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DEVELOPMENT AND APPLICATION OF AN AGRICULTURAL INTENSITY INDEX TO INVERTEBRATE AND ALGAL METRICS FROM STREAMS AT TWO SCALES[1]

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ABSTRACT: Research was conducted at 28-30 sites within eight study areas across the United States along a gradient of nutrient enrichment/agricultural land use between 2003 and 2007. Objectives were to test the application of an agricultural intensity index (*AG-Index*) and compare among various invertebrate and algal metrics to determine indicators of nutrient enrichment nationally and within three regions. The agricultural index was based on total nitrogen and phosphorus input to the watershed, percent watershed agriculture, and percent riparian agriculture. Among data sources, agriculture within riparian zone showed significant differences among values generated from remote sensing or from higher resolution orthophotography; median values dropped significantly when estimated by orthophotography. *Percent agriculture* in the watershed consistently had lower correlations to invertebrate and algal metrics than the developed *AG-Index* across all regions. *Percent agriculture* showed fewer pairwise comparisons that were significant than the same comparisons using the *AG-Index*. Highest correlations to the *AG-Index* regionally were −0.75 for Ephemeroptera, Plecoptera, and Trichoptera richness (EPTR) and −0.70 for algae Observed/Expected (O/E), nationally the highest was −0.43 for EPTR *vs*. total nitrogen and −0.62 for algae O/E *vs*. *AG-Index*. Results suggest that analysis of metrics at national scale can often detect large differences in disturbance, but more detail and specificity is obtained by analyzing data at regional scales.

(KEY TERMS: agricultural intensity index; macroinvertebrate and algal indicators; national and regional scales; riparian assessment.)

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INTRODUCTION

Nutrients are a major cause of impairment in streams, rivers, lakes, and estuaries, and agricultural land use practices are a leading contributor of nutrients to these receiving waters (Allan, 2004; Herlihy and Sifneos, 2008; Stevenson *et al.*, 2008; Van Sickle and Paulsen, 2008; Dubrovsky *et al.*, 2010; Munn *et al.*, 2010). The loss of riparian areas and wetlands along stream corridors has further exacerbated nutrient input from agricultural lands by reducing or eliminating nutrient uptake, denitrification, and sedimentation of adsorbed phosphorus (Verhoeven *et al.*, 2006). As a result, the United States Environmental Protection Agency (USEPA), with assistance from states and tribes, has been working to establishing nutrient criteria. The present approach is to

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divide the United States (U.S.) into 14 nutrient ecoregions in recognition of the inherently large spatial variability in natural and anthropogenic influences across the U.S. Numerous studies have assessed the effects of nutrients on biological integrity in streams, with an emphasis on macroinvertebrates and periphyton assemblages (Waite *et al.*, 2004; Smith *et al.*, 2007; Lucke and Johnson, 2009; Black *et al.*, 2011; Yuan, 2010). Most of this research has involved a specific study in one region or at one spatial scale; few studies have been conducted across various spatial scales to better understand the effects of different agricultural practices and natural influences on biological assemblages across these scales (U.S. Environmental Protection Agency, 2006; Potapova and Charles, 2007).

Recently there has been increased interest in developing optima and tolerances for individual taxa for both diatoms (Potapova and Charles, 2007) and invertebrates (Carlisle *et al.*, 2007; Smith *et al.*, 2007) related to nutrients. Carlisle *et al.* (2007) developed national indicator values at the genus level for a combination of ammonia, nitrite + nitrate, and total phosphorus (TP), but did not find a strong relationship of the mean indicator values to land use in Alabama despite concluding that nutrients were strongly associated with biological impairment. Smith *et al.* (2007) developed optima for TP and nitrate for invertebrate taxa within the state of New York then developed a three-tiered scale of eutrophication for both, but did not relate the information to land use or other sources. Potapova and Charles (2007) identified 371 taxa as either low or high nutrient indicators and concluded that it was important to develop and apply indicator metrics at the regional scale rather than combining them at the national scale. Though there have been increased efforts to develop individual indicator taxa, Yuan (2010) suggested that there has been insufficient effort towards better understanding of the effects of nutrients on aggregate measures of invertebrates, such as richness metrics and functional traits. He found that total nitrogen (TN) was highly correlated with agriculture and urban watershed land use, grazing intensity, and riparian agriculture disturbance, yet total invertebrate richness initially increased along the TN continuum before significantly decreasing by 6-10 taxa per unit increase in TN (log). Carleton *et al.* (2009) found a good relationship of percent blue-green algae to TP concentration and a threshold at 0.1 mg/l TP. They also found an interaction among TN, TP, total suspended solids (TSS), and benthic and sestonic chlorophyll a concentration: as TSS increased, benthic biomass decreased, sestonic increased, likely since blue-green algae can float (Carleton *et al.*, 2009).

When interpreting water quality and biological responses to agricultural practices, most studies have used single variables of the total amount of agricultural land use within either the watershed or riparian zones to represent the environmental continuum posed by agricultural intensity. However, the amount, type, and intensity of agricultural practices vary within basins, particularly when viewed across regional or national scales. Therefore, some measure is needed to account for these differences among agricultural regions. Whittier and Van Sickle (2010) developed a human disturbance gradient (index) based on nine variables representing three components: water quality, physical habitat, and human activity in the watershed. This index is likely an effective measure of human disturbance; however, it includes water quality and habitat measures that potentially vary in response to agricultural practices and therefore could not be applied to unsampled sites. McMahon and Cuffney (2000) developed an urban index that can be applied to unsampled sites by combining measures of land cover, infrastructure, population, and socioeconomic characteristics to give an overall measure of the amount of urban intensity within a watershed. Following this concept, and expanding on my previous attempts in other agricultural areas, I wanted to develop an agricultural index across all sites that would use only landscape-based variables to effectively describe an environmental continuum that integrates the combined effects of agricultural practices within the watershed and riparian zone. An agricultural intensity index (AG-Index) not only can act as an explanatory variable, but can also allow comparison among sites using a common currency across diverse regions even though the sites may vary in their amount and type of agricultural practices. In addition, I worked with other U.S. Geological Survey (USGS) researchers to expand the AG-Index developed for the 8 study areas in this study to a national coverage for the lower 48 states by using 19,420 watersheds based on HUC-10 coverage. This was important, since it is usually cost prohibitive to conduct research across all agricultural land use types and regions; therefore, by developing a national agricultural intensity index (National AG-Index), we could answer the question of how the sites assessed in this study compared to the national population. Were the 229 sites from this study biased toward one end or the other of the national agriculture gradient or did they represent the full range of conditions that agriculturally influenced streams experience?

Macroinvertebrates are the most commonly used biological assemblage in bioassessments nationally and internationally, but they are not expected to be directly affected by nutrients and as a result may be less sensitive than algal assemblages to the effects of

agricultural land use and nutrient enrichment in particular (Wang *et al.*, 2007; Stevenson *et al.*, 2008; Yuan, 2010). Many researchers are concerned that by relying primarily on macroinvertebrates for these types of bioassessments, lower-level impacts may not be detected (Brown *et al.*, 2009; Friberg *et al.*, 2010; Munn *et al.*, 2010). As a result, further research is needed comparing macroinvertebrates and periphyton as indicators of the effects of agricultural land use and nutrient enrichment in a variety of land use settings and geographic regions. This is the objective of this research: to develop and test the application of an AG-Index at various spatial scales, and to test and compare among various macroinvertebrate and algal metrics to determine the best indicators of nutrient enrichment for each assemblage nationally and regionally.

METHODS

Study Area

This study consisted of eight study areas characterized by extensive agricultural land use across the U.S. (Figure 1); the research was funded as part of the USGS National Water Quality Assessment Program (NAWQA). Previous research has shown that it is important to take into account regional differences, due to both natural and anthropogenic influences, when conducting bioassessments (Herlihy *et al.*, 2008). Therefore, I aggregated the study area data sets into (1) a national data set and (2) three regional data sets based on proximity and similarities in climate and physiography (Cushing and Allan, 2001). The Columbia Plateau (CCYK) and the Upper Snake River study areas (USNK) are arid to xeric and were grouped as the Western region; all sites were within the Columbia River basin. Agriculture is heavily dependent on irrigation, while forests and wetlands are relatively rare. The Upper Mississippi (UMIS), Central Nebraska (CNBR), White-Miami (WHMI), and the Ozarks (OZRK) study areas are located in the semi-humid plains and were grouped in the Central region; all sites were located in the Mississippi River drainage. Wetlands were more abundant in the Central than in the Western region. The Georgia Coastal Plain (GCP) and the Delmarva Peninsula (DLMV) study areas were grouped in the Eastern region; all sites located along the coast and drained to the Atlantic Ocean. Coastal Plain streams typically had riparian zones with forested floodplains and abundant canopy cover and the highest amount of wetlands. Analyses were conducted at both national (across all eight areas) and three regional scales.

