitrogen, and organic phosphorus for watersheds upstream of the fish and invertebrate sample sites are provided in Table 1.

There was a significant association between benthic macroinvertebrate IBI metrics and SWAT model estimates after removing confounding effects of spatial proximity among sample sites (partial CCA; 499 Monte Carlo runs; all canonical axes $F = 1.90$; $P = 0.046$, axis 1 $F = 4.54$; $P = 0.018$). More specifically, %TNUTOL showed a significant positive relationship with organic nitrogen and phosphorus yield, whereas %CLINGER was negatively associated with organic nitrogen and phosphorus (Figure 3). Sediment yield had a significant negative relationship with %CLINGER, TR, and EPT (Figure 3). With respect to fish IBI metrics, there was also a significant association with SWAT model estimates after accounting for spatial position of sample sites (partial CA; 499 Monte Carlo runs; total inertia = 0.39; cumulative explained variance in fish metric-SWAT variable for axes 1 and 2 = 95.5%). A significant positive association was detected between %HYBRID and CPUE and all three SWAT model variables (Figure 4). In contrast, native species richness (NATIVE) was negatively associated with non-point sources of sediment, nitrogen and phosphorus yields (Figure 4).

TABLE 2. Summary of abundance and frequencies of benthic macroinvertebrate taxa collected using semi-quantitative kick nets in riffle habitats in the Nolichucky River watershed, Tennessee, during 2014–2016. Counts reflect the combined numbers of each taxon for 21 samples.

Class/order	Family	Genus	Species/ species group	Count	% Occurrence	% Composition
Amphipoda	Crangonyctidae	*Crangonyx*		1	4.8	0.01
	Crangonyctidae			1	4.8	0.01
Coleoptera	Chrysomelidae			7	4.8	0.06
	Curculionidae	*Stenopelmus*	*rufinasus*	1	4.8	0.01
	Dryopidae	*Helichus*	*fastigiatus*	5	4.8	0.05
	Elmidae	*Dubiraphia*		1	4.8	0.01
		Gonielmis		3	4.8	0.03
		Macronychus	*glabratus*	13	9.5	0.12
		Macronychus		3	9.5	0.03
		Microcylloepus	*pusillus*	10	9.5	0.09
		Microcylloepus		16	19.0	0.15
		Optioservus	*ovalis*	26	4.8	0.24
		Optioservus		55	4.8	0.51
		Oulimnius	*latiusculus*	40	4.8	0.37
		Oulimnius		9	4.8	0.08
		Promoresia	*tardella*	17	4.8	0.16
		Promoresia	*elegans*	32	19.0	0.3
		Promoresia	*tardella*	1	4.8	0.01
		Promoresia		10	19.0	0.09
		Stenelmis	*mera*	247	14.3	2.28
		Stenelmis		414	81.0	3.82
		Stenelmis	*sandersoni*	5	4.8	0.05
	Gyrinidae	*Dineutus*		6	14.3	0.06
		Gyretes		2	4.8	0.02
	Helophoridae	*Helophorus*		3	4.8	0.03
	Hydrochidae	*Hydrochus*		2	9.5	0.02
	Hydrophilidae	*Cerycon*		7	4.8	0.06
	Psephenidae	*Psephenus*	*herricki*	210	38.1	1.94
		Psephenus		69	19.0	0.64
	Ptilodactylidae	*Anchytarsus*	*bicolor*	3	4.8	0.03
	Scirtidae	*Sacodes*		7	4.8	0.06
	Scirtidae			2	4.8	0.02
	Hydrphilidae	*Sperchopsis*	*tesselata*	1	4.8	0.01
Diptera	Athericidae	*Atherix*	*lantha*	39	23.8	0.36
		Atherix		13	9.5	0.12
	Ceratopogonidae	*Prionocera*		2	4.8	0.02
	Chironomidae	*Ablabesmyia*	*mallochi*	8	19.0	0.07
		Cardiocladius	*albiplumus*	17	14.3	0.16
		Cardiocladius	*obscurus*	173	19.0	1.6
		Cladotanytarsus	*davesi*	1	4.8	0.01
		Cladotanytarsus		3	4.8	0.03
		Cricotopus	*absurdius*	1	4.8	0.01
		Cricotopus	*fugax*	12	19.0	0.11
		Cricotopus	*politus*	3	4.8	0.03
		Cricotopus	*tibialis*	1	4.8	0.01
		Cricotopus	*triannulatus*	2	4.8	0.02
		Cricotopus	*trifascia*	1	4.8	0.01
		Cricotopus		16	19.0	0.15
		Cryptochironomus		1	4.8	0.01
		Demicryptochironomus		2	9.5	0.02
		Eukiefferiella	*brehmi*	1	4.8	0.01
		Eukiefferiella	*claripennis*	1	4.8	0.01

