Multistressor predictive models of invertebrate condition in the Corn Belt, USA

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Abstract: Understanding the complex relations between multiple environmental stressors and ecological conditions in streams can help guide resource-management decisions. During 14 weeks in spring/summer 2013, personnel from the US Geological Survey and the US Environmental Protection Agency sampled 98 wadeable streams across the Midwest Corn Belt region of the USA for water and sediment quality, physical and habitat characteristics, and ecological communities. We used these data to develop independent predictive disturbance models for 3 macroinvertebrate metrics and a multimetric index. We developed the models based on boosted regression trees (BRT) for 3 stressor categories, land use/land cover (geographic information system [GIS]), all in-stream stressors combined (nutrients, habitat, and contaminants), and for GIS plus in-stream stressors. The GIS plus in-stream stressor models had the best overall performance with an average cross-validation R^2 across all models of 0.41. The models were generally consistent in the explanatory variables selected within each stressor group across the 4 invertebrate metrics modeled. Variables related to riparian condition, substrate size or embeddedness, velocity and channel shape, nutrients (NH_3), and contaminants (pyrethroid degradates) were important descriptors of the invertebrate metrics. Models based on all measured in-stream stressors performed better than models based on GIS landscape variables, suggesting that the in-stream stressor characterization reasonably represents the dominant factors affecting invertebrate communities and that GIS variables are acting as surrogates for in-stream stressors that directly affect in-stream biota.

Key words: multiple stressors, modeling, macroinvertebrates, streams, watershed disturbance, agricultural land use, statistical asses sment, boosted regression trees

Understanding the effects of human landuse modification on stream biota, including the effects of specific chemical and physical stressors, is a fundamental goal of bioassessment in stream ecology (Allan 2004, Sundermann et al. 2013). The use of models to elucidate these effects has increased markedly in the past decade in all areas of ecology, partly to address the complexity in natural stressor–response relationships. Major advances in conceptual models and statistical techniques (Leathwick et al. 2005, Austin 2007, Clapcott et al. 2011, Cuffney et al. 2011, Bailey et al. 2014, Waite et al. 2014) have helped practitioners develop response models that better support the needs of bioassessment programs. The expansion and application of models in stream ecology are helping researchers develop a broader understanding of the complexity of ecological responses to multiple stressors (Cabecinha et al. 2007, Kennen et al. 2010, Turak et al. 2010, Waite 2014, May et al. 2015).

Human actions on the landscape are a principal threat to the ecological integrity of river ecosystems, can adversely affect habitat, water quality, and the biota via numerous and complex pathways and multiple stressors (Allan 2004, Wiley et al. 2010, Clapcott et al. 2011, Coles et al. 2012, Waite et al. 2014). Numerous researchers have used spatial (GIS-based) explanatory variables (e.g., land use and land cover) as stream bioassessment stress indicators (Carlisle et al. 2009, Maloney et al. 2009, Waite et al. 2010, Brown et al. 2012, Villeneuve et al. 2015). In general, national- or large regional-scale models typically focus on large-scale natural landscape features, such as climate, topography, elevation, and geology, as the most important discriminating variables, with disturbance variables, such as land use, nutrients, sediments, and contaminants, usually of lower predictive importance. Many previous models of stream ecology were focused on the effects of land use in general or, at best, incorporated 1 or 2 types of in-stream

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stressors (Carlisle et al. 2009, Maloney et al. 2009, Clapcott et al. 2011, Brown et al. 2012, Waite et al. 2014).

Large data sets recently have become available. These data sets enable researchers to build stream bioassessment models across a range of spatial scales (Feld 2013, Waite et al. 2014, May et al. 2015) and to incorporate more types of stressors, such as hydrological alteration and water quality (Norton et al. 2002, Kennen et al. 2010, Wiley et al. 2010, Riseng et al. 2011, Esselman et al. 2015, Villeneuve et al. 2015). However, we know of no stream ecology models that incorporate detailed assessment of all of the following stressors at the regional scale simultaneously: nutrients, sediment, flow alteration, physicochemical properties (e.g., dissolved O_2 [DO], pH, and temperature), and contaminants (e.g., pesticides, trace elements, and organic hydrocarbons).

In 2013, personnel from the US Geological Survey (USGS) National Water Quality Assessment Program (NAWQA) began conducting large-scale regional studies to evaluate the effects of multiple stressors on stream biota. In the first such regional study (2013), scientists from NAWQA and USGS Columbia Environmental Research Center (CERC) collaborated with the US Environmental Protection Agency (EPA) National Rivers and Streams Assessment (NRSA) to assess stream quality at 100 sites across the Corn Belt region in the midwestern USA. The Midwest Stream Quality Assessment (MSQA) covered parts of 12 states (Van Metre et al. 2016, Garrett et al. 2017).

We analyzed data collected during the MSQA with the aid of models we developed to explore the influence of multiple physical and chemical stressors on macroinvertebrates in streams across this large, predominantly agricultural region. We compared the overall performance (cross-validation R^2) of models developed using Boosted Regression Tree (BRT) techniques for predicting the responses of 3 common macroinvertebrate metrics—richness of the orders Ephemeroptera, Plecoptera, and Trichoptera [EPTR] and 2 tolerance-value metrics (average tolerance of all taxa [RICHTOL], richness of intolerant taxa [INTOL_RICH])—and a multimetric index (MMI). Potential explanatory variables in the models include common landscape-based disturbance variables and natural landscape factors (GIS-based variables) and, at the scale of the stream reach, detailed measures of nutrients, physiochemical properties, flow alteration, sediment, habitat, and contaminants measured over a 14-wk index period prior to ecological sampling. Our primary objective was to identify the most important stressors predicting stream invertebrate condition. A secondary objective was to compare the stressors identified in models developed with only landscape GIS variables as predictors to models developed with the in-stream stressors.

METHODS

Study area

The MSQA region lies within the Midwestern agricultural region dominated by corn and soybean cultivated crops and comprises parts of 12 states, with an area of ~600,000 km^2 (Fig. 1). The MSQA study area is formed by the combination of all or part of 6 EPA Level III Ecoregions (https://www.epa.gov/eco-research/level-iii-and-iv-ecoregions-continental-united-states). A more detailed description of the region was given by Garrett et al. (2017).

Study design

The design of the NAWQA regional studies (also called Regional Stream Quality Assessments [RSQA]) relies on the concept of a water-quality index period during which it is assumed that stream biota are affected by the chemistry, flow condition, and habitat of the streams. The index period is assumed to extend for several weeks to months prior to the ecological assessment and corresponds to the time that researchers have observed is necessary for stream benthic invertebrate communities to recover from stressor effects (Moulton et al. 2002). A total of 100 wadeable stream sites were selected for the MSQA study, 98 of which were included in this analysis. Basin area for the 98 streams ranged from 3.5 to 2865 km^2 (median = 167 km^2). Of the 98 sites sampled, 49 were RSQA-targeted sites (37 selected across a gradient of agricultural land use and 12 across a gradient of urban land use) and 49 were NRSA probabilistic (randomly selected) sites. The 37 targeted sites were situated along an agricultural gradient that was defined by a combination of the % row crop agriculture and toxicity-weighted pesticide use (TWU) within the basin. The 12 targeted urban sites were in or near 7 major cities: Chicago, Illinois; Dayton and Cincinnati, Ohio; Indianapolis, Indiana; Kansas City, Missouri; Milwaukee, Wisconsin; and Omaha, Nebraska. Two of the 100 streams sampled for water quality were dropped for this analysis. The ecological survey was not done at one stream because it was too deep, and the other was considered an outlier based on basin size. The site-selection process was described in detail by Garrett et al. (2017).