Site Selection

In each study area, sites were selected to represent a nutrient concentration gradient ranging from minimally to highly impacted as mediated by agricultural land use. The initial selection of sites relied partially on modeled estimates of nitrogen and phos-

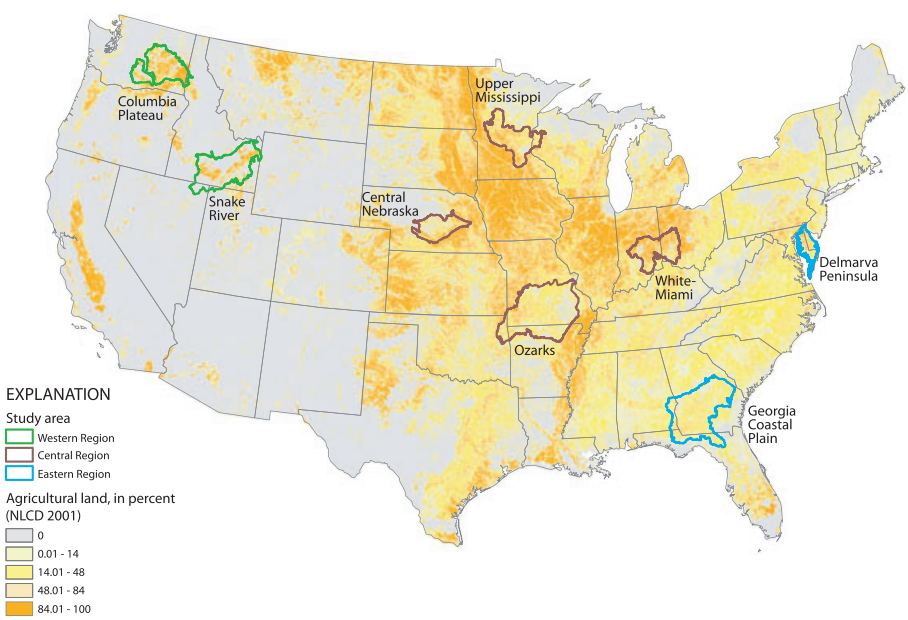

FIGURE 1. Map Showing the Eight Study Areas Coded by Region: Western (green outline), Central (red), and Eastern (blue).

phorus input to each of the independent watersheds derived from county-level fertilizer sales, atmospheric deposition (nitrate), and livestock data (Ruddy *et al.*, 2006). National-scale analysis of NAWQA data has demonstrated that nitrogen input to the land surface was significantly related to nitrogen yields to streams (Fuhrer *et al.*, 1999) and could be used as a surrogate for nutrient concentration in streams with sparse water quality data. Final selection of sites was based upon modeled nutrient input to the watershed, existing USGS nutrient data, and similarity of stream habitat/geomorphology with other sites within the study area as determined by general reconnaissance. This approach yielded 28-30 wadeable sites within each study area that spanned greatest range in nutrient concentrations possible, given similar stream habitats within the study area. A total of 232 sites were sampled between 2003 and 2007. Means and ranges of select descriptive and environmental variables for each of the eight study areas are provided in Table 1.

Watershed and Riparian Characterization

Geographical information system (GIS)-derived watershed land cover, soil characteristics, topographic features, runoff estimates, and riparian land cover were calculated for each watershed upstream of the study site. Calculations were made using the National Hydrography Dataset (NHD 100K) (U.S. Geological Survey, 2003a), the National Land Cover Dataset (NLCD2001) (U.S. Geological Survey, 2003b), and digitized orthophoto quarter quadrangles to characterize riparian conditions, as described in Johnson and Zelt (2005). Watershed boundaries were derived from digital elevation model data obtained from the USGS Elevation Derivatives for National Applications project. Riparian variables were determined from GIS land cover (NLCD) at the reach scale for 90 m on either side of the stream for a set distance upstream (base-10 logarithm of the upstream drainage area) using methods outlined in Johnson and Zelt (2005) and Zelt and Munn (2009). Because of concerns that riparian land use estimates might be redundant with

TABLE 1. Summary of Important Environmental Variables by Study Area; Numeric Variables Presented as Average Values, Range in Parentheses (TN and TP Input in Units of km/km^2).

Region	Study Area	Study Area Code	Mean Annual Precipitation (cm)	Regional Crops	Percent Agriculture	TN Input in Watershed	TP Input in Watershed	Agricultural Intensity Index
Western	Columbia Plateau	CCYK	40 (22-91)	Orchards, vineyards, wheat/small grains, alfalfa, dried beans	36 (0-91)	4,100 (330-15,000)	510 (40-2,200)	49 (0-100)
	Upper Snake River	USNK	45 (22-75)	Potatoes, sugar beets, alfalfa, wheat/small grains	19 (0-92)	3,200 (200-17,000)	670 (20-3,600)	35 (0-95)
Central	Central Nebraska	CNBR	68 (59-78)	Corn, soybeans, alfalfa	47 (14-91)	8,600 (3,600-15,000)	1,530 (640-2,600)	80 (45-100)
	Ozarks	OZRK	122 (116-130)	Alfalfa, wheat/small grains, grass hay, soybeans	38 (1-81)	4,600 (700-14,000)	1,100 (80-3,800)	49 (0-85)
	Upper Mississippi	UMIS	78 (69-88)	Corn, soybeans, alfalfa, wheat/small grains	56 (2-89)	4,700 (600-10,000)	870 (40-2,000)	68 (20-100)
	White-Miami	WHMI	108 (98-115)	Corn, soybeans	83 (67-92)	8,400 (6,000-13,000)	1,590 (1,100-3,200)	91 (80-100)
Eastern	Georgia Coastal Plains	GCP	123 (117-132)	Cotton, peanuts, soybeans, wheat/small grains	32 (5-65)	2,800 (600-6,500)	750 (80-1,800)	46 (10-80)
	Delmarva Peninsula	DLMV	114 (113-116)	Corn, soybeans, wheat/small grains	61 (23-92)	8,000 (2,300-16,000)	1,470 (190-3,600)	82 (40-100)

TABLE 2. Description, Variable Code, and Definition of Explanatory Environmental (Landscape) and Biological Response (Invertebrate and Algae Metrics) Variables Used for Data Analysis.

Explanatory Variables: Landscape		
Description	**Variable Code**	**Definition**
Watershed scale variables		
Percent agricultural land use	WS_Ag	Percent watershed area in agricultural land use (NLCD 2001 category 81, 82)
Percent forest	WS_Forest	Percent watershed area in forest land use (NLCD 2001 categories 41-43)
Mean annual precipitation	WS_MnAnnPrecip	Mean annual precipitation (cm)
Soil infiltration rate	Soil_Mod-Infil	Hydrologic soil group B, moderate infiltration rate (min. infiltration rate 4-8 mm/h)
Total nitrogen input	TN Input	Input to the watershed of total nitrogen from fertilizer application, atmospheric deposition and animal manure (kg/km^2)
Total phosphorus input	TP Input	Input to the watershed of total phosphorus from fertilizer application, atmospheric deposition, and animal manure (kg/km^2)
Riparian scale variables		
Percent agricultural land use	Rip_Ag	Percent buffer area in agricultural land use (NLCD 2001 category 81, 82)
Percent forest	Rip_Forest	Percent buffer area in forest land use (NLCD 2001 categories 41-43)
Percent agricultural land use in 100 m riparian buffer (orthophotography)	Ag_Buf_100	Percent agricultural land use (Cropland + Farmstead) within 100 m riparian buffer for the segment based on orthophotography
Response variables: invertebrate metrics		
Observed/expected	O/E	Ratio of number of observed macroinvertebrate taxa at a site over the expected taxa based on null model of reference sites from each region
Abundance-weighted tolerance	AbundTOL	Abundance-weighted EPA tolerance value for a sample
Percent abundance of intolerant taxa	Intol_Abundp	Percent of the total abundance within the intolerant class ($\leq$ 4) based on USEPA tolerance values for a sample
Richness-weighted tolerance	Intol_rich	Richness-weighted EPA tolerance value for a sample
Total sample richness	Richness	Total number of taxa in a sample
Ephemeroptera, Plecoptera, and Trichoptera richness	EPTR	Richness composed of mayflies, stoneflies, and caddisflies for a sample
Algae metrics		
Percent eutrophic diatoms plus soft algae	% Eutrophic	Percentage of diatoms and soft algae classified as eutrophic
Percent less tolerant diatoms	% Less tolerant	Percentage of diatoms classified as less tolerant (3b) based on Lange-Bertalot (1979)
Low nitrogen autotrophic diatoms	Low inorganic N diatoms	Percentage of diatoms classified as low organic nitrogen autotrophs based on Van Dam *et al.* (1994)
Observed/expected	O/E	Ratio of number of observed algal taxa at a site over the expected taxa based on null model of reference sites from each region

watershed NLCD data and to test how well the NLCD riparian estimates characterized riparian land cover, additional measurements were estimated using orthophotography calculated within 100 m buffers adjacent to the study reaches (Zelt and Munn, 2009). The specific watershed and riparian characteristics metrics used in this study are identified in Table 2. A base flow index, the component of streamflow that can be attributed to groundwater discharge into streams, was estimated for watersheds from the national 1 km resolution data set developed by Wolock (2003).