TABLE 2. Continued.

Class/order	Family	Genus	Species/ species group	Count	% Occurrence	% Composition
		Eukiefferiella	*devonica*	5	4.8	0.05
		Eukiefferiella	*gracei*	9	9.5	0.08
		Euryhapsis		1	4.8	0.01
		Microtendipes	*pedellus*	2	4.8	0.02
		Nanocladius	*downesi*	7	9.5	0.06
		Nanocladius		3	4.8	0.03
		Natarsia		12	28.6	0.11
		Orthocladius	*annectens*	4	14.3	0.04
		Orthocladius	*annulator*	9	4.8	0.08
		Orthocladius	*dubitatus*	90	28.6	0.83
		Orthocladius	*frigidus*	1	4.8	0.01
		Orthocladius	*lignicola*	5	4.8	0.05
		Orthocladius	*luteipes*	3	9.5	0.03
		Orthocladius	*obumbratus*	17	4.8	0.16
		Orthocladius	*oliveri*	3	9.5	0.03
		Orthocladius	*rivicola*	1	4.8	0.01
		Orthocladius	*robacki*	1	4.8	0.01
		Orthocladius	*rubicundus*	1	4.8	0.01
		Orthocladius	*saxosus*	6	9.5	0.06
		Orthocladius	*thienemanni*	4	14.3	0.04
		Orthocladius		1	4.8	0.01
		Pagastia		2	4.8	0.02
		Polypedilum	*aviceps*	11	28.6	0.1
		Polypedilum	*fallax*	1	4.8	0.01
		Polypedilum	*flavum*	6	19.0	0.06
		Polypedilum	*illinoense*	3	9.5	0.03
		Polypedilum	*nubeculosum*	3	4.8	0.03
		Polypedilum	*scalaenum*	1	4.8	0.01
		Polypedilum	*tritum*	12	9.5	0.11
		Potthastia	*goedii*	4	4.8	0.04
		Rheotanytarsus	*exiguus*	33	19.0	0.3
		Rheotanytarsus		2	9.5	0.02
		Stempellinella		6	4.8	0.06
		Stenochironomus		2	9.5	0.02
		Sublettea	*coffmani*	1	4.8	0.01
		Tanytarsus	*brundini*	3	4.8	0.03
		Tanytarsus		6	9.5	0.06
		Thienemanniella	*similis*	1	4.8	0.01
		Thienemannimyia		2	4.8	0.02
		Tokunagaia		1	4.8	0.01
		Tvetenia	*vitracies*	3	4.8	0.03
		Tvetenia		21	23.8	0.19
		Dicrotendipes		2	4.8	0.02
		Apedilum		1	4.8	0.01
		Rheocricotopus	*unidentatus*	1	4.8	0.01
	Empididae	*Hemerodromia*		6	14.3	0.06
	Simuliidae	*Cnephia*		244	38.1	2.25
		Simulium		450	76.2	4.15
	Tabanidae	*Haematopota*		1	4.8	0.01
	Tanyderidae	*Protoplasa*	*fitchii*	1	4.8	0.01
		Protoplasa		1	4.8	0.01
	Tipulidae	*Antocha*		60	42.9	0.55
		Hexatoma		9	19.0	0.08
		Limonia		1	4.8	0.01

TABLE 2. Continued.