Data collection and chemical analysis

Water and sediment chemistry Twelve water samples were collected from each site over 14 wk from early May to early August 2013. The sampling period coincided with the season of high agrochemical use and runoff (Gilliom et al. 2006). All samples were collected using standard USGS depth-integrated methods (USGS 2009) across the stream channel during daylight hours. Temperature and water level were monitored continuously (15-min intervals) at all sites for at least a month before and after the index period. Basic water-quality variables (dissolved O_2 [DO], pH, conductivity, and temperature) were measured during each water-quality sampling trip and summarized for analysis (minimum, mean, median, and maximum). One composite bed-sediment sample was collected from each site at the end of the index period, coincident with ecological sampling. Subsamples of bed sediment were analyzed for contaminants or used for whole-sediment toxicity testing. Samples from all sites were

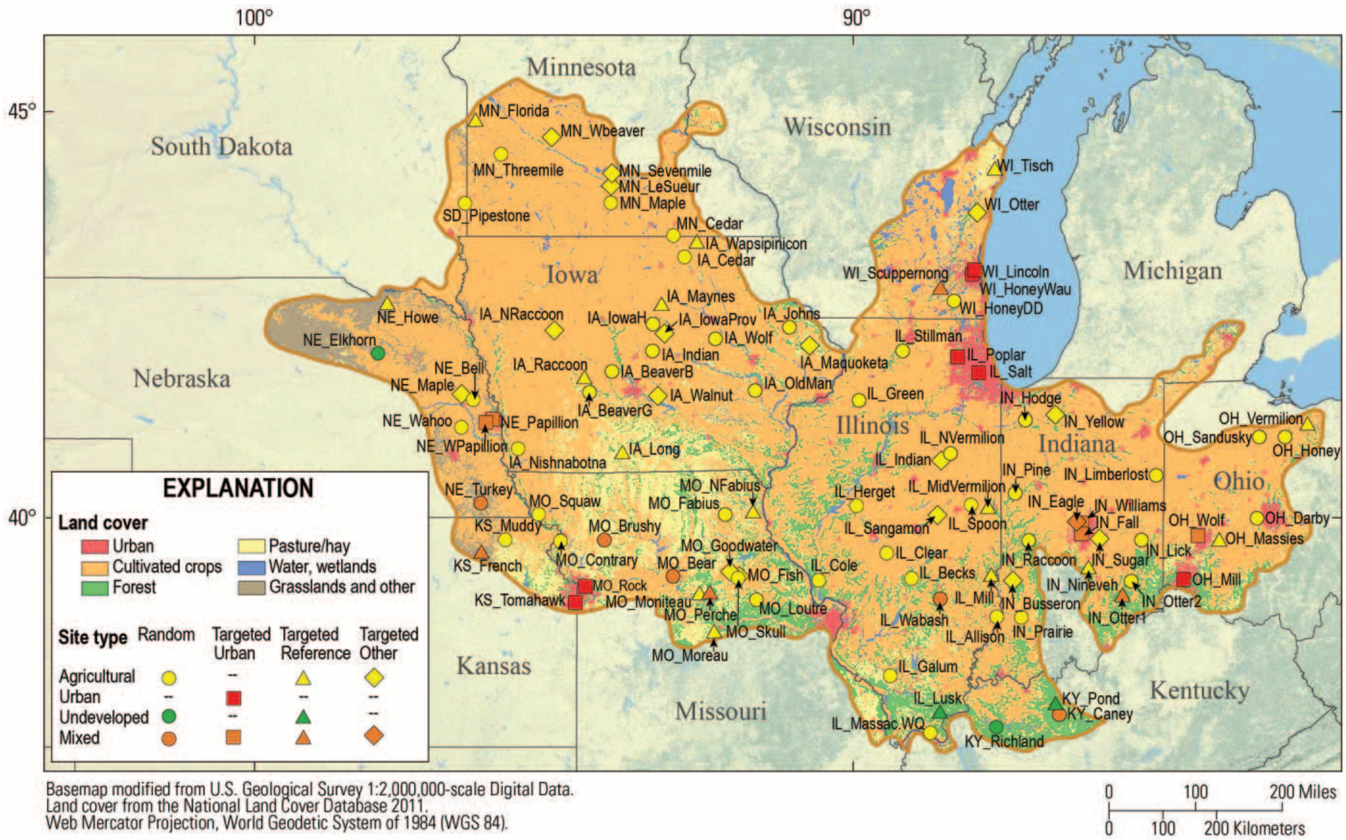

Figure 1. Map showing the Midwest Stream Quality Assessment (MSQA) study region and sampling sites.

analyzed for water chemistry (nutrients, Cl^- and SO_4^{2+}, dissolved organic C [DOC], and dissolved pesticides), phytoplankton chlorophyll *a*, suspended sediment concentration and grain size, and sediment chemistry (trace and major elements, radionuclides, currently used pesticides). Polar Organic Chemical Integrative Samplers (POCIS) were deployed in all streams for ~5 to 6 wk (37 ± 7 d) before the ecological survey. These samplers accumulate water-soluble contaminants from the water column during the deployment period (Alvarez 2010). Extracts were analyzed for 227 current-use pesticides and degradates (Van Metre et al. 2016).

Unless otherwise noted, chemical analyses were done at the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado. Briefly, pesticides in water (except for glyphosate) and in POCIS extracts were analyzed using direct aqueous-injection liquid chromatography–tandem mass spectrometry (DAI LC-MS/MS) (Sandstrom et al. 2016). For POCIS, a mixture of DCM : methyl-tert-butyl ether (80 : 20, v : v) was used to elute and concentrate the extract from the samplers prior to analysis by DAI LC-MS/MS (Sandstrom et al. 2016). Glyphosate was analyzed at the USGS Texas Water Science Center by enzyme-linked immunosorbent assay (ELISA) (Mahler et al. 2016). Samples for nutrients and major ions were analyzed as described by Fishman (1993) and Patton and Kryskalla (2011).

Streambed sediment samples were analyzed for pesticides by gas chromatography with mass spectrometry (GC/MS) following accelerated solvent (ASE) extraction and cleanup (Hladik and McWayne 2012) at the USGS Pesticide Fate Research Group Laboratory in Sacramento, California. Sediment samples were extracted with ASE and analyzed for polycyclic aromatic hydrocarbons (PAHs) and other semivolatile compounds by gas chromatography mass spectrometry (GC/MS) (Zaugg et al. 2006). A separate method using ASE and GC/MS was used to measure organo-chlorine insecticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in sediment (Counihan et al. 2014). Elemental concentrations were estimated on a 0.2-g freeze-dried and ground sample digested on a hotplate in a mixture of hydrochloric–nitric–perchloric–hydrofluoric acids, with analysis by inductively coupled plasma–mass spectrometry (Briggs and Meier 2003).