Agricultural Intensity Index

The percentage of agricultural land use in the watershed or riparian zone above a stream site has been a common measure of disturbance due to agricultural activities. However, over large spatial areas the type of agricultural practices can vary widely often resulting in dramatic changes in the influences from these practices that can directly and indirectly affect the water quality and physical habitat. Therefore, in addition to these single variables, I developed an index that combines four landscape-based variables to attempt to assess the overall agricultural intensity within the watershed. The four variables selected were *percent agricultural land use in the watershed* based on NLCD 2001 data, *percent agricultural land use in the riparian zone* based on orthophotography-interpreted data, and *TN and TP individual input to the watershed* (Zelt and Munn, 2009). Each variable was given the following scores based on these percentile ranges across all 232 sites for that specific variable: 0-10% = 0, 11-30% = 2, 31-50% = 3, 51-75% = 4, >75% = 5. The scores were then summed, divided by 20 (the total possible score), then multiplied by 100 to convert the scores to a percentage from 0 to 100, with a score of 100 being high agricultural intensity. To place this agricultural data

set into a national perspective, the index was also calculated for all HUC-10 basins nationwide. The percentiles used were shifted and an additional scoring interval was added (0-10% = 0, 11-39% = 2, 40-59% = 4, 60-74% = 6, 75-89% = 8, and $\geq$ 90% = 10) to account for the skewness of the data due to a large number of watersheds with zero percent agriculture or high amounts of forested land cover. In addition, the amount of agriculture in the riparian zone was based on NLCD 2001 data for a 2 km-long section since orthophotography data was not available for all 19,000 plus HUC-10 basins. Distributions and medians for the index developed for the agricultural sites in this study were compared to those from the nationally derived index.

Habitat Characterization

A study reach of approximately 20-30 wetted channel widths in length based on average width measurements was established at each site. Reach-scale data were collected along 11 transects distributed proportionally within the study reach. Habitat measures for each study reach included an assessment of instream and bank features such as water velocity, depth, width, substrate size (based on Wentworth substrate classes), substrate embeddedness, channel bankfull width, bank vegetation cover, and estimates of canopy closure (made using a spherical densiometer). All width and depth measurements were to the nearest 0.1 m. Instantaneous stream discharge was measured following standard USGS methods (Rantz, 1982). Habitat characterizations were performed during low-flow conditions. A detailed description of the reach-scale habitat and riparian delineation methods is available in Fitzpatrick *et al.* (1998) and Biggs and Kilroy (1994). Study reach habitat variables that were collected or derived for data analysis are shown in Table 2.

Water Quality/Chemistry

Water chemistry samples were collected twice at all sites: once ca. 30 days prior to the habitat characterization and once during the habitat assessments. Samples were collected using depth-integrated equal-width increment sampling methods unless the stream was too shallow or water velocity was insufficient, in which case, samples were collected as multi-vertical grab samples (U.S. Geological Survey, 2006). Field water quality properties were measured during each sampling event and included water temperature, dissolved oxygen, specific conductance, and pH using laboratory meters that were calibrated daily prior to use. Water chemistry analysis included nutrients (TN and TP plus dissolved species), alkalinity, and dissolved organic carbon. Samples also were collected for the determination of suspended sediment. Once a chemical sample was appropriately collected and processed, it was stored on ice and shipped to the laboratory within 24 h, where they were maintained at 4°C prior to analysis (Patton and Gilroy, 1998). All laboratory analyses for chemical constituents were conducted at the USGS National Water Quality Laboratory in Denver, Colorado, using methods by Fishman (1993) and Patton and Kryskalla (2003). Nutrient and dissolved organic carbon samples were analyzed within 30 and 28 days, respectively. Table 2 includes the water quality variables that were collected or derived for data analysis.

Algae and Macroinvertebrate Sampling

Algae and macroinvertebrate samples were collected once from coarse or fine-grained substrate during the summers of 2003 and 2007. Coarse substrate was defined as either gravel or cobble typical of riffles or large woody debris where sand substrates dominated. A detailed discussion of algal and macroinvertebrate sampling protocols can be found in Moulton *et al.* (2002) and Cuffney (2003). Where coarse substrates occurred they were sampled throughout each study reach from five locations and composited into one sample per site. Coarse substrates were sampled for algae by scrapping the biological material off rock or wood from a measured area. The material was transferred into a container by washing the substrate with a known volume of water. The samples were preserved with 5% formalin for identification (Moulton *et al.*, 2002). At each location where biological samples were collected, depth, velocity, and substrate characteristics were recorded. Velocity measures were taken as close to the stream bottom as possible. All algal community samples were shipped to the Academy of Natural Science in Philadelphia for algal identification, density, and biovolume calculations (Charles *et al.*, 2002). Algae data for all sites were collected during stable low-flow periods, typically between July and September. Algae and diatoms were identified to the lowest taxonomic level possible, usually species or sub-species. A minimum of 100 cells were counted and identified. Algal taxonomic resolution varied slightly among sites and over time, so the data were modified before data analysis to establish a consistent level of taxonomy across all sites and time. For macroinvertebrates, five composited samples were collected with a 0.5-m^2 area 500 μm mesh net from coarse substrates where possible (total area 1.25 m^2), otherwise from submerged snags. Invertebrates were identified to the lowest taxonomic level possible,

typically the genus level. A minimum of 300 organisms were counted and identified for each site. Three sites were dropped from the original 232 list, one due to no algal data (not sampled), the second due to no invertebrates found in the sample, and another due to no habitat data; final site list for analysis was 229.

Over 150 metrics were calculated for each of the algal and macroinvertebrate assemblages using the USGS developed programs Invertebrate and Algae Data Analysis System (ADAS and IDAS) (Cuffney, 2003). Algal metrics included indicators of nitrogen metabolism, trophic condition, organic enrichment, dissolved oxygen concentrations, physical conditions, and nutrient optima. Autecological characterization of algae was based on Prescott (1962), Lowe (1974), Lange-Bertalot (1979), Bahls (1993), Van Dam *et al.* (1994), Potapova and Charles (2007), and Porter *et al.* (2008). For macroinvertebrates, the metrics describe abundance and richness of the invertebrate assemblage, dominance, functional feeding group, behavior, and tolerance. Ecological tolerance values that indicate how well each taxon is expected to tolerate pollution were obtained from the USEPA or regional biologists (Wisseman, 1996; Barbour *et al.*, 1999; Cuffney, 2003). Tolerance values ranged from 1 to 10; low values indicate intolerant taxa (1-4) and high numbers indicate tolerant taxa (7-10). I calculated average tolerance values using both national and region-specific tolerance estimates; national tolerances were an average of all the regional tolerances (Cuffney, 2003). Final algal and invertebrate metrics used in the data analysis are shown in Table 2. For more detailed information on algal metrics see Porter *et al.* (2008) and for invertebrate metrics see Cuffney (2003); final metric selection is described below in Data Analysis. The observed/expected ratio (O/E) metric was calculated following Van Sickle *et al.*'s (2005) null model method using the low AG-Index category as the reference sites or expected condition. Only taxa that were identified to at least the genus level and occurred at greater than 65 and 45% of the sites in the low AG-Index category for invertebrate and algal data, respectively, were used. A lower percentage was used for algae in order to include sufficient number of expected taxa in the null models. O/E values were generated for each region separately.