Class/order	Family	Genus	Species/ species group	Count	% Occurrence	% Composition
		Pedicia		3	4.8	0.03
		Tipula		25	14.3	0.23
Ephemeroptera	Baetidae	*Acentrella*		11	19.0	0.10
		Acerpenna		17	4.8	0.16
		Baetis		504	81.0	4.65
		Heterocloeon	*jubilatum*	1	4.8	0.01
		Heterocloeon		268	52.4	2.47
		Iswaeon		42	23.8	0.39
		Plauditus		74	4.8	0.68
		Procloeon	*nelsoni*	1	42.9	0.01
		Pseudocentroptiloides		1	4.8	0.01
	Caenidae	*Caenis*		12	23.8	0.11
	Ephemerellidae	*Drunella*		2	9.5	0.02
		Ephemerella	*excruciens*	3	4.8	0.03
		Serratella	*deficiens*	57	19.0	0.53
		Serratella	*frisoni*	10	9.5	0.09
		Serratella	*serrata*	14	9.5	0.13
		Serratella	*serratoides*	3	4.8	0.03
		Serratella		59	23.8	0.54
		Teloganopsis	*deficiens*	1	4.8	0.01
		Teloganopsis		5	14.3	0.05
	Ephemeridae	*Ephemera*		2	4.8	0.02
	Heptageniidae	*Epeorus*	*rubidus/subpallidus*	1	4.8	0.01
		Epeorus	*vitreus*	10	4.8	0.09
		Epeorus		19	14.3	0.18
		Leucocruta	*aphrodite*	3	4.8	0.03
		Leucocruta		64	19.0	0.59
		Maccaffertium	*carlsoni*	1	4.8	0.01
		Maccaffertium	*ithaca*	1	4.8	0.01
		Maccaffertium	*mediopunctatum*	133	52.4	1.23
		Maccaffertium	*modestum*	39	19.0	0.36
		Maccaffertium	*pudicum*	11	9.5	0.10
		Maccaffertium	*terminatum*	3	4.8	0.03
		Maccaffertium		585	52.4	5.40
		Rhithrogena		1	4.8	0.01
		Stenacron	*interpunctatum*	2	9.5	0.02
		Stenacron		46	23.8	0.42
		Stenonema	*femoratum*	17	19.0	0.16
		Stenonema		182	28.6	1.68
		Heptagenia		2	4.8	0.02
	Isonychidae	*Isonychia*	*bicolor*	3	4.8	0.03
		Isonychia		1,026	76.2	9.47
	Leptohyphidae	*Tricorythodes*		45	23.8	0.42
	Leptophlebiidae	*Leptophlebia*		17	4.8	0.16
		Paraleptophlebia		19	14.3	0.18
	Polymitarcydae	*Ephoron*	*leukon*	29	4.8	0.27
		Ephoron		47	19.0	0.43
Megaloptera	Corydalidae	*Corydalus*	*cornutus*	108	61.9	1.00
		Nigronia	*serricornis*	41	33.3	0.38
		Nigronia		6	9.5	0.06
	Sialidae	*Sialis*		9	4.8	0.08
Odonata	Aeshnidae	*Triacanthagyna*		5	4.8	0.05
	Calopterygidae	*Hetaerina*	*americana*	1	4.8	0.01
		Hetaerina		1	4.8	0.01

TABLE 2. Continued.