Contaminant and nutrient metrics We computed contaminant metrics by chemical class and medium: pesticides in water, pesticides in POCIS extracts, and 5 classes of contaminants in bed sediment: metals, currently used pesticides, organochlorine pesticides, PCBs, and PAHs. Briefly, we obtained metrics for each contaminant class and medium (with the exception of pesticides in POCIS extracts) by calculating a benchmark quotient (the ratio of the contaminant concentration to an effects-based benchmark) for each contaminant at each site and averaging benchmark quotients for all constituents in that class (Nowell et al. 2013, 2014).

For pesticides in water, we divided the concentration of each pesticide in a sample by its relative toxicity concentration and summed these toxicity quotients to represent all pesticides in the sample. We computed multiple metrics based on various toxicity concentrations to yield Pesticide Toxicity Index values for fish, cladocerans, and benthic invertebrates (Nowell et al. 2014) and benchmark quotients based on USEPA Office of Pesticide Programs aquatic-life benchmarks for invertebrates and plants (Nowell et al. 2016). We computed additional metrics by considering different concentration statistics (maximum, mean) and different exposure periods for the concentration used in the computation (entire 14-wk index period, last ½ of the study period, last sample collected).

For bed sediment, we computed metrics for 5 classes of contaminants (metals, PAHs, PCBs, organochlorine pesticides, currently used pesticides) and combined these metrics into aggregated metrics of sediment contamination (e.g., mean for all 5 classes and mean for the 4 classes of organic contaminants). We calculated metrics for individual contaminants by dividing the environmental concentration by the sediment benchmark. We used Probable Effect Concentrations (PECs) (MacDonald et al. 2000) for metals, total PAHs, total PCBs, and organochlorine pesticides and Likely Effect Benchmarks for currently used pesticides (Nowell et al. 2016, Moran et al. 2017). Then we computed metrics for each class by averaging (metals) or summing (pesticides) benchmark quotients for contaminants within the class. PAHs, PCBs, and some organochlorines in sediment were measured at only 27 sites (including the 12 urban sites), so we estimated concentrations at the remaining 72 rural sites with regression methods (PAHs) or by substituting the median value for PCBs and organochlorines, concentrations of which were uniformly low (Moran et al. 2017). We estimated the sum of PAHs used for the PEC at 72 rural sites with a regression equation based on population density in the watershed because of the strong relation of PAHs to urban land use ($r^2 = 0.87$ for the 27 sites with PAH data).

We computed metrics for pesticides in POCIS extracts by summing concentrations of pesticides in POCIS extracts by use type or by pesticide chemical subgroup (e.g., sum of insecticides, sum of insecticide degradates, and sum of pyrethroid degradates). Use of a unit of mass/POCIS (rather than concentration in water) was necessary because only 52 of the 227 compounds analyzed in POCIS have available POCIS–water sampling rate factors, which are required for estimation of water concentrations (Van Metre et al. 2016). Therefore, we chose to use sums of POCIS concentrations (i.e., ng/POCIS) by chemical subgroup as indicators of relative pesticide occurrence among sites.

Nutrient metrics consisted of median and maximum concentrations of the N and P compounds measured for the sampling period (12 samples) and for the last 6 samples leading up to the ecological survey (Van Metre et al. 2015). We included the latter to test whether the shorter time period before ecological sampling was a better indicator of ecological stress than the longer time period.

Geospatial data We developed a geospatial database for the MSQA containing stream characteristics at the sampling reach and watershed (using each sampling site as the pour point for watershed delineation). Watershed delineation and sources of geospatial variables were published by Nakagaki et al. (2016a, b). Along the ecological survey reach, the riparian buffer was delineated by digitizing the centerline of the stream starting at the downstream end of the ecological survey reach and extending upstream for a distance in kilometers equal to the $\log_{10}$ of the watershed area (Zelt and Munn 2009). The mean length of the riparian reach based on this approach was 2.2 ± 0.6 km. A 50-m buffer was applied to the stream centerline and the resulting polygon, the riparian buffer, was overlaid with various geospatial data layers. Geospatial variables also were estimated for the watershed, and we included both riparian and watershed variables as potential explanatory variables in ecological models. Geospatial variables included land use (e.g., % crop land), management practices (e.g., subsurface tile drains), and soil properties (e.g., % sand, permeability).

Macroinvertebrate and habitat data Habitat was surveyed and biological assemblages (algae, benthic macroinvertebrates, and fish) were sampled at 98 sites during July, August, and September 2013, with most sites (83) sampled between 22 July and 8 August. Sampling was done concurrently with the collection of bed and bank sediment samples and timed to be as close as was feasible to the end of the water-chemistry index sampling period during the week of 5–9 August. Algae and macroinvertebrate samples were collected following NRSA protocols along multiple transects across each stream reach (USEPA 2007). Benthic macroinvertebrate samples were collected with a D-frame net with 500-μm mesh openings at 11 equally spaced transects at one of the assigned sampling stations (left, center, right) on the transect. Organisms were separated from the substrate and preserved in 95% ethanol, with larger predaceous invertebrates preserved immediately to reduce the chance of consumption of or damage to other specimens. Specimens were identified to lowest practical taxon following a 500-specimen count based on EPA protocols (USEPA 2007).

Habitat data were collected along 11 evenly spaced transects within the stream reach. The physical habitat was characterized by measuring substrate, canopy cover, in-stream fish cover, bankfull condition, riparian vegetation, evidence of human disturbances, discharge, and channel measurements, such as depth, width, stream slope, and bank angle. The 6 components of the physical-habitat characterization are described in the NRSA field operations manual (USEPA 2013b).

We used the 11 transect measurements to calculate median values of channel width, bankfull depth (bank height + wetted depth), wetted widths, and thalweg depths. We calculated the median and 84th percentile of the particle-size distribution (d84), % fines, and % alluvium from the 105 bed-material measurements in the reach. We calculated water surface gradient over the entire survey reach. We calculated sinuosity (stream length/valley length) and flow line gradient in a GIS with the segment from National Hydrography Dataset medium resolution (1: 100,000) hydrography segment containing the survey reach.

We used the 105 point measures of depth to calculate the reach-mean depth at the time of the habitat survey. Stage was collected continuously (15-min interval) at the water-sampling site, and we used changes in stage as estimates of the change in depth during the 6 wk prior to biological sampling. Water temperature was collected continuously (15-min interval).