Data Analysis

Evaluation and reduction of environmental variables followed a common step-wise approach; variables were assessed for general distribution and possible outliers via scatter plots and correlation statistics to remove redundant or surrogate variables. This process was also assessed through the use of Primer's BIO-ENV procedure (PRIMER, version 6: Clarke and Gorley, 2006) which calculates a hierarchical subset of variables that explain the greatest portion of the variance in the original full data set. This was done component by component (i.e., habitat, water chemistry, and riparian and watershed land cover) before the final subset of variables was selected. This reduced subset of environmental variables was then compared to the full suite of metrics via Spearman rank correlation statistics. Metrics with consistently high correlation values to the agricultural land use variables across the regions were selected as the final metrics for assessment. The AG-Index was also broken into four intensity categories (0-30 = Low Intensity, 35-50 = 2, 55-75 = 3, and >75 = High Intensity), and the top metrics tested against the categories via ANOVA and Tukey pairwise comparisons. The AG-Index was also evaluated against percent agricultural land use in the watershed and TN and TP concentration. All subsequent analyses and graphical displays except noted above were completed in R (R Development Core Team, 2006, version 2.13.1).

RESULTS

Table 1 provides a comparison of important descriptor and environmental variables (mean and range) for each of the eight study areas. To smooth possible single year anomalies, the TN and TP watershed input data presented in Table 1 are annual averages for 2001-2004 (Ronald Zelt, U.S. Geological Survey, 2011, personal communication). The two study areas in the Western region have the lowest average precipitation, more diverse crop types (e.g., orchards, vineyards, potatoes, etc.), relatively low nutrient input values, and lower average AG-Index than the other six study areas. The DLMV in the Eastern region, and WHMI, UMIS, and CNBR areas in the Central region had higher average agriculture and AG-Index values plus higher minimum values, resulting in smaller ranges than in the other four study areas (Table 1). In contrast, the CCYK, USNK, OZRK, and GCP study areas had lower average agriculture intensity but a larger gradient or total range in nutrients and agriculture intensity than the other study areas (Table 1).

Estimates of the amount of agricultural land use within the riparian zone showed significant differences among data sources based on whether the values were generated from 30 m resolution NLCD remote sensing data or from higher resolution orthophotography; the median values dropped dramatically across most study areas (Figure 2) when estimated by

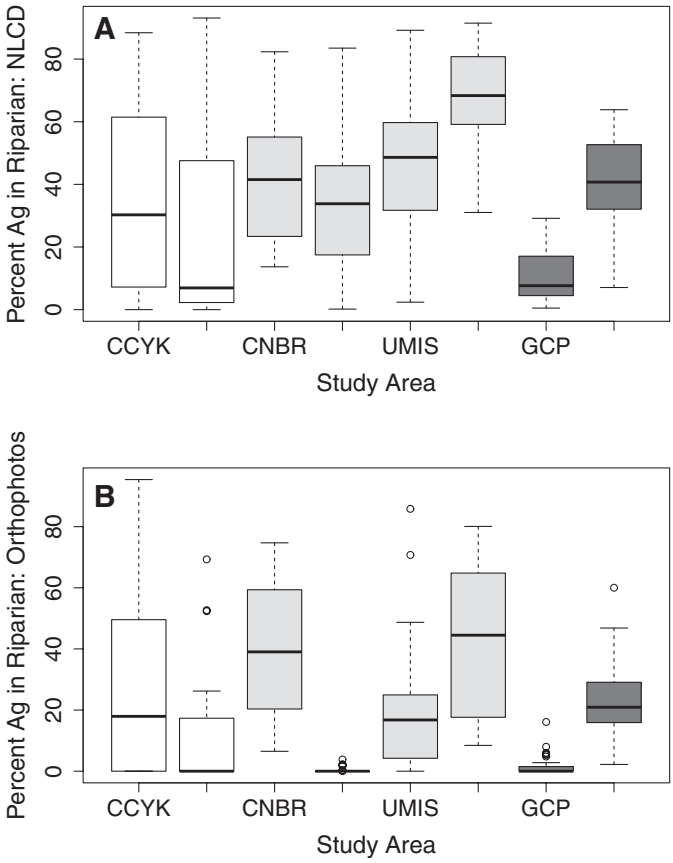

FIGURE 2. Box Plots of (A) Percent Agricultural Land Use in Riparian Buffer Based on NLCD Data and (B) Percent Agricultural Land Use in Riparian Buffer Based on Orthophotography, Ordered and Shaded by Region (Western: white, Central: light grey, Eastern: dark grey). See text for study area codes.

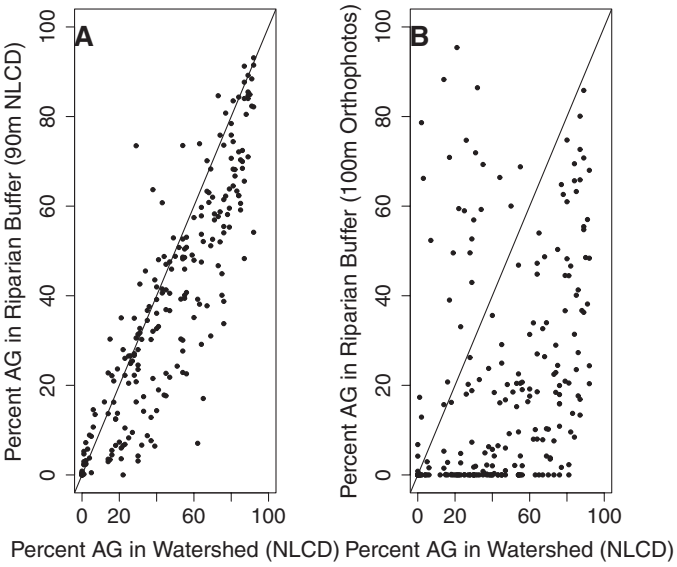

FIGURE 3. Scatter Plots of Percent Agricultural Land Use in Watershed *vs*. (A) Riparian Buffer Based on NLCD 2001 Data and *vs*. (B) Riparian Buffer Based on Orthophotography, 1-1 Line Added to Aid Visual Interpretation.

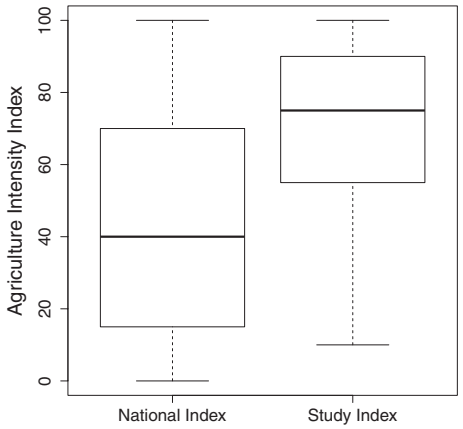

FIGURE 4. Box Plot of the National Agricultural Intensity Index Scores (left) for All 19,420 Watersheds (HUC-10) and Scores for the 229 Sites in This Study (right). Lower whisker = 10%, lower boundary of box = 25%, line in box = median, upper boundary of box = 75%, upper whisker = 90%.

orthophotography. The amount of agriculture in the watershed and riparian zones both based on NLCD data were highly correlated and followed the 1:1 line fairly closely (Figure 3A); however, when watershed agriculture based on NLCD was compared to riparian estimates from orthophotography, there were dramatic deviations from the 1:1 line (Figure 3B). Some riparian values had much greater values than their corresponding watershed value (points above the 1:1 line) while most of the points had lower riparian agriculture compared to the watershed estimates.

A comparison of the AG-Index developed from the 229 sites in this study to the national AG-Index we developed from all 19,420 HUC-10 watersheds showed that the sites sampled for this study were found along most of the entire range of the national index except for values below a score of 10 (Figure 4). However, the median values differed considerably between the two: 75 for the 229 sites in this study *vs*. 40 for the national data set that includes a large number of watersheds from non-agricultural regions.

With few exceptions, the variable percent agriculture in the watershed consistently had lower correlations to invertebrate and algal metrics than the AG-Index across all regions (Table 3), and there were a few metrics where the difference in percent correlation was greater than 17 points (Spearman rho). The highest correlations to the AG-Index was −0.75 for EPTR (Ephemeroptera, Plecoptera, and Trichoptera richness: Western region) and −0.70 for O/E (algae: Eastern region). The AG-Index also had higher correlations in general to invertebrate and algal metrics than correlations with stream TN and TP concentrations for the Western and Eastern regions, but TN and TP concentrations had higher correlations to the biotic metrics than the AG-Index in the Central

TABLE 3. Spearman Rank Correlations Among Select Macroinvertebrate and Algal Metrics and Agriculture Related Variables (Agricultural Intensity Index [AG-Index], % Agriculture in the Watershed Based on NLCD (% AG), TN and TP Instream Concentration [mg/l]) by Region.