Class/order	Family	Genus	Species/ species group	Count	% Occurrence	% Composition
	Coenagrionidae	*Argia*		10	14.3	0.09
	Gomphidae	*Arigomphus*		8	4.8	0.07
		Hetaerina	*americana*	1	4.8	0.01
		Lanthus	*vernalis*	2	4.8	0.02
		Lanthus		2	9.5	0.02
		Stylogomphus		14	23.8	0.13
		Ophiogomphus	*mainensis*	3	4.8	0.03
	Macromiidae	*Macromia*		1	4.8	0.01
Plecoptera	Chloroperlidae	*Haploperla*		1	4.8	0.01
		Suwallia	*marginata*	1	4.8	0.01
	Leuctridae	*Leuctra*		123	19.0	1.14
	Peltoperlidae	*Tallaperla*		148	14.3	1.37
		Peltoperla		1	4.8	0.01
	Perlidae	*Acroneuria*	*abnormis*	15	9.5	0.14
		Acroneuria		35	23.8	0.32
		Agnetina		29	23.8	0.27
		Neoperla		6	9.5	0.06
		Paragnetina	*ichusa*	12	4.8	0.11
		Paragnetina	*media*	7	4.8	0.06
		Paragnetina		1	4.8	0.01
		Perlesta	*frisoni*	5	4.8	0.05
		Perlesta		47	14.3	0.43
		Hansonoperla		2	4.8	0.02
	Pteronarcyidae	*Pteronarcys*	*proteus*	25	4.8	0.23
		Pteronarcys		9	14.3	0.08
	Perlodidae	*Diploperla*	*duplicata*	1	4.8	0.01
	Taeniopterygidae	*Strophopteryx*	*fasciata*	8	4.8	0.07
Trichoptera	Brachycentridae	*Brachycentrus*	*nigrosoma*	188	23.8	1.74
		Brachycentrus	*numerosus*	14	23.8	0.13
		Brachycentrus	*spinae*	1	4.8	0.01
		Brachycentrus		199	9.5	1.84
		Micrasema	*rusticum*	1	4.8	0.01
		Micrasema	*wataga*	5	14.3	0.05
		Micrasema		2	9.5	0.02
	Glossosomatidae	*Glossosoma*	*nigrior*	3	9.5	0.03
		Glossosoma		4	9.5	0.04
		Protoptila		1	4.8	0.01
	Goeridae	*Goera*		1	4.8	0.01
	Helicopsychidae	*Helicopsyche*		1	4.8	0.01
	Hydropsychidae	*Ceratopsyche*	*etnieri*	7	9.5	0.06
		Ceratopsyche	*morosa*	255	23.8	2.35
		Ceratopsyche	*sparna*	83	19.0	0.77
		Ceratopsyche		412	42.9	3.80
		Cheumatopsyche		1,482	100.0	13.68
		Diplectrona	*modesta*	30	14.3	0.28
		Hydropsyche	*alvata*	17	4.8	0.16
		Hydropsyche	*betteni/depravata*	10	4.8	0.09
		Hydropsyche	*mississippiensis*	1	4.8	0.01
		Hydropsyche	*phalerata*	1	4.8	0.01
		Hydropsyche	*venularis*	45	14.3	0.42
		Hydropsyche		514	66.7	4.75
		Psychomyia	*flavida*	1	4.8	0.01
		Psychomyia		41	33.3	0.38
	Hydroptilidae	*Hydroptila*		36	14.3	0.33

TABLE 2. Continued.