We resolved all taxonomic issues (taxonomic identification level and nomenclature, i.e., taxonomic harmonization), removed ambiguous taxa (Cuffney et al. 2007), and generated invertebrate metrics with the Invertebrate Data Analysis System (IDAS) software (Cuffney 2003). In general, we resolved data for dominant aquatic insect orders at genus level. Less common orders were sometimes aggregated to family level. Rare organisms or those with difficult taxonomy were sometimes aggregated to order or higher. We resolved ambiguous taxa sample by sample by combining parents into ambiguous children (e.g., Baetidae combined into *Baetis* if both occurred in the same sample) (Cuffney et al. 2007). We used IDAS to generate ~120 macroinvertebrate richness and abundance metrics, including tolerance and functional-group metrics, which were calculated based on values published by Barbour et al. (1999). We obtained a reduced set of metrics with a variety of exploratory analysis techniques including evaluation of scatter plots for outliers and range, correlation of metrics to core watershed disturbance variables, correlation among macroinvertebrate metrics and professional judgment based on results of previous metric analyses in different regions of the USA (Kennen et al. 2010, Waite et al. 2010, Cuffney et al. 2011, Brown et al. 2012). From the reduced set, we selected 3 macroinvertebrate metrics and a multimetric index (MMI) as response variables for model development and validation: EPTR, RICHTOL, INTOL_RICH, and MMI (for simplicity, we refer to all 4 as macroinvertebrate metrics hereafter). The 4 metrics represent different aspects of the invertebrate assemblage: richness of the 3 dominant sensitive aquatic insect groups (EPTR); tolerance (RICHTOL: average tolerance of all taxa in a sample based on supplemented EPA tolerance values; Cuffney 2003) and INTOL_RICH (richness of intolerant taxa), and an MMI based on NRSA methods (Table S1) that includes 6 individual invertebrate metrics: % EPT, Shannon diversity, scraper richness, clinger richness, Ephemeroptera richness, and % richness of tolerant taxa (scores 8–10) (Stoddard et al. 2008). Data are available in USGS ScienceBase-Catalog for all 98 sites (https://doi.org/10.5066/F71V5C57).

Model development

Regression trees fall within the commonly used classification and regression-tree (CART) or decision-tree family of techniques. Numerous descriptions of their use and technical details (e.g., Breiman et al. 1984, De'ath and Fabricius 2000, Prasad et al. 2006) and applications (Aertsen et al. 2010, Clapcott et al. 2011, Leclere et al. 2011, Brown et al. 2012, and Waite et al. 2010, 2012, and 2014) are available in the literature. Therefore, we provide only a brief description here. Boosted regression trees (BRTs) are among a family of techniques used to advance single-classification or regression trees by combining the results of sequentially fit regression trees to reduce predictive error and improve overall performance (De'ath 2007, Elith et al. 2008).

We developed BRT models for 4 macroinvertebrate metrics for 3 model categories: 1) GIS-based variables, 2) variables from the 3 in-stream stressor categories combined (habitat, nutrients, and contaminants; Env.All), and 3) the 4 stressor categories combined (GIS+Env). We developed the BRT models based on a multistage process. BRT models were run individually for each stressor category (habitat, nutrients, and contaminants individually; Tables S2, S3, Figs S1–S4). Next, we used only the variables retained in each of these final models of the 4 invertebrate metrics as input in the Env.All and GIS+Env models. We reduced explanatory variables in each final BRT model by using a combination of variable importance (VI) scores, evaluation of interactions and partial dependency responses, and *gbm* simplify scores from R (see below) following the approach outlined by Elith et al. (2008) to minimize overfitting. All variables with VI < 8 were deleted to reduce the variables to a core set. The remaining variables were then used to develop the final BRT model. We selected the final model by sequentially deleting variables, evaluating the effects on cross-validation R^2 (CV R^2), and examining partial dependency plots to evaluate the response form. This stepwise process was repeated until further pruning of variables would have reduced the performance of the model beyond what we deemed reasonable. We completed the selection process independently for each invertebrate metric for each stressor category. Additional information on the BRT model methodology is provided in Appendix S1.

We used partial dependency plots to visualize the effect of explanatory variables on the response variable after accounting for the average effects of all other explanatory variables (De'ath 2007, Elith et al. 2008). BRT models were run using the *gbm* library in R (version 3.1.3; R Project for Statistical Computing, Vienna, Austria) and specific code from Elith et al. (2008). This code optimizes the number of trees run in each model and, thus, it varies for each model. We used CV R^2 values to compare performance of BRT models because CV R^2 values are more conservative and less likely to be inflated by potential overfitting than R^2. We calculated CV R^2 values by holding 25% (bag fraction) of the sites out for each regression tree split then used the withheld sites to test the percentage of deviance explained by the split (Elith et al. 2008).

RESULTS

Invertebrate community condition across the MSQA region spanned a wide range, as indicated by the MMI range of 6 to 87. EPTR showed a more restricted range of values with sites having from 0 to 21 and a median of only 8 EPT taxa, which is low compared to other geographic regions in the USA (e.g., median EPTR from our Southeastern USA regional study was 15, range 1–39). INTOL_RICH also showed a restricted range, with a maximum of 12 and a median of 5.8 intolerant taxa. RICHTOL, which is the average tolerance for all taxa at a site, ranged from 4.7 to 7.8. The minimum average of 4.7 was higher than the cutoff of 0 to 3 for intolerant taxa, suggesting that the lower end of the scale for RICHTOL was missing because on average few intolerant and more tolerant taxa were present. RICHTOL and INTOL_RICH are opposite in sign, so higher values of INTOL_RICH and lower values of RICHTOL are generally associated with lower levels of human disturbance. The 3 individual metrics all indicated that true reference conditions are lacking in the region, making the absolute evaluation of condition on the basis of MMI difficult.

The GIS+Env models, which used variables from geospatial and in-stream explanatory variable categories combined, were the most successful in explaining variability in the 4 invertebrate metrics (CV R^2 range = 0.29–0.55, mean = 0.41; Table 1) and required 4–6 explanatory variables (Table 2). The model fit for all 3 model categories was best for the MMI, and the GIS+Env model had the highest overall model performance (CV R^2 = 0.55; Table 1). The Env.All models, which used only in-stream variables, also had relatively good performance. Two of the 4 models performed as well as the GIS+Env models. GIS only models had relatively poor performance except for the MMI (Table 1), so we concluded that prediction of invertebrate condition at unsampled sites was not realistic with these models.

Many explanatory variables were retained consistently in the BRT models of the 4 invertebrate metrics within a model category (Tables 2, 3, S3). For example, Pasture Hay (% watershed in pasture or hay, a surrogate for non-row crop and non-urban) and Rip (riparian) Canopy (% 50-m stream buffer that is canopy) appeared in all 4 GIS models. Sand Content (% sand in watershed soils) and Rip (riparian) Crops, appeared in 3 of the 4 GIS models. In general, the partial dependency plots showed that streams draining watersheds with higher Sand Content, Pasture Hay, Riparian Canopy, or Base Flow Index had better invertebrate metric

Table 1. Comparison of model evaluation statistics of boosted regression tree (BRT) models for 4 macroinvertebrate metrics by stressor category. n = number of initial variables entered into model development. The number of variables in the final model is given in parentheses. GIS = geographic-information system; Env.All = all in-stream environmental stressors; GIS+Env = GIS + environmental variables; CV R^2 = cross-validation R^2; EPTR = total taxon richness of Ephemeroptera, Plecoptera, and Trichoptera; RICHTOL = average tolerance of all taxa; INTOL_RICH = richness of intolerant taxa; MMI = macroinvertebrate multimetric index.

