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Complex mixtures of dissolved pesticides show potential aquatic toxicity in a synoptic study of Midwestern U.S. streams

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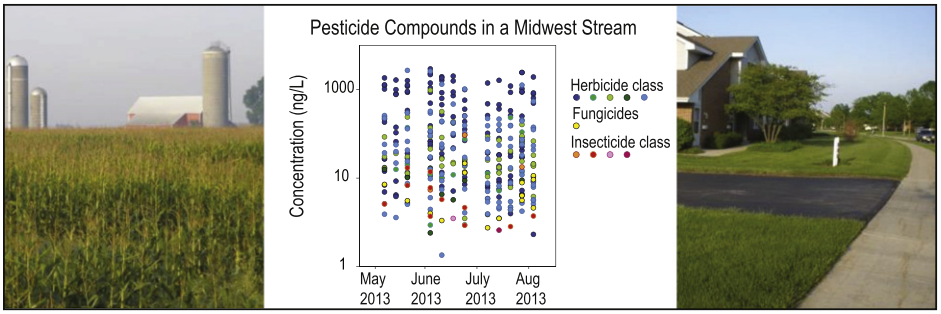
HIGHLIGHTS

- Complex pesticide mixtures detected in 100 Midwestern streams
- Median of 25 pesticides detected in weekly water samples from Midwest streams
- Benchmarks and a Pesticide Toxicity Index used to screen for potential toxicity
- Screening predicted effects on invertebrates and acute, reversible effects on plants.
- Pesticide stressors were significantly related to invertebrate community condition.

GRAPHICAL ABSTRACT



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ABSTRACT

Aquatic organisms in streams are exposed to pesticide mixtures that vary in composition over time in response to changes in flow conditions, pesticide inputs to the stream, and pesticide fate and degradation within the stream. To characterize mixtures of dissolved-phase pesticides and degradates in Midwestern streams, a synoptic study was conducted at 100 streams during May–August 2013. In weekly water samples, 94 pesticides and 89 degradates were detected, with a median of 25 compounds detected per sample and 54 detected per site. In a screening-level assessment using aquatic-life benchmarks and the Pesticide Toxicity Index (PTI), potential effects on fish were unlikely in most streams. For invertebrates, potential chronic toxicity was predicted in 53% of streams, punctuated in 12% of streams by acutely toxic exposures. For aquatic plants, acute but likely reversible effects on biomass were predicted in 75% of streams, with potential longer-term effects on plant communities in

Abbreviations: CA, concentration addition; CAAT, didealkylatrazine; CEAT, deisopropylatrazine; CIAT, deethylatrazine; DAR, deethylatrazine/atrazine concentration ratio; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MoA, mode of action; MRL, method reporting level; MSQA, Midwest Stream Quality Assessment; NAWQA, National Water Quality Assessment; NRSA, National Rivers and Streams Assessment; NWQL, National Water Quality Laboratory; NWQN, National Water Quality Network; OEAT, 2-hydroxy-6-ethylamino-4-amino-*s*-triazine; OIET, 2-hydroxyatrazine; OP, organophosphate; PTI, Pesticide Toxicity Index; QC, quality control; RA, response addition; TM, thiophanate-methyl; TU, Toxic Unit; TUmax, maximum Toxic Unit, i.e., toxic unit for the pesticide that makes the single largest contribution to the Pesticide Toxicity Index; USEPA, U.S. Environmental Protection Agency; USGS, U.S. Geological Survey.

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9% of streams. Relatively few pesticides in water—atrazine, acetochlor, metolachlor, imidacloprid, fipronil, organophosphate insecticides, and carbendazim—were predicted to be major contributors to potential toxicity. Agricultural streams had the highest potential for effects on plants, especially in May–June, corresponding to high spring-flush herbicide concentrations. Urban streams had higher detection frequencies and concentrations of insecticides and most fungicides than in agricultural streams, and higher potential for invertebrate toxicity, which peaked during July–August. Toxicity-screening predictions for invertebrates were supported by quantile regressions showing significant associations for the Benthic Invertebrate-PTI and imidacloprid concentrations with invertebrate community metrics for MSQA streams, and by mesocosm toxicity testing with imidacloprid showing effects on invertebrate communities at environmentally relevant concentrations. This study documents the most complex pesticide mixtures yet reported in discrete water samples in the U.S. and, using multiple lines of evidence, predicts that pesticides were potentially toxic to nontarget aquatic life in about half of the sampled streams.

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1. Introduction

About 70 million kg of pesticides were applied annually during 2000–2013 in the Midwestern U.S. (Baker and Stone, 2015; Stone, 2013). Widespread occurrence of pesticides in Midwestern streams has been reported for decades, especially for triazine and acetanilide herbicides used on corn and soybeans (e.g., Kalkhoff et al., 2003; Scribner et al., 2005; Thurman et al., 1992). However, pesticide use patterns change as new pesticides are registered, new uses are approved, and other uses are discontinued or restricted. On average, 9 new pesticides were applied each year in 11 Midwestern States during 2000–2013 (Baker and Stone, 2015; Stone, 2013). Recent studies have focused on pesticides of toxicological interest and(or) increased use, such as glyphosate (Battaglin et al., 2005; Battaglin et al., 2014; Mahler et al., 2016), fungicides (Battaglin et al., 2011; Reilly et al., 2012), and neonicotinoid insecticides (Hladik and Kolpin, 2015; Hladik et al., 2014; Schaafsma et al., 2015). In 2013, the U.S. Geological Survey (USGS) National Water Quality Assessment (NAWQA) Project investigated the occurrence of dissolved pesticides in weekly water samples collected over a 14-week period (May–August 2013) in 100 wadeable streams in the Midwestern U.S. (Van Metre et al., 2012). This effort was part of the Midwest Stream Quality Assessment (MSQA), a multistressor study conducted by NAWQA in collaboration with the U.S. Environmental Protection Agency (USEPA) National Rivers and Streams Assessment (NRSA) Program. The MSQA study analyzed 228 pesticide compounds in water, substantially expanding on previous NAWQA pesticide assessments during 1992–2001 (83 pesticides) and 2002–2011 (123 pesticides) (Gilliom et al., 2006; Stone et al., 2014a; Stone et al., 2014b).