Class/order	Family	Genus	Species/ species group	Count	% Occurrence	% Composition
		Leucotrichia	*pictipes*	1	4.8	0.01
		Oxyethira		155	9.5	1.43
		Stactobiella		3	9.5	0.03
	Lepidostomatidae	*Lepidostoma*		6	19.0	0.06
	Leptoceridae	*Nectopsyche*		1	4.8	0.01
		Oecetis	*avara*	1	4.8	0.01
		Oecetis	*persimilis*	10	14.3	0.09
		Oecetis		9	14.3	0.08
		Setodes		4	14.3	0.04
	Limephilidae	*Pycnopsyche*		1	4.8	0.01
	Odontoceridae	*Psilotreta*		43	4.8	0.4
	Philopotamidae	*Chimarra*	*aterrima*	18	4.8	0.17
		Chimarra		6	9.5	0.06
		Dolophilodes	*distincta*	15	4.8	0.14
		Dolophilodes		67	9.5	0.62
		Wormaldia		3	4.8	0.03
	Polycentropodidae	*Cernotina*		1	4.8	0.01
		Cyrnellus		2	9.5	0.02
		Neureclipsis	*crepuscularis*	2	9.5	0.02
		Neureclipsis		3	9.5	0.03
	Psychomyiidae	*Lype*	*diversa*	1	4.8	0.01
		Psychomyia	*flavida*	3	4.8	0.03
		Psychomyia		2	9.5	0.02
	Rhyacophilidae	*Rhyacophila*	*formosa*	1	4.8	0.01
		Rhyacophila	*fuscula*	2	4.8	0.02
		Rhyacophila		7	9.5	0.06
	Thremmatidae	*Neophylax*	*etnieri*	2	9.5	0.02
		Neophylax		5	19.0	0.05
Limnophila	Ancylidae	*Ferrissia*	*fragilis*	3	9.5	0.03
Veneroida	Corbiculidae	*Corbicula*	*fluminea*	64	52.4	0.59
Lumbriculida	Lumbricidae			2	4.8	0.02
	Lumbriculidae	*Eclipidrilus*	*lacustris*	7	14.3	0.06
		Lumbriculis	*variegatus*	6	9.5	0.06
		Lumbriculis		8	9.5	0.07
		Stylodrilus	*wahkeenensis*	8	9.5	0.07
	Naididae			1	4.8	0.01
	Sparganophilidae	*Sparganophilus*		2	9.5	0.02
	Tubificidae			1	4.8	0.01
Mesogastropoda	Pleuroceridae	*Leptoxis*	*praerosa*	87	19.0	0.80
		Pleurocera	*clavaeformis*	90	33.3	0.83
		Pleurocera	*troostiana*	2	4.8	0.02
Decapoda	Cambaridae	*Cambarus*	*girardianus*	10	9.5	0.09
		Cambarus	*longirostris*	16	9.5	0.15
		Cambarus		5	14.3	0.05
		Faxonius	*juvenilis*	11	4.8	0.10
		Faxonius		4	4.8	0.04
Lepidoptera	Pyralidae	*Petrophila*		11	14.3	0.10
Acarina	Acarina	*Acarina*		5	19.0	0.05
Tricladida	Dugesiidae	*Dugesia*		5	4.8	0.05
	Planaridae			1	4.8	0.01
Isopoda	Asselidae	*Lirceus*		19	14.3	0.18

TABLE 3. Summary of fish species from 21 samples collected by backpack electroshocking and seining in riffle, run, and pool habitats in the Nolichucky River watershed, Tennessee, during 2014–2016.