	Western				Central				Eastern			
Metrics	**AG-Index**	**% AG**	**TN**	**TP**	**AG-Index**	**% AG**	**TN**	**TP**	**AG-Index**	**% AG**	**TN**	**TP**
Invertebrates												
Intol_rich	**−0.74**	**−0.60**	**−0.59**	−0.23	**−0.40**	−0.17	**−0.46**	**−0.73**	**0.46**	**0.47**	**0.62**	0.03
AbundTOL	**0.67**	**0.51**	**0.60**	**0.41**	0.31	0.20	0.35	**0.53**	**−0.42**	**−0.55**	**−0.67**	0.07
Intol_abundp	**−0.59**	**−0.47**	**−0.47**	−0.21	**−0.40**	−0.28	**−0.46**	**−0.62**	**0.43**	**0.53**	**0.64**	0.16
Richness	**−0.58**	**−0.47**	**−0.47**	0.01	−0.35	−0.13	**−0.45**	**−0.59**	**−0.47**	**−0.48**	−0.37	−0.32
EPTR	**−0.75**	**−0.63**	**−0.57**	−0.05	**−0.42**	−0.20	**−0.50**	**−0.68**	**−0.46**	−0.35	−0.32	**−0.53**
O/E	**−0.50**	−0.38	−0.29	0.23	**−0.46**	−0.20	**−0.55**	**−0.77**	**−0.63**	**−0.57**	**−0.60**	−0.27
Algae												
O/E	−0.31	−0.26	−0.26	0.24	**−0.59**	−0.33	**−0.63**	**−0.78**	**−0.70**	**−0.60**	**−0.67**	−0.34
Eutrophic	−0.17	−0.20	−0.14	**0.41**	**0.58**	**0.40**	**0.62**	**0.71**	**0.55**	**0.49**	**0.46**	**0.40**
Less tolerant	**−0.53**	−0.33	−0.32	−0.36	**−0.49**	**−0.44**	**−0.55**	**−0.41**	**−0.61**	**−0.61**	**−0.51**	−0.28
Low inorg. N	**−0.56**	−0.33	**−0.41**	**−0.53**	**−0.60**	**−0.46**	**−0.67**	**−0.62**	**−0.64**	**−0.65**	**−0.48**	−0.36

Notes: Values over 0.40 are in bold, TN and TP were log-transformed (see Table 2 for metric definitions).

region. This was likely due to the relatively large nutrient gradient in the Central region; median TN concentrations in CNBR and WHMI were the two highest of all the regions and median TP concentration in CNBR was almost three times higher than that in the next highest study area, while the nutrient concentrations in the OZRK, also in the Central region, were the lowest. Correlations of TP to the O/E metric for both invertebrates and algae were −0.77 or above in the Central region. For invertebrate metrics in the Western region, EPT richness had the highest correlation to AG-Index, and abundance of tolerant taxa (AbundTOL) to TN and TP. In the Central region, O/E invertebrates had the highest correlation to AG-Index, TN, and TP. In the Eastern region, the highest correlations were O/E invertebrates to AG-Index, AbundTOL to TN, and EPTR to TP (Table 3). The algal metrics with the highest correlations to agricultural intensity indicator variables (Table 3) by region were percent low inorganic N diatoms (Low Inorg. N) *vs*. AG-Index, TN, and TP in the Western region; low inorganic N diatoms *vs*. AG-Index and TN and O/E algae *vs*. TP in the Central region; and O/E algae *vs*. AG-Index and TN, and percent eutrophic *vs*. TP in the Eastern region (Table 3).

The AG-Index was divided into four intensity categories (Low Intensity, 2, 3, and High Intensity), which were used to test the response of the invertebrate and algal metrics. TN concentration was also tested to determine if nutrient concentrations and not just biota changed along the four agriculture intensity categories. There was significant separation in TN concentration for the Low Intensity and categories 2 and 3 *vs*. High Intensity for most of the three regions but not for many of the other more subtle pairwise comparisons. The three invertebrate metrics shown in Table 4 were able to significantly distinguish the largest pairwise difference, Low Intensity *vs*. High Intensity, in all three regions. No metrics were able to distinguish among all intensity category comparisons, but EPTR and O/E metrics in the Western and Central regions, respectively, were able to significantly distinguish all pairwise comparisons except High *vs*. 3 (Table 4). The percent abundance of intolerant invertebrate taxa (Intol_abundp) separated most categories in the Western and Central regions but showed minimal separation in the Eastern region (Table 4). Figure 5 shows bar plots following a decreasing stair-step response from Low to High Intensity categories for average EPTR in Western region and O/E metrics in the Central and Eastern regions. EPTR did the best separating the different AG-Index categories in the Western region, but the O/E had the most significant comparisons in the Central and Eastern regions and nationally (Table 4).

Comparison among means (Tukey pairwise comparisons, Table 5) between levels of the AG-Index for the algal metric percent low inorganic N diatoms had the clearest differences nationally and across the regions among index categories, the other three metrics showed clear differences in the Central region but were less defined in the Western and Eastern regions (Table 5). Figure 6 shows the plots of percent low inorganic N diatoms for each region; the Western region showed a large difference between the Low Intensity category and all the others, but little difference among 2, 3, and High Intensity categories, while the Eastern region showed an increase from Low Intensity to category 2, followed by a large decrease to categories 3 and High Intensity. Only the Central region showed the dramatic decreasing stair-step response across all categories. Means between levels of the AG-Index of three out of the four algal metrics were able to significantly separate all the pairwise index comparisons

TABLE 4. Tukey Pairwise Comparisons Among the Four Agricultural Intensity Index Categories (6 Pairs) by National and Regional Scales for Three Macroinvertebrate Metrics and Instream Total Nitrogen Concentration (TN).

		Macroinvertebrate Metrics			
	Pairwise Groups	Observed/ Expected	EPTR	% Intolerant Abundance	TN
National	Low – 2	ns	**	**	ns
n = 229	Low – 3	****	***	***	ns
	Low – High	****	****	****	****
	2 – 3	***	ns	ns	ns
	2 – High	****	ns	ns	****
	3 – High	*	ns	ns	****
Western	Low – 2	**	**	**	ns
n = 59	Low – 3	**	****	***	***
	Low – High	**	****	****	****
	2 – 3	ns	**	ns	**
	2 – High	ns	**	**	***
	3 – High	ns	ns	ns	ns
Central	Low – 2	**	ns	ns	ns
n = 116	Low – 3	****	ns	**	ns
	Low – High	****	**	**	***
	2 – 3	**	ns	*	ns
	2 – High	**	**	**	****
	3 – High	ns	ns	ns	**
Eastern	Low – 2	ns	ns	ns	ns
n = 54	Low – 3	**	ns	ns	ns
	Low – High	****	**	*	**
	2 – 3	ns	ns	ns	ns
	2 – High	**	**	*	**
	3 – High	ns	ns	ns	ns

Notes: ns, not significant.
*$p \leq 0.10$, **$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

except for High Intensity *vs*. 3 nationally and in the Central region, but the various metrics did not do as well in the other two regions. Nevertheless, three out of the four algal metrics showed significant pairwise comparisons for all but the Low Intensity — 2 and High Intensity — 3 comparisons in the Eastern region. On the other hand, only two out of the four algal metrics in the Western region were able to significantly distinguish the most obvious pairwise difference, Low Intensity *vs*. High Intensity.

To look at the covariation and interaction of the AG-Index and nutrient enrichment (i.e., nutrient concentrations), I generated plots of EPTR *vs*. the AG-Index as an example for each of the three regions and then coded the points into categories of TN concentration of low, medium, and high (Figure 7). Category levels were chosen by dividing the full data set for TN into three percentage classes (0-33, 33-66, and >66%) which translated into TN class values of 0.88 mg/l (low), 0.88-2.65 (medium), and >2.65 mg/l (high) which is similar to other values in the literature for low and high nutrient classes (Potapova and Charles, 2007; Smith *et al.*, 2007; Munn *et al.*, 2010). Figure 7 shows that nutrient values follow the general gradient of the AG-Index for each region but that there are fewer low TN values in the Central and Eastern than the Western region, and this translates into weaker relationships between EPTR and the AG-Index in these regions (linear regression $R^2 < 0.2$ for Central and Eastern, 0.55 for Western) (see also Table 3). Furthermore, there is a stronger interaction and slopes at low TN values for all regions, but response or slope levels off as nutrient values increase to the medium and high categories. Therefore, Figure 7 suggests that factors other than nutrients are likely affecting or controlling the response of biota at medium to high nutrient enrichment.