Pesticides are biologically active and are applied to kill or control targeted pest species. Pesticides are typically observed in the environment as mixtures (Belden et al., 2007a; Gilliom et al., 2006; Moschet et al., 2014; Reilly et al., 2012; Schäfer et al., 2013; Smalling and Orlando, 2011; Smiley et al., 2014; von der Ohe et al., 2009), and they are associated with a range of unintended adverse effects on nontarget stream communities, including direct toxicity; indirect effects on predators, prey, and competitors; and broad-scale effects on community structure and function (e.g., Beketov et al., 2013; Gibbons et al., 2015; Macneale et al., 2014; Peters et al., 2013; Schäfer et al., 2007; Schäfer et al., 2012). The cumulative toxicity of environmental pesticide mixtures has been estimated using various models, including concentration addition (CA) (Bliss, 1939); response addition (RA) (de Zwart and Posthuma, 2005); the maximum Toxic Unit (TUmax) (Liess and von der Ohe, 2005; Schäfer et al., 2011); and a hybrid approach in which CA is applied to chemicals with the same mode of action (MoA) and RA is applied to chemical groups with different MoAs (de Zwart and Posthuma, 2005; Schäfer et al., 2013). Such models typically are based on acute toxicity, such as short-term LC50 values, or (for cladocerans) short-term EC50 values for immobilization. Here we assessed mixtures using the Pesticide Toxicity Index (PTI), which is a CA model (Munn et al., 2006; Nowell et al., 2014). Although CA is strictly appropriate only for compounds with a common MoA, synergism is relatively rare (Cedergreen, 2014), and CA-predicted and observed toxicities typically agree within a factor of 2–3, regardless of MoA (Belden et al., 2007b; Deneer, 2000; Faust et al., 2003; Warne, 2003).

The primary objectives of the present study are to 1) characterize mixtures of currently used pesticides and their degradates in water from Midwestern streams, including weekly temporal changes; 2) screen these mixtures for potential aquatic toxicity by using a combination of aquatic-life benchmark comparisons and the PTI; 3) identify pesticides that are major contributors to potential toxicity; and 4) test screening predictions for susceptible taxonomic group(s) by using multiple lines of evidence.

2. Methods

The MSQA methods are summarized here, with more detail provided in online Supplementary material on the study design and site selection (Appendix A), chemical analysis (Appendix B), potential aquatic-toxicity screening (Appendix C) and benthic invertebrate sampling (Appendix D).

2.1. Study area and site selection

The study area covered parts of 11 Midwestern States, overlying the corn and soybean agricultural region (Fig. 1; Appendix Table A.1). The 100 sampling sites had drainage basins ranging in size from 3 to 2870 km^2 (except for 1 site at 6350 km^2). Of the 100 basins, 88 had land use representing an agricultural gradient (ranging from 3 to 95% cropland, with urban land <8%, in the basin) and 12 had substantial urban influence (ranging from 18 to 88% urban land, with 0–55% cropland in the basin). Agricultural-gradient sites were sub-divided into sites with <20% cropland ("Low Ag" sites) and sites with >25% cropland ("Med-High Ag" sites).

2.2. Water sampling

Twelve water samples were collected at each site over 14 weeks (May 7–August 9) in 2013; samples were collected weekly, except that one sample was collected during each of two 2-week periods (May 27–June 6 and July 1–10). The sampling period spanned typical application periods for herbicides (mid-April to early May), insecticides (which can be applied any time, but generally later in the growing season after infestation occurs), and fungicides (mid-July to early August). Cool wet weather delayed the planting of corn throughout much of the Midwest in 2013, and likely also delayed pesticide application (Van Metre et al., 2016b).

Weekly water samples were depth- and width-integrated (U.S. Geological Survey, 2006). Equipment was rinsed with native water. Verticals were collected along a cross-section and combined in a methanol-