Scientific name	Common name	Count	% Occurrence	% Composition
Notropis micropteryx	Highland Shiner	1,126	66.7	9.87
Etheostoma acuticeps	Sharphead Darter	932	47.6	8.17
Etheostoma blennioides	Greenside Darter	905	85.7	7.93
Notropis telescopus	Telescope Shiner	897	66.7	7.86
Campostoma anomalum	Central Stoneroller	854	95.2	7.48
Cottus carolinae	Banded Sculpin	699	90.5	6.12
Etheostoma camurum	Bluebreast Darter	685	57.1	6.00
Etheostoma zonale	Banded Darter	660	71.4	5.78
Cyprinella spiloptera	Spotfin Shiner	434	66.7	3.80
Notropis rubricroceus	Saffron Shiner	392	23.8	3.43
Etheostoma simoterum	Snubnose Darter	368	76.2	3.22
Notropis volucellus	Mimic Shiner	356	42.9	3.12
Cottus bairdi	Mottled Sculpin	353	28.6	3.09
Notropis photogenis	Silver Shiner	343	47.6	3.01
Hypentelium nigricans	Northern Hogsucker	273	90.5	2.39
Cyprinella galactura	Whitetail Shiner	263	38.1	2.30
Luxilus chrycocephalus	Striped Shiner	257	9.5	2.25
Rhinichthys atratulus	Blacknose Dace	203	33.3	1.78
Nocomis micropogon	River Chub	165	71.4	1.45
Percina evides	Gilt Darter	158	52.4	1.38
Luxilus coccogenis	Warpaint Shiner	147	33.3	1.29
Semotilus atromaculatus	Creek Chub	147	9.5	1.29
Etheostoma rufilineatum	Redline Darter	129	28.6	1.13
Moxostoma breviceps	Smallmouth Redhorse	107	9.5	0.94
Notropis straminea	Sand Shiner	83	4.8	0.73
Oncorhyncus mykiss	Rainbow Trout	79	28.6	0.69
Micropterus dolomieu	Smallmouth Bass	52	33.3	0.46
Notropis leuciodes	Tennessee Shiner	49	23.8	0.43
Hybopsis amblops	Bigeye Chub	45	23.8	0.39
Phenacobius uranops	Stargazing Minnow	44	42.9	0.39
Pimephales vigilax	Bullhead Minnow	41	23.8	0.36
Rhinichthys cataractae	Longnose Dace	36	9.5	0.32
Percina squamata	Olive Darter	18	14.3	0.16
Moxostoma erythrurum	Golden Redhorse	17	4.8	0.15
Etheostoma vulneratum	Wounded Darter	14	14.3	0.12
Moxostoma carinatum	River Redhorse	10	4.8	0.09
Moxostoma duquesnei	Black Redhorse	9	4.8	0.08
Percina aurantiaca	Tangerine Darter	9	23.8	0.08
Noturus eleutherus	Mountain Madtom	8	4.8	0.07
Percina caprodes	Common Logperch	7	14.3	0.06
Gambusia affinis	Western Mosquitofish	6	4.8	0.05
Erimystax insignis	Blotched Chub	5	4.8	0.04
Ambloplites rupestris	Rock Bass	4	14.3	0.04
Ictiobus bubalus	Smallmouth Buffalo	4	4.8	0.04
Ichthyomyzon bdellium	Ohio Lamprey	3	4.8	0.03
Lepomis auritus	Redbreast Sunfish	3	4.8	0.03
Lepomis cyanellus	Green Sunfish	3	4.8	0.03
Lepomis macrochirus	Bluegill	3	4.8	0.03
Etheostoma chlorobranchium	Greenfin Darter	2	4.8	0.02
Etheostoma kennicotti	Stripetail Darter	2	9.5	0.02
Ameiurus natalis	Yellow Bullhead	1	4.8	0.01
Catastomus commersoni	White Sucker	1	4.8	0.01
Etheostoma jessiae	Blueside Darter	1	4.8	0.01
Notemigonus chrysoleucas	Golden Shiner	1	4.8	0.01
Pylodicitis olivaris	Flathead Catfish	1	4.8	0.01

Discussion

Regulatory agencies that assess the biological health of streams as mandated by the USA Clean Water Act of 1972 utilize multi-metric techniques like the IBI to assess a stream's ability to mirror the biological condition it would exhibit in its "natural", unimpaired setting (Karr et al., 1986; Karr, 1991; Lyons et al., 1996; Hughes et al., 2004). Metrics that comprise an IBI, and the total score, should be sensitive to anthropogenic changes to water chemistry and physical habitat, as well as external sources of pollution from fecal coliforms (Stoddard et al., 2008; Hawkins et al., 2008; Ruaro and Gubiani, 2013). However, given the stochastic nature of stream fish and invertebrate populations (e.g., reproductive success, recruitment of juveniles to maturation), and their ability to disperse and colonize other nearby patches of suitable habitat, it can be challenging to demonstrate strong cause-effect relationships between some site-specific IBI metrics and physicochemical properties of streams, such as biological oxygen demand (BOD), sediment load, daily/instantaneous dissolved oxygen concentration, or nitrogen/phosphorus concentrations (Rowe et al., 2009). At any assessed stream reach, water chemistry, habitat, and pollutants are often measured as snapshots in time that may reflect disturbances that are occurring at larger spatiotemporal scales outside the stream reach (Hitt and Roberts, 2012; Alford, 2014; Midway et al., 2014). Cumulative impacts of land use change and natural geomorphic or hydrologic inputs farther up a stream's watershed may influence the water quality at a particular point in a stream (Johnson at al., 1997; Allan, 2004; Helms et al., 2009; Blevins et al., 2013). In addition, these disturbances can occur prior to the time when water or biological samples are taken.