Macroinvertebrate variables	GIS (n = 46) CV R^2	Env.All (n = 34) CV R^2	GIS+Env (n = 44) CV R^2
EPTR	0.32 (5)	0.40 (6)	0.39 (6)
RICHTOL	0.26 (6)	0.40 (6)	0.40 (6)
INTOL_RICH	0.21 (6)	0.25 (6)	0.29 (4)
MMI	0.46 (6)	0.44 (8)	0.55 (6)
Average	0.31	0.37	0.41

scores than streams draining watersheds with other characteristics (Figs 2A–H, 3A–F). The only variable retained in all 4 Env.All models was Mean NH_3 (mean NH_3 concentration at a site) (Table 2, Fig. 2E). Other variables were retained in only 1 or 2 models. In general, habitat variables appeared most frequently (3–5 retained in each model). GIS variables appeared the most frequently in the GIS+Env models, followed by, in order, habitat variables, nutrient variables, and contaminant variables (Table 2, Fig. 3A–F). Sand Content, NH_3, and measures of channel morphology (median wetted width [Wet Width Med], wetted width variation [Wet Width Var], or Sinuosity) appeared in all models. Streams with a combination of more fine substrate (low d84 values), high Embeddedness, narrow Wetted Width, low Sinuosity, high Mean NH_3 and high Rip (riparian) Disturbance (low complexity) had poorer invertebrate communities (low EPTR, INTOL_RICH, or MMI values and high RICHTOL) (Figs 2A–H, 3A–F).

Because of the strong geomorphic signal in these models (e.g., velocity, wetted width variation, and substrate), we separated sites into groups with % fines < 0.50 and with % fines ≥ 0.50 (n = 40 and n = 54, respectively, with 4 sites lacking % fines data). The MMI model of sites with % fines > 0.50 had explanatory variables similar to those in the Env.All MMI model of all 98 sites. This submodel had the following variables (in order of importance): Wetted Width Var (variation), d84 Substrate, Velocity, Pyrethroid_deg (Pyrethroid degradates), and Embeddedness (results not shown in Tables 2, 3). The MMI model with % fines < 0.50 had 3 explanatory variables: Rip (riparian) Complexity, Pyrethroid_deg, and DO Daily Minimum. Both sub-models had CV R^2 = 0.34.

The success of the models in predicting metric scores and bias are illustrated in observed vs predicted plots (Fig. 4A–C). The 3 models all had CV R^2 > 0.4 showing a relatively

Table 2. Comparison of explanatory variables for boosted regression tree (BRT) models for 4 macroinvertebrate metrics by stressor category. Variables are presented in descending order of variable importance (VI) in each model. All variables are based on mean values of multiple samples, unless otherwise indicated. GIS = geographic-information system; Env.All = all in-stream environmental stressors; GIS+Env = GIS + environmental variables; EPTR = total taxon richness of Ephemeroptera, Plecoptera, and Trichoptera; Rip = riparian; Med = median; Max = maximum; Var = standard deviation, d84 = 84th percentile of substrate particle diameter; Pyrethroid.deg = concentration of pyrethroid degradates; Wet Width Var = wetted width variability, DO = dissolved O_2; sCIBQ 14 = Pesticide Toxicity Index (PTI) based on chronic invertebrate benchmark quotient; 4Org bs mPECQ = aggregated mean benchmark quotient (ABQ) for 4 classes of organic sediment contaminants. See Table S1 for complete variable definitions. n = number of initial variables entered into model development.

	GIS (n = 46)		Env.All (n = 34)		GIS+Env (n = 44)	
Invertebrate metrics	Variable	VI	Variable	VI	Variable	VI
EPTR	Sand Content	24	NH_3	21	NH_3	21
	Rip Crops	23	d84 Substrate	18	Sand Content	18
	Pasture Hay	20	Pyrethroids.deg	16	Sinuosity	17
	Base Flow Index	18	Stream Flow	16	Coarse Substrate	16
	Rip Canopy	16	Sinuosity	15	Permeability	14
			Wet Width Var	14	Pyrethroids.deg	14
RICHTOL	Rip Canopy	18	NH_3	22	NH_3	23
	SubDrain	18	Velocity	21	Velocity	17
	Pasture Hay	17	Wet Width Med	18	Rip Canopy	17
	Rip Wetlands	16	Rip Disturbance	15	Wet Width Med	16
	Rip Crops	15	Embeddedness	13	Pasture Hay	15
	Canal-Ditch	15	DO Min Daily	11	Sand Content	13
INTOL_RICH	Sand Content	22	NH_3	25	Sand Content	28
	Base Flow Index	20	Rip Disturbance	20	Rip Disturbance	26
	Pasture Hay	20	sCIBQ 14	16	Wet Width Var	25
	Rip Canopy	18	Sinuosity	14	NH_3	21
	Permeability	11	4Org bs mPECQ	13		
	Rip Impervious	9	Bank Undercut	12		
MMI	Rip Canopy	21	Velocity	15	Pasture Hay	21
	Sand Content	19	Rip Complexity	14	Rip Canopy	18
	Pasture Hay	18	d84 Substrate	14	Sand Content	17
	Elevation	15	Embeddedness	13	d84 Substrate	15
	SubDrain	15	NH_3	13	NH_3	15
	Rip Crops	12	DO Max Daily	12	Base Flow Index	14
			Pyrethroids.deg	10		
			Wet Width Var	8		

strong 1 : 1 relationship, with only minor bias or deviation at the high and low ends of the MMI scale.

DISCUSSION

A major objective of the MSQA study and of our analysis was to evaluate the importance of a wide range of stressors in explaining invertebrate community health. When variables that describe spatial, habitat, nutrient, and contaminant characteristics of each watershed and stream were combined (GIS+Env model), 1 GIS variable (Sand Content), 1 nutrient variable (NH_3), and some measure of stream geomorphology (Wet Width Med, Wet Width Var, or Sinuosity) were retained in the model for each of the 4 invertebrate measures. The importance of these variables indicates that the overall habitat of the streams, particularly as controlled by hydrology, exerts a strong influence on invertebrate condition in the Midwest.

Sand Content describes the amount of sand in watershed soils, and increased Sand Content was related to better invertebrate community health as measured by the metrics (an example for MMI is shown in Fig. 3C), with the greatest effect for 0 to ~20% Sand Content. We hypothesize that increasing Sand Content positively affects invertebrate community health by causing greater water infiltration leading to less overland runoff of sediments and contaminants and in-

Table 3. Summary of explanatory variables for boosted regression tree (BRT) models across the 4 macroinvertebrate metrics by stressor category. Variables are presented in descending order of number of times they were included (TI) in the 4 invertebrate metric models. All variables are based on average values of multiple samples, unless otherwise indicated. See Table S1 for complete variable definitions.