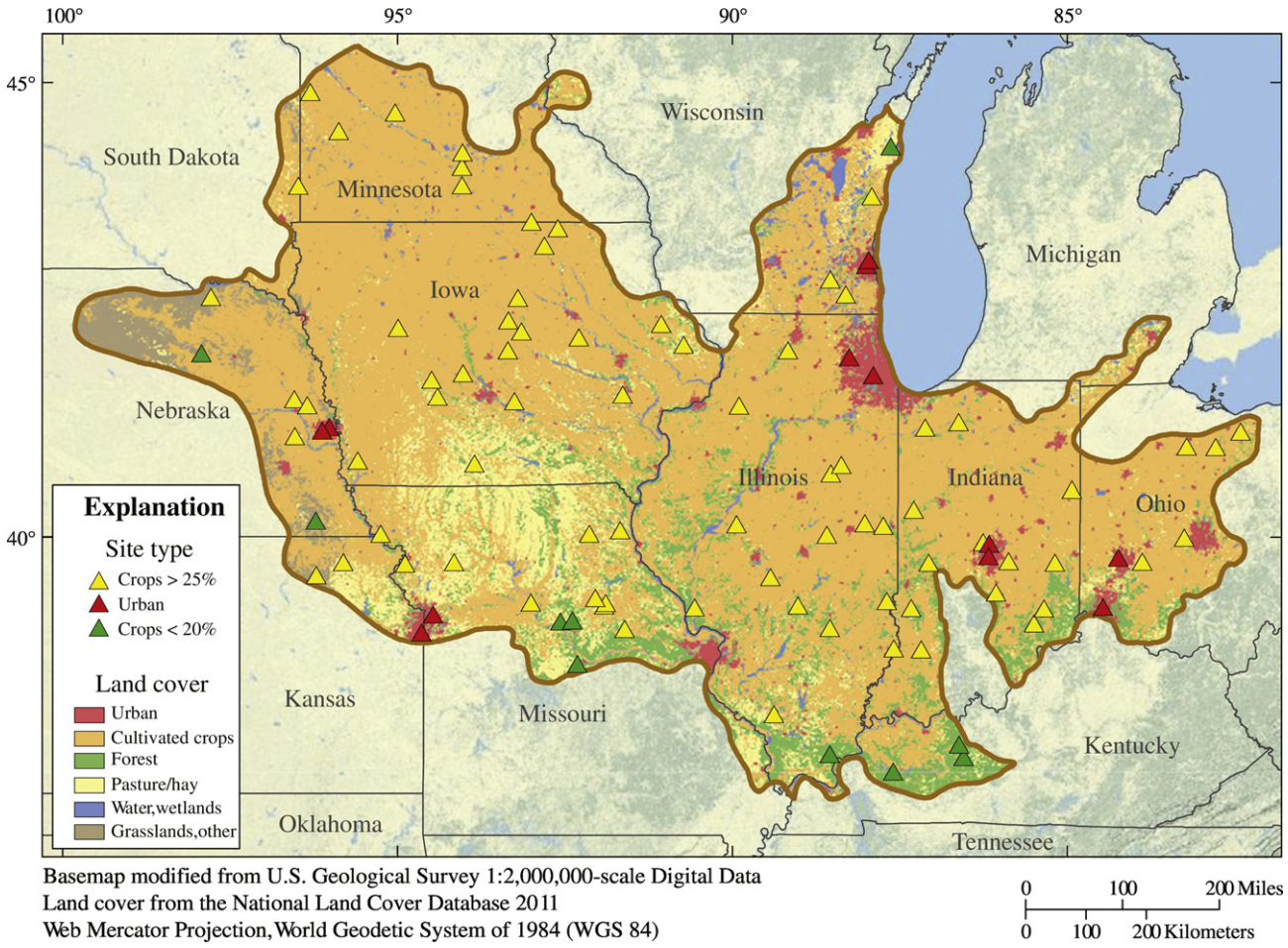

Fig. 1. Sampling sites and land cover in the Midwest Stream Quality Assessment study area.

rinsed Teflon churn-splitter, from which subsamples of the composited water were withdrawn for various analyses. A large-bore syringe and disk filter (0.7 μm) were used to collect 20 mL of filtered sample for analysis of pesticides (Sandstrom and Wilde, 2014). Subsamples were chilled at 4 °C and shipped overnight to the laboratories for analysis.

2.3. Chemical analysis and quality control

2.3.1. Broad-spectrum method for 227 pesticide compounds

A total of 227 pesticide compounds (111 parent pesticides and 116 degradates) were analyzed in filtered water samples at the USGS National Water Quality Laboratory (NWQL) in Denver, CO, using direct-aqueous injection liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Sandstrom et al., 2015). Method reporting levels (MRL) were in the 2 to 500 ng/L range (<50 ng/L for over 80% of analytes). Analytes, MRLs, pesticide class, and (for degradates) parent pesticides are listed in Appendix Table B.1. Target analytes for method development (Sandstrom et al., 2015) were selected by prioritizing pesticides that were frequently detected in past studies, and(or) were expected to be present at concentrations potentially toxic to human health or aquatic life, or were degradates of pesticides that met these criteria (Norman et al., 2012).

Laboratory quality control (QC) at the NWQL consisted of reagent blank and laboratory reagent spike (LRS) samples, as well as the addition of 21 surrogate compounds (isotopically labeled internal standards) to every environmental and QC sample. Field QC consisted of blanks and replicates. Analytical method performance in field matrices was further evaluated on the basis of field matrix spikes from 207 streams and rivers nationwide (prepared during December 2012–September 2015). Results of the QC analyses for the 227 compounds analyzed at the NWQL are summarized here and described more fully in Appendix B.

About 0.6% of MSQA environmental sample results had contamination observed in one or more laboratory blanks analyzed in the same set, so were reported as less than a raised reporting level (e.g., <12 ng/L, for an analyte with a normal MRL of 5 ng/L) or as detections with estimated concentrations (only if the concentration measured in the environmental sample was 3–10 times higher than the highest concentration in the associated laboratory blanks; see Appendix Sec. B.1.2 for more information). In addition, about 10% of results were reported as censored data (i.e., less-than values) with raised reporting levels because of interference, loss of instrument sensitivity, sample dilution, or partial qualitative identification (i.e., some but not all criteria for identifying the analyte were met). Censored results with raised reporting levels were retained in most statistical analyses, where the procedures used could accommodate multiple censoring levels. In some types of data analysis (e.g., calculation of total detection frequencies), censored results with raised reporting levels were dropped from the dataset (i.e., considered to be missing data) if the raised reporting level was greater than the next highest calibration standard above the MRL, because the analysis did not meet the desired laboratory analytical resolution (see Sec. 2.7 and Appendix B.1.2 for more information).

For the 21 isotopically labeled surrogate compounds added to all samples prior to analysis, the mean recoveries ranged from 83% to 124% (Appendix Table B.2). In laboratory and field validation studies (Sandstrom et al., 2015), 19 analytes had variable or biased recoveries in one or two of the matrices tested, so all detected concentrations were reported as estimated. Of these 19 analytes, which are designated

with a validation qualifier code of "E" in Appendix Table B.1, only 3 were frequently detected in this study (flumetsulam, carbendazim and 1H-1,2,4-triazole). Percentile concentrations were not computed for 1H-1,2,4-triazole because over 80% of results were censored with raised reporting levels, and the remaining 20% of results may not be representative. Carbendazim and flumetsulam had 6% and 10% of results (respectively) reported as censored with raised reporting levels; summary statistics were calculated and concentrations were designated as estimated (Appendix Tables E.1, E.2).