Among spatial scales, the regional correlations were stronger than those at the national scale; the highest correlation nationally for invertebrates was −0.43 for EPTR *vs*. TN and −0.62 for O/E for algae *vs*. AG-Index. On the other hand, regional correlation values summed across the four agricultural indicator variables (AG-Index, % AG, TN, and TP) had four values over 0.7 for invertebrates and two for algae, plus numerous values over 0.6 (Table 3). The Tukey pairwise comparisons revealed patterns not evident in the correlations findings. In general, the regional comparisons showed more significant pairwise comparisons than when the data were combined at the national scale for EPTR and intolerant taxa; however, O/E did as well or better at the national scale than at the three regional scales for invertebrates. For the

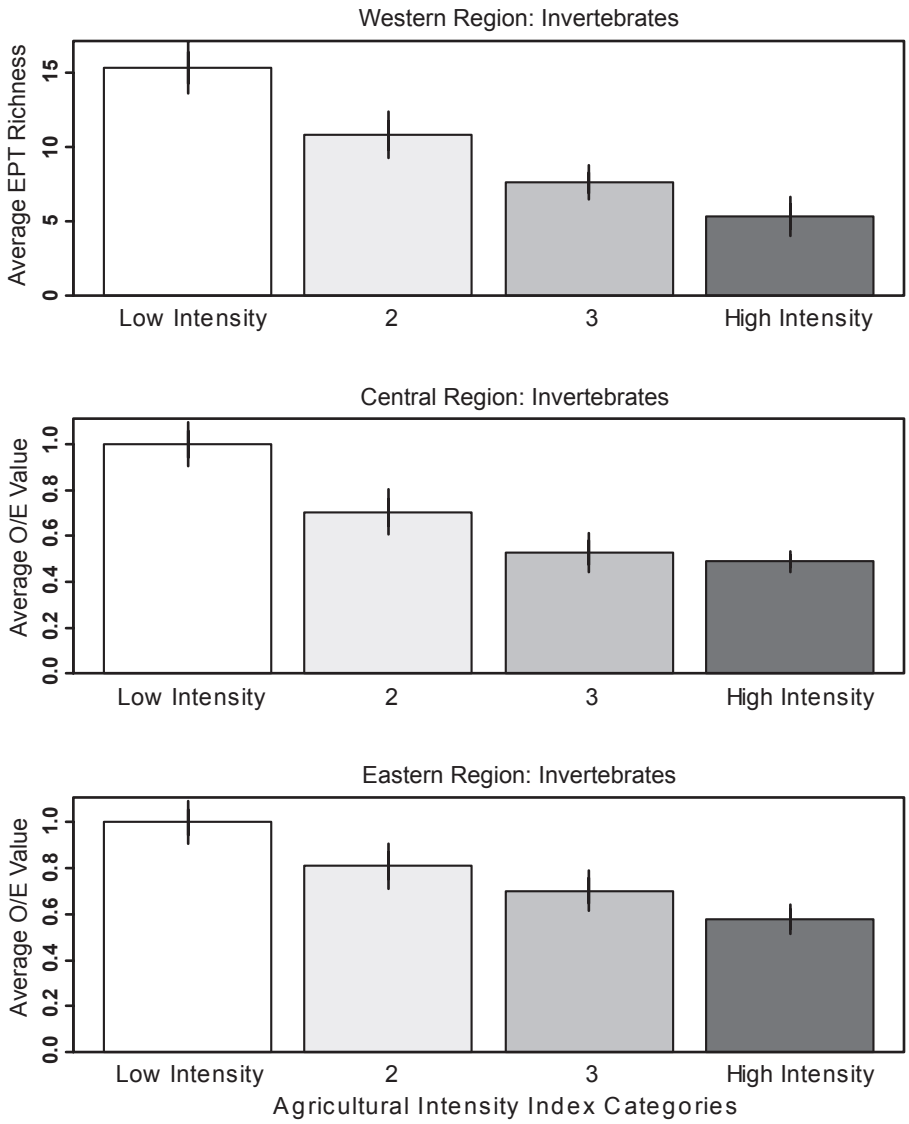

FIGURE 5. Plots of Invertebrate Metrics (EPTR — Western Region; Observed/Expected Ratio — Central and Eastern Region) by Agricultural Intensity Index Categories, Vertical Bar Is the 90% Standard Error.

algae metrics, data scaled nationally had more significant comparisons than the individual regions for all the metrics except for percent eutrophic. The national algal O/E metric was the only metric that was able to significantly distinguish all six pairwise comparisons of the AG-Index categories for either algae or invertebrates and showed good distinction among categories in the Central and Eastern regions, though relatively poor in the Western region.

DISCUSSION

The agriculture intensity index, composed of four variables indicative of agricultural land use and fertilizer input, consistently acted as a better explanatory variable of biological metrics and TN concentration than the standard, single variable of percent agriculture in the watershed (Table 3). Comparison of the AG-Index to the individual variable percent agriculture in their ability to show significant distinction among four agricultural index categories for TN concentration and invertebrate metrics showed that the AG-Index generally had more significant comparisons (data not shown for AG-Index category comparisons for percent agriculture; Table 4 for AG-Index) and of higher strength than percent agriculture (Table 3). Nevertheless, the overall trends were similar for the two variables as one would expect since percent agriculture in the watershed was one of the four variables making up the AG-Index. I believe that by including variables on nutrient input in the index, an important

TABLE 5. Tukey Pairwise Comparisons Among the Four Agricultural Intensity Index Categories (6 Pairs) by National and Regional Scales for Four Algal Metrics.

	Pairwise Groups	Algal Metrics			
		Observed/ Expected	% Eutrophic Diatoms	% Less Tolerant Diatoms	% Low Inorganic N Diatoms
National	Low – 2	***	ns	**	**
n = 229	Low – 3	****	**	****	****
	Low – High	****	****	****	****
	2 – 3	***	ns	**	***
	2 – High	****	**	****	****
	3 – High	**	ns	ns	ns
Western	Low – 2	**	ns	**	****
n = 59	Low – 3	**	ns	**	***
	Low – High	ns	ns	**	****
	2 – 3	ns	ns	ns	ns
	2 – High	ns	ns	ns	ns
	3 – High	ns	ns	ns	ns
Central	Low – 2	**	**	ns	**
n = 116	Low – 3	*	****	***	****
	Low – High	****	****	****	****
	2 – 3	***	**	**	**
	2 – High	****	****	***	****
	3 – High	ns	ns	ns	ns
Eastern	Low – 2	ns	ns	ns	ns
n = 54	Low – 3	**	**	*	**
	Low – High	****	***	***	***
	2 – 3	*	*	ns	****
	2 – High	***	**	**	****
	3 – High	ns	ns	ns	ns

Notes: ns, not significant.
*$p \leq 0.10$, **$p \leq 0.05$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

agriculture signature was revealed that could have been missed by percent agriculture alone, especially in areas like the Ozarks, where the amount of agriculture as row crops is relatively low but the land receives a relatively high amount of nutrients added via animal manure (fourth highest in both TN and TP input, Table 2). Lastly, though it takes more effort to generate, percent agriculture in the riparian zone from orthophotographs showed significant improvement over NLCD estimates of agricultural land use in the riparian zone, and this was an important addition to the index. Depending on the region and farming practices, the orthophotography either showed higher amounts of agriculture in the riparian zone than NLCD estimates or lower amounts, but rarely the same amount (Figure 3) (Zelt and Munn, 2009). As a result, orthophotography riparian estimates provided new information and explained more variation than NLCD riparian estimates, or, in other words, NLCD riparian estimates were largely redundant with NLCD percent agriculture in the watershed estimates; this was also found by Zelt and Munn (2009) when analyzing five of the same study areas from this study.