GIS (n = 46)		Env.All (n = 34)		GIS+Env (n = 44)	
Variable	TI	Variable	TI	Variable	TI
Rip Canopy	4	NH_3	4	NH_3	4
Pasture Hay	4	Pyrethroids.deg	2	Sand Content	4
Rip Crops	3	Rip Disturbance	2	Pasture Hay	2
Sand Content	3	Sinuosity	2	Rip Canopy	2
Base Flow Index	2	Wet Width Med	2	Wet Width Var	2
Rip Wetlands	1	Wet Width Var	2	Wet Width Med	1
Rip Impervious	1	Embeddedness	2	Sinuosity	1
Permeability	1	d84 Substrate	2	Coarse Substrate	1
Canal–ditch	1	Velocity	2	d84 Substrate	1
SubDrain	1	4Org bs mPECQ	1	Velocity	1
Elevation	1	sCIBQ 14	1	Rip Disturbance	1
		DO Min Daily	1	Base Flow Index	1
		DO Max Daily	1	Permeability	1
		Stream Flow	1	Pyrethroids.deg	1
		Undercut Banks	1		
		Rip Complexity	1		

creased groundwater recharge contributing to more stable base flow. We found some support for this hypothesis in that Sand Content was positively correlated to Base Flow Index for the MSQA sites (Spearman's $\rho = 0.50$, $p < 0.001$).

Stream geomorphology in this region is strongly influenced by changes to natural stream channels by dredging, straightening, and resulting incision of the channel, slowing and deepening of the water, and fining of substrate (Hadish et al. 1994). These factors often occur in agricultural landscapes individually and in combination and are regarded as detrimental to stream biota (Allan 2004, USEPA 2006, Edwards 2014, Waite 2013, 2014). The 3 measures of stream geomorphology noted above all reflect the complexity, or lack thereof, of the channel.

NH_3 concentrations were negatively associated with invertebrate community health, which is not surprising because the un-ionized form is toxic to invertebrates at low concentration (USEPA 2013a). NH_3 is generated by the decomposition of manure and sewage and is relatively rapidly transformed to NO_3^- in aerated streams. NH_3 in this model might be a general indicator of recent runoff, eutrophication, and potential contamination by manure and sewage. The partial dependency plots indicate that MMI decreases rapidly as NH_3 concentrations increase from 0 to 0.1 mg/L (Figs 2E, 3E). Similar responses of invertebrates to nutrients in agricultural streams in the Central USA have been reported (USEPA 2006, Yuan 2010, Waite 2014), but most previous investigators have not found NH_3 to be an important explanatory variable (Brown et al. 2009, Clapcott et al. 2012, Waite 2014). In many settings, NH_3 is rarely above detection levels because it is used so rapidly biologically or is transformed to NO_3^-, and thus, the resulting data often do not provide a strong signal. However, in our study, where relatively high NH_3 values occurred across the range of sites, the NH_3 might be toxic or simply a detectable indicator of nutrient enrichment in the streams.

A key objective of our analysis was to test the effects of the measured in-stream stressors (i.e., habitat, nutrients, and contaminants) on invertebrate community health separately from landscape effects. Presumably, alteration of the landscape by human development introduces specific stressors, such as contaminants and habitat alteration, to streams, and these stressors, not the presence or absence of development in the watershed, directly affect stream biota. If we adequately characterized the important in-stream stressors, then models based on those stressors should perform as well as or better than models based on landuse variables to describe differences in stream macroinvertebrate community health. The relevance of these in-stream-stressor models is that they can shed light on which specific aspects of human development are adversely affecting stream health. On the basis of the CV R^2, the Env.All models performed better than the GIS models. Thus, we conclude that the combination of stressors characterized at the stream-reach scale represents invertebrate

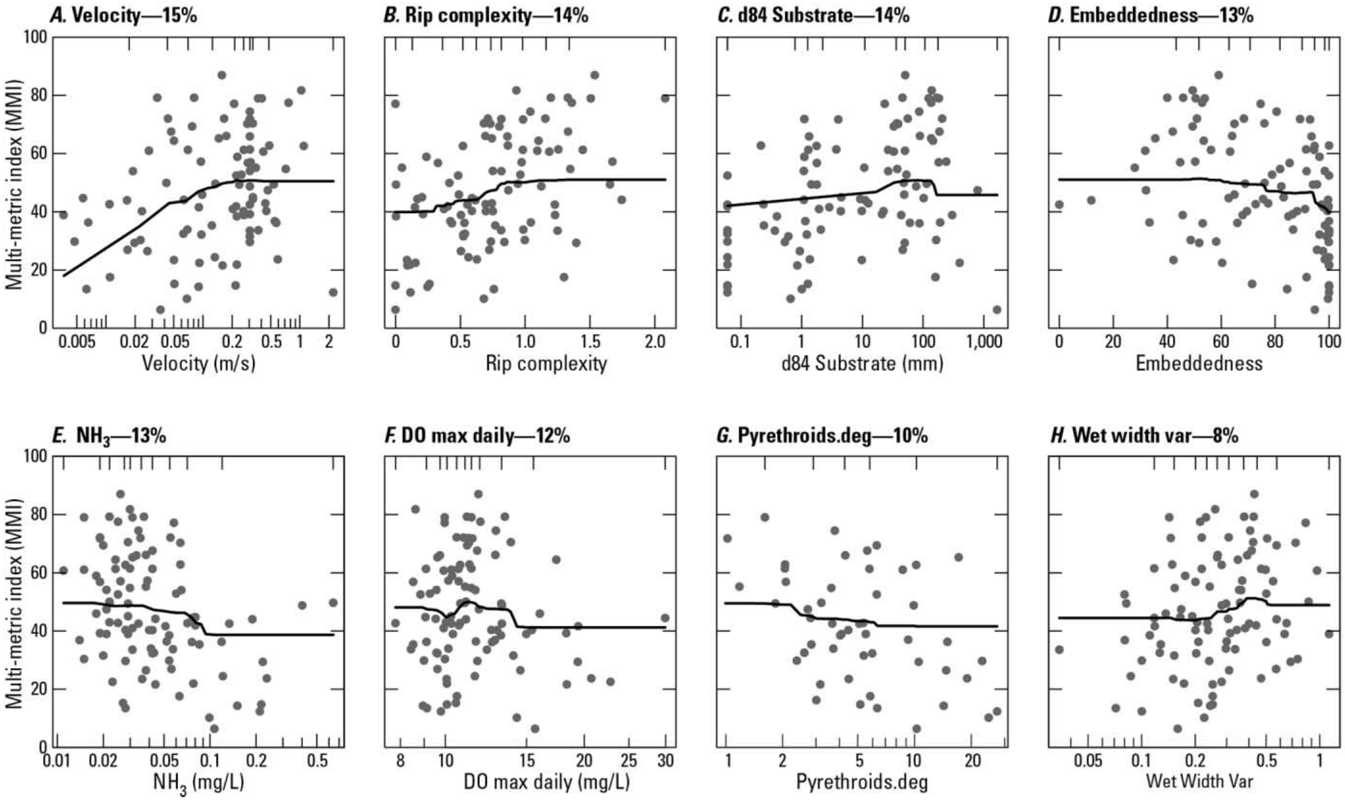

Figure 2. Partial dependency plots for mean velocity (A), riparian (Rip) complexity (B), 84th percentile of substrate diameter (d84 substrate) (C), embeddedness (D), mean NH_3 (E), maximum dissolved O_2 (DO) (F), concentration of pyrethroid degradates (Pyrethroid.deg) (G), and wetted width variability (Wet Width Var) (H), the top variables included in the final boosted regression tree model developed for macroinvertebrate multimetric index (MMI) for 3 stressor categories (data set Env.All). The *y*-axis fitted function represents the effect of the selected variable on MMI. The relative contribution of each explanatory variable is reported as a percentage following the variable name. Each tick mark at the top of each graph represents the 10th percentile of the data.