Because metolachlor was detected at concentrations below the MRL in 19% of field blanks (Appendix Tables B.3, B.4), metolachlor concentrations in all environmental samples were censored at the MRL. Results for 2,4-D and triclopyr in one environmental sample were deleted because the corresponding field blank showed possible evidence of incidental contamination with these herbicides (Appendix Table B.3), which are commonly used on roadsides and rights-of-way. In 50 paired replicate field samples, the mean relative percent difference (RPD) ranged from <1% to 62%, but was ≤30% for 75 of the 86 analytes with at least 1 pair of detections (Appendix Table B.5).

Field matrix spikes collected for the MSQA study were prepared with a faulty spike solution (see Appendix sections B.1.2, B.1.3), so were considered to underestimate method performance and are not reported here. Instead, method performance was assessed using 289 field spike samples from other NAWQA studies, which were prepared between December 2012, and September 2015, using water from 207 streams and rivers nationwide (Shoda et al., in press; Appendix Table B.6). These field spikes were prepared using the same water sampling and spiking procedures, and analyzed for pesticides using the same LC-MS/MS method as in the MSQA study. These NAWQA field spikes bracket the MSQA study in terms of sampling date, so they provide supplemental information on method performance in surface water field matrices. Median recoveries were between 70% and 130% for 200 of the 221 analytes with field spike data. Three analytes (the degradates diketonitrile isoxaflutole, OEAT, and CAAT) had median recoveries >130% in field spikes, indicating that measured concentrations may be biased high. Four analytes had median recoveries <50% and an additional 14 analytes had median recoveries <70%; both detection frequencies and concentrations of these 18 analytes may be biased low. Because the field spikes prepared from the MSQA water samples were not valid, matrix effects in MSQA streams could not be evaluated in the present study (see Appendix B.1.3 for more information).

2.3.2. Glyphosate method

Glyphosate was analyzed separately by enzyme-linked immunoassay (ELISA) at the USGS Texas Water Science Center in Austin, TX, at an MRL of 200 ng/L (Mahler et al., 2016). To confirm the ELISA results, a subset (20%) of the samples was analyzed for glyphosate by LC-MS/MS at the USGS Kansas Water Science Center in Lawrence, KS, with an MRL of 20 ng/L (Meyer et al., 2009). There was good agreement between the concentrations measured and the temporal patterns described by the two methods (Mahler et al., 2016). For the ELISA analysis, laboratory QC consisted of laboratory blanks, duplicate analyses of control solution provided by the manufacturer, and laboratory spikes; field QC samples consisted of field blanks and replicates. The QC sampling methods and results for glyphosate are published elsewhere (Mahler et al., 2016), but spike samples had a median recovery of 115% and an interquartile range of 89–119%, and replicates had a median RPD of 8% with an interquartile range of 4–13%.

2.4. Geospatial data

Basin land use information for the 100 MSQA sites is provided in Appendix Table A.1. Additional basin characteristics and basin boundaries for MSQA stream sites are available from Nakagaki et al. (2016a, 2016b). Urban land (defined here as the sum of low, medium and high-density developed land cover) and cropland were from the 2011 National Land Cover Database (Homer et al., 2015). Basin-scale estimates of agricultural pesticide use at MSQA sites are provided in Nowell et al. (2017) and correspond to "Epest-High" and "Epest-Low" values for 2013 from Baker and Stone (2014). Use estimates were not available for nonagricultural applications of pesticides in MSQA basins.

2.5. Screening process to assess potential toxicity

Two types of screening tools were used to assess potential toxicity and to identify pesticides with the largest contributions to potential toxicity: (1) USEPA Office of Pesticide Programs (OPP) aquatic-life benchmarks for individual pesticides (U.S. Environmental Protection Agency, 2017a); and (2) the PTI (Nowell et al., 2014), which characterizes potential toxicity of a pesticide mixture towards a particular taxonomic group and provides supplemental information on major contributors to potential toxicity. Appendix C provides more information on screening procedures.

2.5.1. OPP aquatic-life benchmarks for pesticides

The OPP publishes acute and chronic benchmarks for fish and invertebrates, and acute benchmarks for vascular and nonvascular plants; data underlying these benchmarks are extracted from the most recent OPP ecological risk assessments for pesticides and are based on the most sensitive aquatic toxicity data of the distribution for each taxon (U.S. Environmental Protection Agency, 2017a). Appendix Table B.1 lists benchmark values used in the present study. Acute benchmark exceedances were determined by comparing pesticide concentrations in individual MSQA samples with acute benchmarks. To compute chronic benchmark exceedance frequencies and to identify sites with exceedances, the maximum 60-day and 21-day moving average pesticide concentrations at each site were compared with chronic benchmarks for fish and invertebrates, respectively; these exposure durations are consistent with USEPA procedures for computing chronic risk quotients in screening-level risk assessments (U.S. Environmental Protection Agency, 2017b). To evaluate temporal patterns in potential toxicity, however, both chronic and acute benchmarks were compared to concentrations in individual samples over time. For atrazine, an additional chronic threshold for aquatic plant community structure and function (3400 ng/L) was obtained from the USEPA refined risk assessment for atrazine (Farruggia et al., 2016) and compared with the maximum 60-day moving average atrazine concentration. This chronic plant community threshold considers effects on both structure (e.g., biomass, chlorophyll *a*, species composition) and function (e.g., photosynthesis rate as indicated by ^{14}C uptake and O_2 production) of plant communities, based on published microcosm and mesocosm studies; it defines "recovery" as a return to pre-exposure levels for the affected population or community (Farruggia et al., 2016), not as replacement by a more tolerant community with comparable productivity (DeNoyelles et al., 1982; Fairchild, 2011).