Cuffney *et al.* (2010) found that an urban intensity index developed for nine metropolitan areas was highly correlated with population density, one of the variables in the index; however, the rate at which development occurred and the environmental settings differed among the nine areas, thus necessitating the multiple variable index. Their findings are similar to those found with the application of the AG-Index developed here, yet, it is likely that the type (e.g., crop type, season, timing, etc.) and intensity (e.g., pasture, row crop, tillage or non-tillage, irrigation type, etc.) of agricultural practices vary as much or more than the variables in the urban index (e.g., population density, road density, percent urban and housing density) thus increasing the need for a common index of agricultural disturbance. Stevenson *et al.* (2008) also found that a multiple variable index of watershed disturbance had stronger relationship to diatom indicator optima than the individual variables making up the index, which included agriculture and urban land use and stream nutrients. Whittier and Van Sickle (2010) developed an overall human disturbance index that included nine variables in three components of lotic ecosystems — water quality, instream physical habitat, and human activity — that were strongly related to macroinvertebrate indicator indices. The agriculture index developed here does not include instream water quality or habitat since

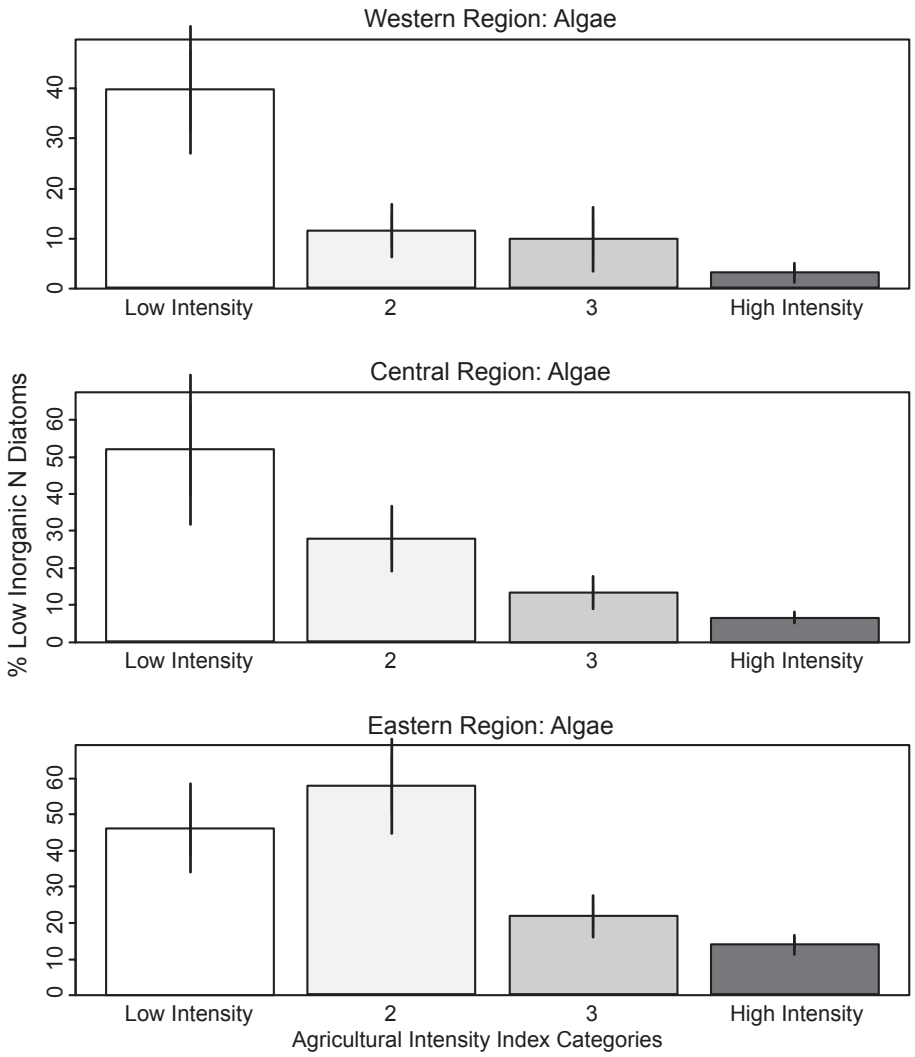

FIGURE 6. Plot of the Algal Metric % Abundance of Low Inorganic N Diatoms by Agricultural Intensity Index Categories for Three Regions (Western, Central, Eastern), Vertical Bar Is the 90% Standard Error.

they can be response variables to human disturbance on their own and because I wanted to separate the disturbance that occurs on the landscape from any ensuing or direct disturbance in the stream to try to better understand and separate the causal pathways that occur from this type of disturbance. The 229 sites sampled from agriculturally influenced regions for this study spanned the entire range of the national agriculture intensity index except for watersheds with a value less than 10 (minimally impaired or reference sites). The national index was developed from all HUC-10 watersheds (n = 19,420) in the mainland U.S.; as a result, a high number of sites were from the forested regions that have little or no agriculture. These types of sites were not the focus of this study other than our need for a small number of minimally impacted sites to represent a full range in agricultural disturbance. The national index allowed the assessment of the range of conditions in our study, but it did not include the orthophotograph based riparian variable that was included in the AG-Index. However, I believe the national index could be further improved with the incorporation of some measure of the amount of grazing within the watershed, but, at this time, no data set exists that documents the amount or type of grazing at the state or national scale, though some researchers have attempted to estimate potential grazing through models (John Van Sickle, U.S. EPA, 2010, personal communication).

Overall, correlations of invertebrate and algal metrics to agriculture indicators of disturbance were greatest for the AG-Index in the Western and Eastern regions, but the concentrations of TP and TN had the two highest correlations with the biological indicators in the Central region followed by the AG-Index. The Central region with the four study areas combined had a larger number of sites over a larger nutrient gradient, which probably accounts for the

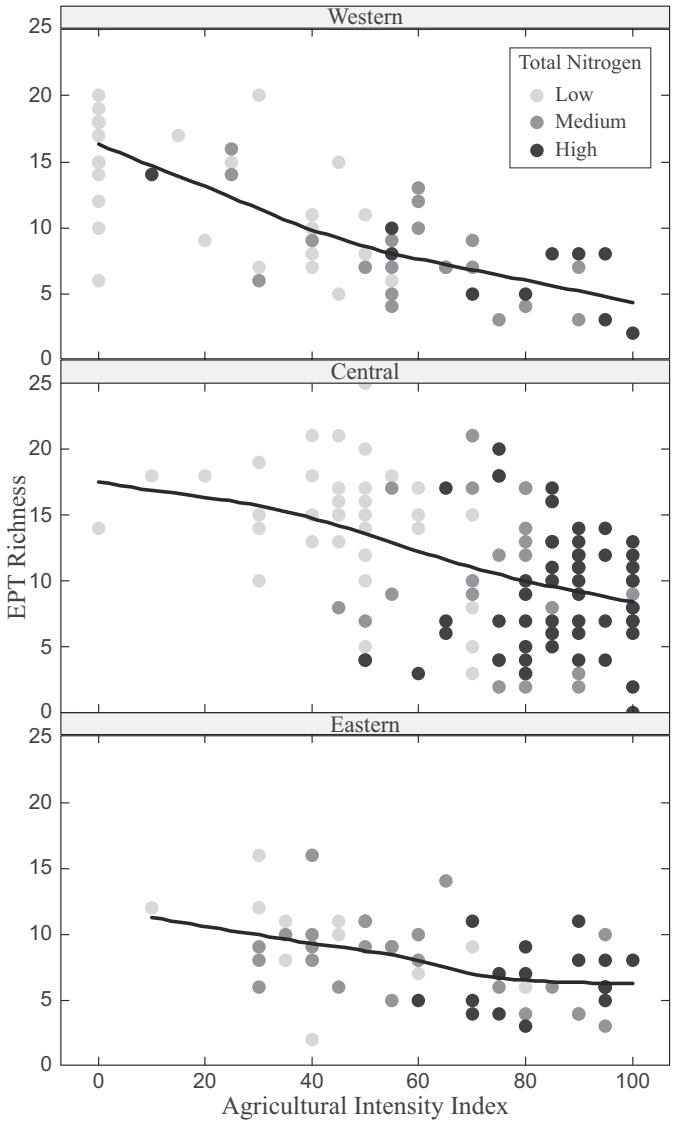

FIGURE 7. Plot of EPT Richness (Ephemeroptera, Plecoptera, and Trichoptera) *vs*. the Agricultural Intensity Index (AG-Index) for the Western, Central, and Eastern Regions. Points for each region are coded as low, medium, and high by total nitrogen concentration, loess best fit line added for interpretation.

higher correlations to the nutrient concentrations. In the Western region, all six invertebrate and three out of the four algal metrics had higher correlations to the AG-Index than either nutrient, and in the Eastern region only two of the invertebrate metrics but all four algal metrics had the same pattern. However, in the Central region the AG-Index never had the highest correlation for either the invertebrate or algal metrics and frequently was the third lowest. The highest correlation of any invertebrate metric to AG-Index was 0.75 in the Western region, 0.63 in the Eastern region, and only 0.46 in the Central. This may be due partly to the fact that though the Central region had a large nutrient gradient, it did not have as balanced a distribution of sites along the AG-Index. The Central region had 74% of its sites in the two highest AG-Index categories and only 6% below an AG-Index score of 40, while the Western and Eastern regions had a slightly more balanced distribution of sites along the intensity index, with 42 and 61% in the two highest categories, respectively. Two of the four study areas in the Central region (CNBR and WHMI) averaged 80 and 91 for the AG-Index with a standard deviation of only 15 and 7 points, respectively, suggesting these areas had little range or gradient in agricultural disturbance to relate to biological measures. This pattern also is shown in the plot of EPTR *vs*. the AG-Index where the points are coded by TN categories; the plots show that the Central and Eastern regions had few low nutrient samples and thus a relatively poor relationship to nutrients and to the AG-Index specifically (Figure 7).