We found that the value of some metrics of riverine biotic integrity can be influenced by watershed-scale impacts from non-point sources of pollution, particularly nitrogen, phosphorus, and sediment. For benthic macroinvertebrates in the Nolichucky River watershed, SWAT model estimates of organic nitrogen and phosphorus yield were positively associated with percent nutrient-tolerant taxa, but negatively associated with percent clingers in semi-quantitative kick net samples. Sediment yield was negatively associated with percent clingers, taxa richness, and Ephemeroptera-Plecoptera-Trichoptera (EPT) richness. For fishes, sediment and nutrients were positively associated with % hybrids and catch per unit effort in backpack electrofishing and seine haul samples, but negatively associated with native species richness.

Remote sensing and GIS technology have made it easier for aquatic scientists and water resource managers to assess the physical and chemical conditions and potential non-point pollution sources of whole watersheds that may have occurred over decades (Gardiner et al., 2009). It is evident that land cover change from natural vegetation (e.g., forests, grasslands) to row-crop agriculture or impervious surfaces leads to increased inputs of nutrients, sediments, and organic pollutants (e.g., manure) to streams. The development of SWAT models has enabled aquatic ecologists and resource managers to investigate potential causes of non-point source pollution to specific stream segments at broader spatiotemporal scales than that of a stream reach at a particular point in time. To date, studies incorporating SWAT model estimates of nutrient and sediment yield have not attempted to show a link with biotic integrity. Our study shows that certain benthic macroinvertebrate and fish IBI metrics in the Nolichucky River drainage of Tennessee, using standard IBI sampling protocols developed by state and federal agencies, are associated with SWAT model estimates of organic nitrogen, organic phosphorus, and sediment yield in a stream segment's upstream watershed.

Ideally, any multi-metric IBI will reflect several aspects of an aquatic ecosystem, such as its biodiversity, food web structure, productivity, disease prevalence, hybridization, or invasive species effects. It is beneficial from an assessment perspective if metrics representing all of these attributes exhibit a strong response to chemical, physical and biological stressors. However, this is not always the case (Wellemeyer et al., 2018), especially if natural disturbance regimes are highly variable with respect to flow and runoff (e.g., desert streams), or in landscapes where agricultural land use has been prevalent for hundreds of years (e.g., Mississippi River alluvial valley streams). Nonetheless, the integration of all metrics into a final IBI score tends to provide a general sense of the relative health of a stream reach (Karr, 1991). In our study, only a few metrics of the benthic macroinvertebrate and fish IBI were sensitive to SWAT model estimates of non-point source pollution. Yet, they were metrics that described complementary properties of the aquatic ecosystem that exist in the Nolichucky River watershed. For example, the benthic macroinvertebrate metrics %TNUTOL, %CLINGER, TR, and EPT (4 out of 7) were strongly related to nitrogen, phosphorus, and sediment yield. This is sensible because %TNUTOL is reflective of trophic changes to the ecosystem brought on by inputs of nutrients. The metric %CLINGER is expected to decrease when sediment loads increase the embeddedness and reduce the availability of rocky substrata that are used by invertebrates to hold their position in the current on rocky surfaces. In addition, clingers typically are adapted to living in high-velocity riffle habitats that are dominated by cobble and boulder substrates. Therefore, when riffles are filled in by fine sediments, or when pool and run macrohabitats are prevalent at a reach, the clingers will drift downstream or fail to survive. Clinger taxa can also decrease when high nutrient loads cause benthic algae or macrophytes to increase their production and cover rock surfaces that otherwise would be occupied by clinging invertebrates. The metrics TR and EPT reflect the native biodiversity of the ecosystem. These metrics will decrease in value if water physicochemical properties (e.g., dissolved oxygen, temperature, ammonia, pH) are impaired due to point source or non-point source pollutants. The EPT in particular tend to be the most pollution-sensitive benthic macroinvertebrate taxonomic orders in streams. Thus, this metric should reflect changes in watershed land cover that ultimately impact water quality at a site by increasing the rate of sediment and nutrient inputs.