Because OP insecticides share a common MoA (acetylcholinesterase inhibition) (Barron et al., 2015), cumulative potential toxicity was evaluated for OPs as a class, by calculating summed acute benchmark quotients (BQ) and summed chronic BQs for invertebrates. Similarly, summed Acute-Plant BQs were calculated for each of four MoA classes of herbicides: triazine (Photosystem II inhibitors), acetanilide (very long chain fatty acid inhibitors), sulfonyl urea (acetolactate synthase inhibitors), and acid and benzoic acid herbicides (synthetic auxins) (Battaglin and Fairchild, 2002; Peterson et al., 2015).

2.5.2. Pesticide Toxicity Index

The PTI was used to assess potential toxicity of pesticide mixtures to fish, cladocerans, and benthic invertebrates (Munn et al., 2006; Nowell et al., 2014). The PTI is a screening-level tool in which the concentration of each pesticide in the mixture is divided by its relative acute toxicity towards a specific taxonomic group, and the resulting toxicity quotients, or PTI toxic units (TU), are summed to get the PTI score for that

taxonomic group. The TU for the pesticide that makes the single largest (maximum) contribution to the PTI is called the TUmax. Toxicity concentrations are based on acute standardized bioassays for individual pesticides (typically 24–48 hour EC50s for *Daphnia magna* and 96-hour LC50s for fish and benthic invertebrates). The PTI scores used here are Sensitive-PTI values, which are calculated using the 5th percentile (or minimum, depending on the available data) toxicity concentrations for each pesticide (Nowell et al., 2014).

PTI scores of 1 and 0.1 were used here to estimate thresholds of predicted acute and chronic toxicity, respectively. The acute toxicity threshold of 1 is a theoretical threshold for a CA model, above which a high probability of acute toxicity is expected because the underlying toxicity values are LC50s. The chronic toxicity threshold (0.1) was obtained by dividing the acute threshold by a safety factor of 10; this is analogous to an acute-to-chronic ratio (ACR) of 10, which is intermediate between ACRs found in other studies (e.g., Mount, 2011; TenBrook et al., 2010). Support for these thresholds also comes from data aggregated from published studies that analyzed pesticides in streams and also measured toxicity to *Ceriodaphnia dubia* exposed to ambient stream water for 4 to 8 days; in the aggregated dataset, ≥50% mortality to *C. dubia* was observed in 89% of samples with a PTI score > 1, and in 19% of samples with a PTI score in the 0.1 to 1 range (Nowell et al., 2014). In the present study, the PTI acute threshold of 1 was applied to mixture PTI scores and to individual pesticide TU values to identify sites or samples or pesticides with potential acute toxicity. To identify sites or pesticides with potential chronic effects, the chronic PTI-TU threshold of 0.1 was applied to time-averaged (60-day for fish and 21-day for invertebrates) TU values for individual pesticides and for the OP insecticide class.

2.6. Benthic macroinvertebrate sample collection and processing

Benthic macroinvertebrate communities were sampled once near the end of the water sampling period (described in more detail in Appendix D). Briefly, samples were collected along 11 equally-spaced transects within the stream reach following protocols from USEPA's NRSA Program (U.S. Environmental Protection Agency, 2013) using a Surber sampler and 500 μm mesh net. Macroinvertebrate samples were processed by the Biological Unit of the NWQL according to methods outlined in Moulton et al. (2000). This involves sorting a sample to a minimum of 300 organisms and assigning taxonomy (generally) to the genus or species level, with quality assurance verified in both the sorting and taxonomic assignment steps by a second person on 10% of the organisms (Moulton et al., 2000).

The Invertebrate Data Analysis System (IDAS) software (Cuffney, 2003) was used to resolve taxonomic issues, remove ambiguous taxa (Cuffney et al., 2007), and generate invertebrate metrics. The raw data of species taxonomy and enumeration are available in the USGS BioData Database (U.S. Geological Survey, 2015). Four commonly used metrics of benthic invertebrate community condition (Moran et al., 2017; Waite and Van Metre, 2017) were used in the present study: Ephemeroptera, Plecoptera, Trichoptera (EPT, which consists of mayflies, stoneflies, and caddisflies) richness; EPT abundance as a percentage of sample total; Ephemeroptera (mayfly) abundance; and a macroinvertebrate multimetric index (MMI), which is based on NRSA methods and includes six individual invertebrate metrics (Stoddard et al., 2008; U.S. Environmental Protection Agency, 2016).

2.7. Statistical analysis

Statistical tests were done in R (version 3.3.1) unless stated otherwise, and results were significant at $p < 0.05$. Differences in the numbers of compounds detected among site groups were tested using the nonparametric Kruskal–Wallis test of association and Tukey multiple comparison test using TIBCO Spotfire SPlus (version 8.1). Maximum likelihood (Tobit) regression methods appropriate for data with censoring, implemented in the 'survreg' procedure using R, were used to evaluate the maximum measured pesticide concentration (log-transformed) observed at a site in relation to agricultural pesticide use and land use within the basin. The Wilcoxon rank sum test was used to evaluate differences in individual pesticide concentration distributions at Agricultural-gradient vs. Urban sites. Summary statistics were computed above two assessment thresholds, >10 and >100 ng/L, using the 'ddply' function in the 'plyr' package in R. In addition, total detection frequencies, which included detections below the MRLs, were computed in R two ways using the 'ddply' function in the 'plyr' package: (1) after dropping results (as missing data) with raised reporting levels greater than the next calibration standard above the MRL, so as to minimally diminish the laboratory analytical resolution; and (2) by retaining all results with raised reporting levels and treating them as nondetections in computing detection frequencies. The former may overestimate total detection frequencies (because some of the samples with raised reporting levels that were dropped from the dataset may truly be nondetections), whereas the latter may underestimate detection frequencies (because the pesticide may be present in some of the samples with raised reporting levels that were counted as nondetections). Percentile concentrations were estimated by retaining raised reporting levels and using the nonparametric Kaplan-Meier method, which is applicable for data with multiple censoring levels (Helsel, 2012), in the 'nada' package in R.