The lack of minimally disturbed and/or reference sites in many regions worldwide is an ongoing concern, but especially in agriculture low lands and valleys (Allan, 2004; Herlihy *et al.*, 2008). Herlihy *et al.* (2008) in discussing the difficulty in locating appropriate reference sites, stated that in the agricultural regions of the U.S. there has been such extensive landscape and waterway degradation through time that even the best remaining stream sites are far removed from historical conditions. Cuffney *et al.* (2010), in studying the effects of urbanization across nine metropolitan areas, found that invertebrate metrics from the urban areas in the central U.S. did not respond significantly to the urban intensity index, while metropolitan areas in the western and eastern U.S. did. They concluded that the responses in metropolitan areas in the central region were affected by antecedent agriculture land cover which impacted the biological community before urbanization occurred and thus truncated the potential response gradient. It is possible that some of the sensitive macroinvertebrate taxa may have largely disappeared from streams in the central region or at least in some areas, resulting in a smaller potential biological response gradient to detect changes to streams in this region. However, algal metrics in the Central region from this study did show a strong response to TP and TN concentrations (Table 3) and moderate to AG-Index categories (Table 5). In general this was the opposite response as the invertebrates. This difference in the response of the different biological assemblages suggests that when possible it is important to evaluate more than one indicator in each bioassessment region, which concurs with others (Hughes and Peck, 2008; Brown *et al.*, 2009).

There recently have been published accounts of individual macroinvertebrate and algal taxa indicator values for TN and TP (Carlisle *et al.*, 2007; Potapova

and Charles, 2007) but as Yuan (2010) mentions, little research has been published on aggregate metrics of these assemblages related to nutrients, especially across different spatial scales. In this study I tested numerous aggregate metrics of macroinvertebrates and algae to examine differences in response to agricultural intensity for three regions and at the national scale. Individual correlations of metrics to agricultural indicators of disturbance showed that the data analyzed at the regional scale had much higher correlations than at the national scale for both assemblages. However, when pairwise comparisons of the AG-Index categories were compared for these two scales, a different pattern emerged. The national scale analysis had more significant pairwise comparisons and often at higher significance than that for either the Central or Eastern region for the macroinvertebrate metrics, but not as strong as for the Western region. Across the four algal metrics, the national scale analysis of the comparison among the AG-Index categories had a much stronger response than that for the Western and Eastern regions but not as strong as for the Central region. This suggests that though analysis of metrics at the national scale can often detect large differences in disturbance, more detail and specificity is obtained by analyzing the data at the regional scale. Potapova and Charles (2007) found that development of algal nutrient indicators was improved when completed at the regional scale *vs*. the national scale, and Cuffney *et al.* (2010) found that it was important to take into account regional climate and urban development differences when analyzing the effects of urbanization on macroinvertebrates. The O/E metric seemed to do well at the national scale; however, it is important to note that the metric was first developed at the regional scale using regional reference sites to determine the expected taxa before the data were combined into a national data set, and this may be part of the reason this metric seemed to do almost as well at the national scale compared to the three regions. The O/E metric was able to distinguish the large agriculture disturbance gradient in this study; however, Waite *et al.* (2012) found that development of models using invertebrate O/E did not explain as much variation as invertebrate tolerance metrics, especially when the disturbance gradient was more subtle.

Smith *et al.* (2007) developed nutrient biotic indices for New York state by summing nutrient tolerance scores for each taxa for each sample, yet the nutrient indices only had correlations of 0.68 and 0.57 to TP and nitrate (NO^{-3}), respectively, even though the original tolerance values were based on these nutrients. In this study, correlations above 0.77 to TP and 0.67 to TN were found for both algal and invertebrate metrics in select regions (Table 3). Porter *et al.* (2008) evaluated a large number of algal metrics and found the highest correlation to TN or TP was 0.57 for percent heterotrophic diatoms. Like this study, they found that total nutrients had higher correlations than dissolved nutrients (data not shown) and found higher correlations of algal metrics to nutrients in the western and central regions than eastern regions. Similarly, Black *et al.* (2011) found poor correlation of TN to algal metrics across the western region; however, they did not assess the central region and eastern regions. However, Black *et al.* (2011) were able to develop strong models explaining more than 87% of the variation in high nitrogen and high phosphorus diatom metrics by including habitat and watershed soil variables in addition to stream nutrients. Riseng *et al.* (2011), using the same invertebrate data used in this study, were able to develop structural equation models nationally and for the same three regions. They found that the national model gave a good overview of impacts but missed some important regional distinctions. Percent riparian forested wetland was an important explanatory variable in the Eastern region, but percent agriculture was more important in the Central and Western regions. Causal pathways in their study showed that agricultural land use effects were transmitted almost entirely via particulate water quality (TP, sediment, and organic carbon) in the Central region and via dissolved water quality (sum of nitrate, nitrite, and ammonia and excess conductivity) in the Western region. Like this study, they found the invertebrate metrics EPTR and measures of intolerant and tolerant taxa to be important indicators. Lastly, they found that the causal pathway of effects of agricultural land use was equally split between effects of habitat and water quality on invertebrate metrics (Riseng *et al.*, 2011). Though the data are not presented, I did not find high correlations for any habitat measures when assessing individual invertebrate or algal metrics, but this is probably partially due to the fact that stream sites were screened to have generally similar habitat conditions. Wang *et al.* (2007) developed models of invertebrate metrics in Wisconsin streams and found that of the 53% total variation explained by the models, 42% of this was attributable to watershed land use and instream habitat, and only 22% to nutrients. Future efforts will look to use this data set to develop national and regional models for a variety of agricultural and environmental indicators at watershed and riparian scales for both algal and invertebrate metrics.

Implications

By including variables on nutrient input with variables on agricultural land use together in an AG-Index, an important agriculture signature was

revealed that could have been missed by using the variable percent agriculture alone. However, even though the index developed and tested in this study was effective, I believe it could be greatly improved by the inclusion of variable(s) on the amount of domestic grazing and suggest that assessment of the intensity and quantity of grazing across North American landscapes be made a high priority. In addition, percent agriculture in the riparian zone from orthophotographs showed significant improvement over NLCD estimates of agricultural land use in the riparian zone, and this was an important addition to the index. Overall, invertebrate metrics were not able to separate AG-Index categories as well in the Eastern region, while algal metrics generally performed poorly in the Western region; on the other hand, the invertebrate metrics worked well in the Western and Central, and algal metrics in the Central and Eastern regions. This difference in the response of the different biological assemblages suggests that when possible, it is important to evaluate more than one indicator in each bioassessment region. Lastly, riparian and instream habitat are important components of healthy rivers and streams, and diverse biological communities can likely thrive where there is a high amount of agriculture if the habitat is good. For example, despite having some of the highest AG-Index values, the WHMI sites had relatively high EPT richness, the likely explanation being that the streams have relatively good riparian and instream riffle habitats (Jeffrey Frey, U.S. Geological Survey, 2011, personal communication; Mark Munn *et al.*, U.S. Geological Survey, 2007, unpublished data). I and other researchers have seen this pattern of biological communities characteristic of relatively undisturbed areas in streams with good habitat despite being in areas of high anthropogenic disturbance, and these observations coincide with the results from Riseng *et al.* (2011) that showed that in some regions, roughly 50% of the explanation for invertebrate quality was water quality and 50% habitat.

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