stressors comparably to or better than the GIS variables. However, 2 Env.All models and all GIS models were improved to various degrees when both GIS variables and Env.All variables were considered together (GIS+Env models; Table 2). This result suggests that even with the extensive quantification of a large number of stressors in the MSQA, we still are not capturing the full breadth of environmental variability affecting the invertebrate community. Presumably, the GIS landuse and landcover variables are acting as surrogates for other factors that directly affect stream health that either were not measured at all or were missed because of non-continuous sampling for many of the in-stream stressors. The relatively poor performance of the GIS models based on the more conservative CV R^2 rather than R^2 suggests that these models might not be appropriate for prediction to unsampled sites. The models probably would predict relatively well in the mid-range of the MMI scores, but either over- or under-predict at low and high MMI values (see Observed vs Prediction; Fig. 4A–C).

Resource managers are unlikely to reverse broad patterns of agricultural and urban development, but knowing which specific chemical and physical stressors associated with development are the most harmful to stream ecology in different settings might provide them with information needed to devise the most effective management strategies. The stressors identified as important explanatory variables for models that included multiple stressor categories (Env.All and GIS+Env) generally were consistent (Table 2). The most important single explanatory variable in 5 of the 8 models was NH_3, which also was included in the final variables in the other 3 models though at lower relative importance. None of the watershed-level landuse variables were important in the models, but a variety of riparian land use and general disturbance variables were, suggesting that riparian management may be critical for stream health.

Habitat variables were most important if importance is measured by total number of variables in a category. The important habitat variables were those describing stream geometry (Sinuosity, Wet Width Var, Wet Width Med), sediment substrate (d84, Coarse Substrate, and Embeddedness), flow (Velocity, Stream Flow), and riparian quality (Rip disturbance and Rip Canopy). Stream geomorphology in this re-

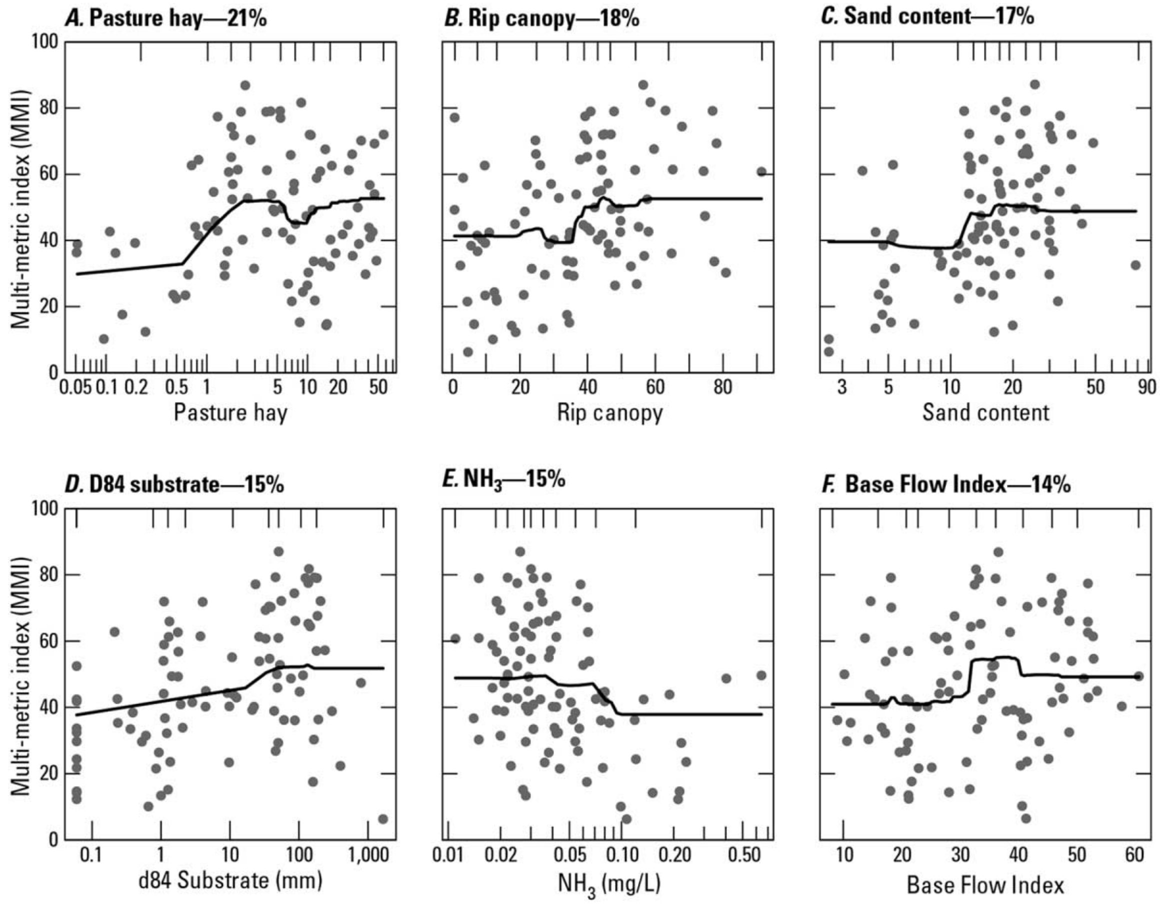

Figure 3. Partial dependency plots for Pasture Hay (A), Rip (riparian) Canopy (B), Sand Content (C), 84th percentile of substrate diameter (d84 substrate) (D), mean NH_3 (E), and base flow index (F), the top variables included in the final boosted regression tree model developed for macroinvertebrate multimetric index (MMI) for 4 stressor categories (data set GIS+Env). The y-axis fitted function represents the effect of the selected variable on MMI. The relative contribution of each explanatory variable is reported as a percentage following the variable name. See Table S1 for variable definitions. Each tick mark at the top of each graph represents the 10th percentile of the data.

gion is strongly influenced by changes to natural stream channels associated with extensive agricultural development and channel modification.

Three contaminant variables were included in the multiple-stressor models (Pyrethroids.deg, 4 Org bs mPECQ [aggregate sediment contaminant measure, see Table S1 for full definition], and sCIBQ 14 [Pesticide Toxic Index]). Of these, Pyrethroids.deg was more important and appeared in 3 of the 8 models (Env.All and GIS+Env). Pyrethroids.deg is the sum of 2 compounds quantified in POCIS samples (cis-cyhalothric acid and 3-phenoxybenzoic acid), which are degradates of bifenthrin, cyhalothrin, tefluthrin, and permethrin, 4 commonly used pyrethroid insecticides. Pyrethroids are highly toxic and have been suggested as important contributors to toxicity in US streams (Kemble et al. 2013, Nowell et al. 2013, Carpenter et al. 2016). We interpret the Pyrethroids.deg variable as an indicator of overall pyrethroid occurrence in the streams. The importance of bifenthrin as a stressor of ecological communities in Midwest streams was recently reported. Rogers et al. (2016) tested the effects of bifenthrin-spiked sediment on invertebrate communities in flowing mesocosm 'streams' and observed a clear dose response at levels of bifenthrin measured in some MSQA stream sediments. They compared the response in the mesocosms to data from MSQA streams and found consistent responses in terms of reduction in selected invertebrate metrics and taxa as bifenthrin concentrations increased.