In potential-toxicity screening calculations, all pesticide nondetections (including raised reporting levels) were assumed equal to zero, so the resulting toxicity quotients are minimum estimates. Several pesticide stressors based on the PTI, Chronic-Invertebrate (Chronic-Invert) benchmarks, or individual pesticide concentrations were tested as explanatory variables in quantile regressions with invertebrate community metrics; variations on these stressors were calculated for different time periods (the entire 14-week study period vs. the last 7 weeks) and statistical values (maximum, median, and 21-day moving average values). The 21-day averaging period was again selected for consistency with USEPA procedures for computing chronic-invertebrate risk quotients in screening-level risk assessments (U.S. Environmental Protection Agency, 2017b).

2.8. Quantile regression

For insecticides with the highest detection frequencies—imidacloprid, fipronil, and total fipronil (the sum of fipronil plus 6 degradates, assuming nondetections were equal to zero)—plots of benthic invertebrate community metrics vs. median and maximum pesticide concentrations in water revealed a "classic wedge-shaped" response, indicating that the pesticide variable(s) may be a limiting factor for the invertebrate metrics (Cade and Noon, 2003; Schmidt et al., 2012; Terrell et al., 1996). To test this, quantile regression was performed in R using the 'quantreg' package, with statistical significance testing following the default, quantile-regression sandwich formula using the Hall-Sheather bandwidth rule (Koenker and Machado, 1999). Six quantiles above the median (70th, 75th, 80th, 85th, 90th, 95th) were modeled for each invertebrate metric in relation to various pesticide stressors. Quantiles above the median are less affected by confounding variables (including natural processes and other stressors) and are more likely to be limited by contaminants in the presence of other stressors (Schmidt et al., 2012). Multiple quantiles were tested to assess the robustness of the relation.

The pesticide stressors evaluated included concentrations of selected pesticides that were predicted by screening analysis to have potential invertebrate toxicity: imidacloprid, fipronil, total fipronil, and total OP insecticides (sum of the 39 detected OP pesticides and degradates). Three additional pesticide stressors represented pesticide mixtures, and were based on the Benthic Invertebrate-PTI, Cladoceran-PTI, or, analogously, the summed Chronic-Invert BQ; in each case, toxicity or benchmark quotients were computed and summed for all pesticides detected in a sample mixture, assuming nondetections equal to zero.

2.9. Mesocosm toxicity test

Because screening predicted that the neonicotinoid insecticide imidacloprid was the largest single contributor to potential invertebrate toxicity of dissolved pesticides, a 10-day mesocosm test was conducted with imidacloprid using naïve natural benthic communities (from Cache La Poudre River, Larimer County, CO) at the USGS Fort Collins Science Center in Fort Collins, CO. Methods followed those previously described (Rogers et al., 2016) but are briefly outlined here. Polyethylene trays filled with large gravel were colonized by macroinvertebrates in the Cache La Poudre River. After 75 days, the trays were removed, transported to the laboratory, and placed in one of 22 experimental streams. Experimental streams were 5.7-L buckets equipped with a pump to recirculate water and simulate a riffle environment. Peristaltic pumps continuously delivered one full volume of river water daily with excess water over-topping a standpipe. Invertebrate colonization trays were acclimated for 24 h, after which a continuous dose of imidacloprid was added for the next 10 days. Seven target imidacloprid concentrations (mean concentrations of 60 to 220,000 ng/L) were applied; the low end of this range overlapped with the range of imidacloprid concentrations observed in MSQA streams (2–2160 ng/L). Water samples were collected from each experimental stream on days 3, 7, and 10, and analyzed for dissolved imidacloprid using methods described in Hladik and Calhoun (2012). Briefly, water samples were filtered using baked 0.7-μm nominal pore size GF/F-grade glass-fiber filters, extracted by solid-phase extraction, and analyzed by LC-MS/MS at the USGS Organic Chemistry Laboratory, Sacramento, CA. Concentrations did not decline over the 10 day exposure period and the mean concentration per treatment was used in data analysis (Nowell et al., 2017). As this was a range-finding mesocosm test, treatment concentrations differed by a factor of about 5, and treatments were not replicated.

On day 10 of the experiment, invertebrates were collected by gently dislodging them from substrates and passing all material through a 500-μm sieve. All invertebrates were preserved in ethanol and identified to genus or species (except for Oligochaeta, which were identified to family). Controls were part of a separate but concurrent study (Rogers et al., 2016), which began at the same as the imidacloprid study but ended 20 days after the imidacloprid study ended. While no-observed-effect concentrations were apparent, effects determined relative to controls may be biased low, as controls degrade with time.

3. Results and discussion

3.1. Pesticide occurrence

A total of 183 pesticide compounds (94 parent pesticides and 89 degradates) were detected in one or more samples, consisting of 98 of the 124 targeted herbicide compounds, 71 of the 88 targeted insecticides and 14 of the 16 targeted fungicides (see Appendix Tables E.1, E.2 for summary statistics). For the most commonly detected pesticides and their degradates, Fig. 2 shows total detection frequencies, as well as detection frequencies at two common assessment thresholds (>10 and >100 ng/L). Table 1 lists use information for the parent pesticides shown in Fig. 2, all of which had a detection frequency of at least 50% (for herbicides) or 10% (for insecticides and fungicides) in one or more site types, or were degradates of parents meeting this threshold, or were parents of degradates meeting this threshold.