For the fish IBI, only 3 out of 12 metrics were associated with SWAT model estimates. The CPUE metric, which is a measure of relative abundance (i.e., reflects population density) is to expected to increase in streams that receive greater nutrient inputs that subsequently increase the primary production at the base of the food web. Fishes like the Bullhead Minnow (*Pimephales vigilax*) and Central Stoneroller (*Campostoma anomalum*) tend to be more abundant and encompass proportionately more of the species composition when benthic algae responds to increased nutrient loads and comprises more of the food base as opposed to allochthonous sources of organic matter. Although greater fish abundance is typically a positive indicator with regard to biotic integrity, when metrics that describe biodiversity (e.g., native species richness) concomitantly decrease in a stream and metrics like percentage omnivores or hybrids increase, then increases in the CPUE metric can be interpreted as a negative indicator of biotic integrity (Sutherland et al., 2002). For example, the CPUE metric utilized by TVA increases in Southern Appalachian streams when primary production increases due to nutrient inputs (pers. comm. D. Matthews, TVA, River and Reservoir Compliance Monitoring). Additionally, the % HYBRID metric tends to increase in streams when sedimentation and nutrient input increase, because fish species are more likely to hybridize with closely related species that exhibit similar nesting behaviors in degraded ecosystems (e.g., high sediment loads). For example, Green Sunfish (*Lepomis cyanellus*) tend to hybridize with other sunfishes, like Bluegill (*L. macrochirus*) or Redbreast Sunfish (*L. auritus*), especially in degraded conditions (Simon and Lyons, 1995). In our study, all hybrids encountered were sunfish hybrids, and these were in streams with high turbidity from sediment runoff. Native species richness (NATIVE) was negatively correlated with sediment and nutrient loads in our study, which is not surprising, since this measure of biodiversity is often used as one of the primary metrics to evaluate biotic integrity in aquatic ecosystems. In North American streams, native species richness tends to decrease as pollution increases, since physiological tolerances are often surpassed for most species, while a few tolerant species will increase in abundance and dominate the assemblage (Zamor and Grossman, 2007; Blevins et al., 2013).

It appears that the benthic invertebrate IBI developed by TDEC was more sensitive to estimates of non-point source pollution than the fish based IBI developed by TVA. This may have occurred because larval benthic invertebrates are more susceptible to changes in sediment load and nutrients (i.e., will move or die sooner than fish), or that the IBI metrics for fish are more sensitive to acute point-source pollution sources and fragmentation from dams that restrict adult dispersal and larval recruitment.

In summation, the SWAT model developed by the U.S.D.A. is an inexpensive GIS tool that aquatic resource managers and researchers can utilize to assess the impacts of non-point source pollution on freshwater ecosystems in the USA. We found that some, but not all, fish and benthic macroinvertebrate IBI metrics in the Nolichucky River watershed are significantly associated with variation in organic nitrogen, phosphorus, and sediment yields estimated from the SWAT model. In general, estimates of non-point source pollution were positively associated with increases in fish production (i.e. CPUE) and percentage of hybrids, but negatively related to metrics of biodiversity like species richness. Similarly, for benthic macroinvertebrates, metrics reflecting biodiversity, such as EPT richness and total taxa richness were negatively associated with non-point source pollution, while behavioral traits like clinging behavior and tolerance to nutrients were positively associated with non-point source inputs. Our findings are limited to the Nolichucky River watershed, but other researchers should use SWAT models and indices of biotic integrity to examine the link between sediment and nutrient loads and aquatic ecosystem health in other regions with different fauna. In addition, our study evaluated only one hydrologic year of non-point source pollution. These estimates will vary with respect to natural changes in precipitation as well as with land use change. A more comprehensive understanding of the influence that sediment and nutrient runoff has on biotic integrity in a watershed will be attained when datasets incorporate a greater variety of hydrologic periods from drought to high flow years.

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