We modeled 2 subgroups of sites defined on the basis of % fines (< or ≥ 50%) in an effort to reduce the effect of stream geomorphology on the effects of the stressors. The median % fines in the ≥50% subgroup was 89%, but the MMI model still had 4 habitat/geomorphic variables (Wet Width Var, d84 Substrate, Velocity, and Embeddedness) and 1 contaminant variable (Pyrethroid.deg). The median %

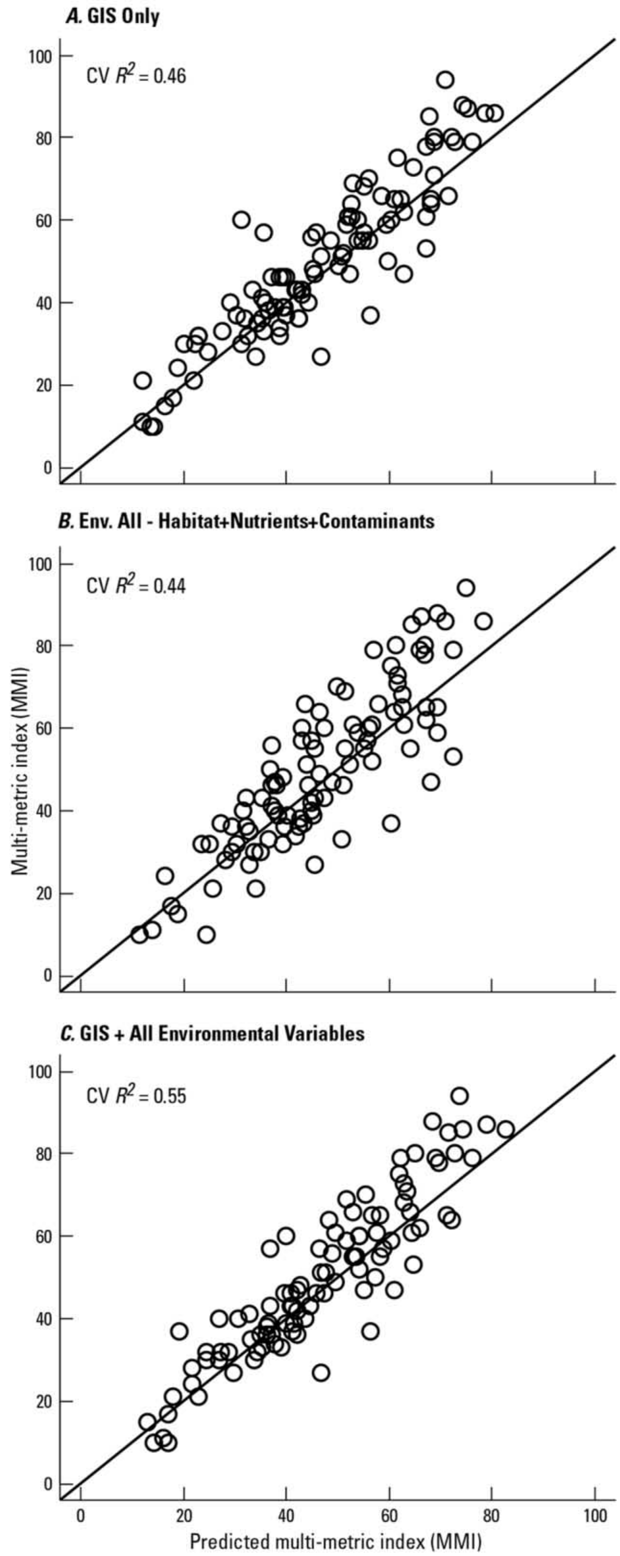

Figure 4. Observed vs predicted plots for boosted regression tree models based on geographic-information system (GIS) stressors only (A), all environmental stressors (Env.All) (B), and all stressors (GIS+Env) for the macroinvertebrate multimetric index (MMI). CV R^2 = cross-validation R^2.

fines in the <50% subgroup was 29%, and the MMI model had only 1 habitat/geomorphic type variable (Riparian Complexity), a water-quality variable (DO Min Daily), and 1 contaminant variable (Pyrethroid.deg). At sites with % fines ≥50%, habitat conditions for invertebrates might be so severely degraded that even minor improvement in substrate, flow, and related geomorphology improves the community. The invertebrate community in this subgroup also might be resistant to additional pressures from other stressors like nutrients and contaminants because it has few sensitive taxa, whereas the invertebrate community in the other subgroup has more sensitive taxa that are more likely to respond to poor water quality and contaminants.

Some of the urban and highly agricultural streams sampled in our study have MMI values indicating good invertebrate condition. This result suggests either that land can be developed in ways that minimize the introduction of ecological stressors or that the MMI is not fully assessing the actual condition of the streams in this region. The importance of several riparian condition metrics in the BRT models (Tables 3, S3) suggests that intact natural buffers can mitigate the adverse effects of development. The mean % developed land in the watersheds (cropland and urban land combined) did not vary much between MMI-condition categories, but the amount of developed and natural area within the riparian buffers did (Table S4). Good MMI-condition streams had ~½ the developed land and 2× the natural land cover in their riparian buffers as did poor MMI-condition streams. Our results suggest that restoration and preservation of intact riparian buffers is a logical strategy for improving stream condition. Many researchers have shown the importance of riparian condition on general stream health, habitat, geomorphology, and water quality (Kiffney et al. 2003, Allan 2004, Death and Collier 2010, Waite et al. 2012, 2014, Feld 2013) and, like us, many have found change points at riparian disturbance as low as 5 to 10% (Carlisle et al. 2009, Maloney et al. 2009, Waite et al. 2010, Brown et al. 2012, Villeneuve et al. 2015).

In summary, the models were generally consistent in the explanatory variables selected within each stressor group across the 4 invertebrate metrics modeled. Variables related to riparian condition, substrate size or embeddedness, velocity and channel shape (natural or channelized, wide or narrow, etc.), nutrients (primarily NH_3), and contaminants (primarily pesticides) were important in describing the invertebrate metrics across the 98 sites in the Midwest Corn Belt region. The model categories that combined the 3 in-stream stressor types and GIS plus the in-stream stressors (Env.All and GIS+Env, respectively) had the best performance. The good performance of the Env.All models indicates that the sampling design did a reasonable job of characterizing important stressors affecting invertebrate communities in the region. Habitat clearly exerts a major influence on invertebrates, but across the range of urban and agricultural land uses in the region, nutrients, contaminants, and natural landscape features (e.g., sand content, base flow index) also clearly influence stream condition.

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