Pesticide occurrence varied with land use and reflected pesticide use patterns. Herbicides were detected more frequently and generally at higher concentrations than insecticides and fungicides, as has been observed previously (Fuhrer et al., 2004; Gilliom et al., 2006; Moschet et al., 2014). Corn and soybean herbicides, especially atrazine, metolachlor, acetochlor, and their degradates tended to have the highest detection frequencies and concentrations (Fig. 2), consistent with past studies in the Midwest (e.g., Battaglin et al., 2000; Kalkhoff et al., 2003; Thurman et al., 1992). In MSQA, the concentrations of atrazine, metolachlor, and acetochlor were significantly higher ($p < 0.001$) at Agricultural-gradient sites than Urban sites (Appendix Fig. E.1, Table E.3), and their maximum concentrations were related to their estimated agricultural use intensity (in kg/km^2), and to the percentage of cropland, in the basin ($p < 0.001$; Appendix Table E.4). Concentrations of these herbicides were higher during the first half of the study period (Appendix Fig. E.2). The median concentration ratio of the degradate CIAT to its parent atrazine (also called the deethylatrazine/atrazine ratio, or DAR) was lowest in late May, with values dropping to ≤0.1 at the majority of sites (Appendix Fig. E.3). The timing of atrazine, metolachlor, and acetochlor occurrence (highest during late May to late June), and the DAR values of <0.1 observed during the first 3 weeks of the study period, are consistent with a spring flush mechanism, in which high concentrations are transported to streams from croplands after late spring and early summer rainfall (Thurman and Fallon, 1996). The observed increase in the DAR after week 8 (late June) at Agricultural-gradient sites is consistent with decreased transport of atrazine to streams and continued degradation later in the study period. Other herbicides frequently detected at Agricultural-gradient sites were dimethenamid and its degradates, sulfentrazone, propazine, 2,4-D, prometon, and glyphosate. Maximum concentrations were significantly related to the percentage of cropland in the basin for propazine ($p < 0.01$), to estimated agricultural use intensity for sulfentrazone ($p < 0.001$), and to both for dimethenamid ($p < 0.01$) and glyphosate ($p < 0.01$) (Appendix Table E.4).

Surprisingly, propazine was detected in about 70% of samples at Agricultural-gradient sites, despite its negligible agricultural use (<0.01 kg/km^2) within the study area in 2013 (Baker and Stone, 2014). However, propazine concentrations were linearly related to atrazine concentrations within the same sample in the present study ($R^2 = 0.95$; Appendix Fig. E.4) and also in a 2012 national-scale study that used the same analytical method (Martin et al., in press), with a common slope (propazine/atrazine mass ratio) of 1% in both studies. Both atrazine and propazine concentrations were much higher in MSQA than in the national study (Appendix Fig. E.4). The frequent occurrence of propazine in agricultural samples from MSQA streams is hypothesized to result from the application in the study area of atrazine formulations containing propazine as an impurity (World Health Organization, 1990).

The herbicides 2,4-D, glyphosate, and prometon were detected at higher concentrations ($p < 0.001$) and more often at Urban than Agricultural-gradient sites (Fig. 2; Appendix Fig. E.1 and Table E.3). Maximum concentrations were significantly related to the percentage of urban land in the basin for 2,4-D ($p < 0.05$) and prometon ($p < 0.001$) (Appendix Table E.4). Glyphosate, which is one of the most heavily used pesticides in both agricultural and nonagricultural applications within the U.S. (Baker and Stone, 2015; Grube et al., 2011), has a higher MRL in the present study (200 ng/L by the ELISA method) than most other herbicides (over 70% have MRLs <20 ng/L; Appendix Table B.1). Compared at a common assessment threshold of 200 ng/L, glyphosate occurrence

Fig. 2. Detection frequencies for the most commonly detected pesticides in MSQA streams, and their degradates, as a function of land use and assessment threshold. Pesticides shown had a detection frequency of at least 50% (for herbicides) or 10% (for insecticides and fungicides) in one or more site types, or were parents of degradates meeting this threshold. Degradates are listed after the parent pesticide, with pesticides in boldface type and degradates indicated with a (D). Asterisk indicates that compound was not analyzed in previous NAWQA pesticide assessments. The dark and intermediate portions of each bar indicate detection frequencies above common assessment thresholds of >100 and >10 ng/L, respectively. The total bar length represents the total detection frequency, except that censored data with reporting levels raised above the next highest calibration standard from the MRL were dropped as missing data, and metolachlor was censored at its MRL because of low-level contamination in some field blanks (Sec. 2.3.1). To compare detection frequencies among compounds, a common assessment threshold should be used. For compounds with reporting levels >100 ng/L, detection frequencies >10 and >100 ng/L thresholds cannot be determined, so only the total detection frequency (lightest portion of bar) is shown.

at Urban sites (detected in 63% of samples) was similar to that of 2,4-D (68%) and much higher than that of prometon (1%), which was the next most frequently detected nonagricultural herbicide; at Agricultural-gradient sites, glyphosate occurrence (41% of samples) was intermediate between that of atrazine (57%), metolachlor (32%) and acetochlor (16%). For comparison, in a subset of MSQA water samples that were

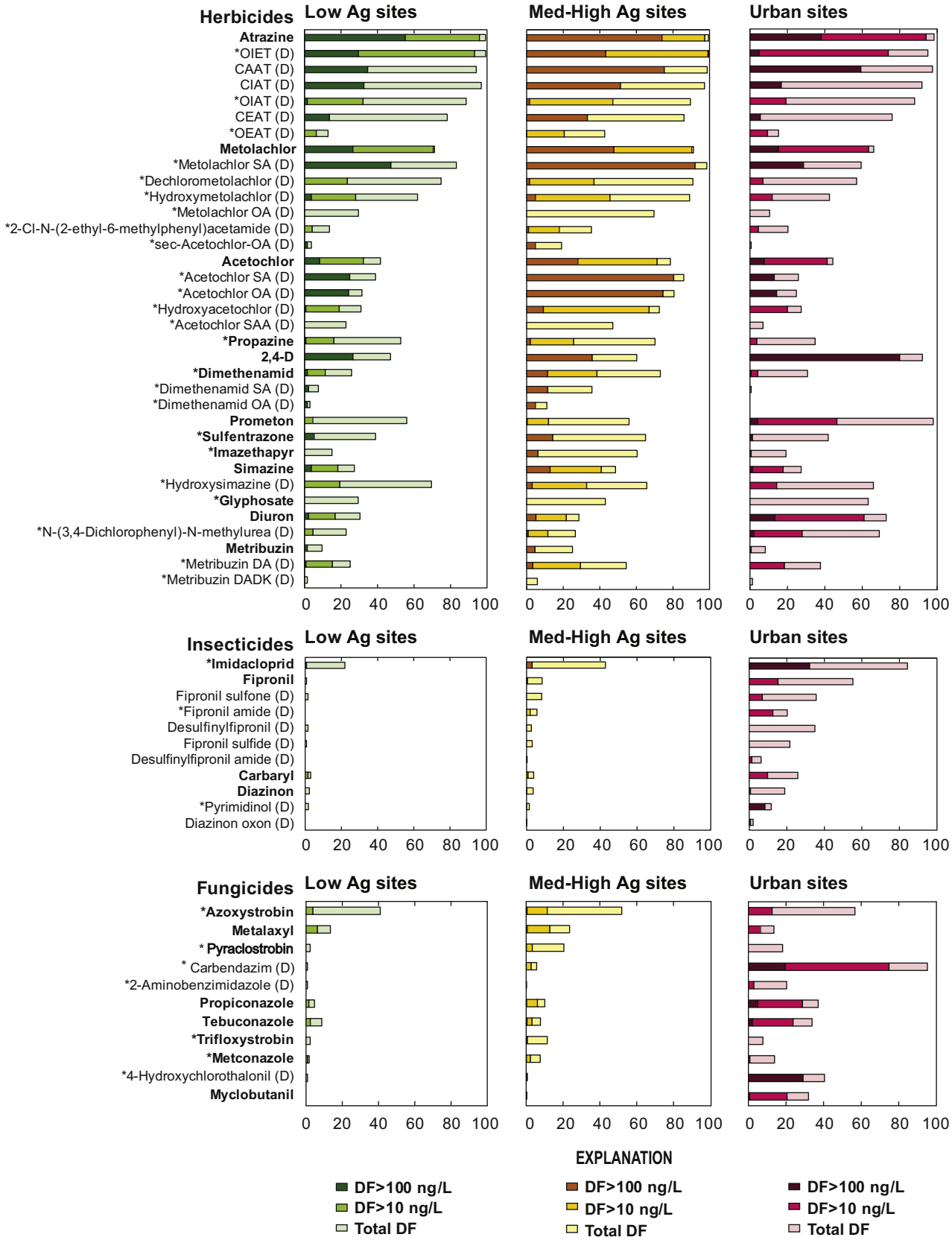

Table 1
Agricultural and nonagricultural use information for the most frequently occurring pesticides in MSQA streams. These pesticides (also shown in Fig. 2) had a detection frequency of at least 50% (for herbicides) or 10% (for insecticides and fungicides) in one or more site types, or were parents of degradates meeting this threshold.

Pesticide	Use group[a]	Chemical class	Agricultural use intensity in study area, 2013 (kg/km^2)[b]	Major agricultural uses in 11 Midwest States, 2013[a,b,c]	Nonagricultural uses in national use profile[d]
2,4-D	H	Acid	5.9	C, S, Wh, Pas, Other	Yes
Acetochlor	H	Acetanilide	21.6	C, S	Yes
Atrazine	H	Triazine	22.3	C, Other	Yes
Azoxystrobin	F	Strobiluron	0.30	C, S, Wh, Veg	Yes
Carbaryl	I	Carbamate	0.013	Veg, Alf, Other, Orch	Yes
Carbendazim	F, FD	Benzimidazole	0.0070[e]	Veg, Other, Orch[e]	Yes[e]
Diazinon	I	Organophosphate	NA[f]	([f])	Yes
Dimethenamid	H	Amide	2.06[g]	C, Other (Veg)	
Diuron	H	Urea	0.00071	Alf, Cot (Orch, Other)	Yes
Fipronil	I	Phenylpyrazole	NA[f]	([f])	Yes
Glyphosate	H	Organophosphate	72.8	S, C (Other, Pas, Wh)	Yes
Imazethapyr	H	Miscellaneous	0.18	S, C (Rice, Veg)	Yes
Imidacloprid	I	Neonicotinoid	0.41	S, Wh (Veg, Other, Cot)	Yes
Metalaxyl	F	Phenylamide	0.077	S (Veg, Wh)	Yes
Metconazole	F	Triazole	12.4	C, Wh	Yes
Metolachlor	H	Acetanilide	17.2[h]	C, S, Other (Veg)	Yes
Metribuzin	H	Triazine	0.53	S,C (Veg, Wh, Alf)	Yes
Myclobutanil	F	Conazole	NA[f]	([f])	Yes
Prometon	H	Triazine	NA[f]	([f])	Yes
Propazine	H	Triazine	0.0000088	Other	
Propiconazole	F	Triazole	0.25	C, Wh, S (Rice, Veg, Other)	Yes
Pyraclostrobin	F	Strobiluron	0.73	C, S, Wh (Other, Veg)	Yes
Simazine	H	Triazine	2.8	C (S)	
Sulfentrazone	H	Sulfonyl urea	0.78	S, Other (C)	Yes
Tebuconazole	F	Triazole	0.084	Wh, S, C	Yes
Trifloxystrobin	F	Strobilurin	0.25	C, S, Wh (Other)	Yes

[a] Alf, alfalfa; C, corn; Cot, cotton; F, fungicide; H, herbicide; I, insecticide; Orch, orchards and grapes; S, soybeans; Veg, vegetables and fruit; Wh, wheat. Crops accounting for >5% of use are listed in order of relative use, with crops accounting for 1–4% in parentheses.
[b] From Baker and Stone (2014).
[c] Midwestern States are those partly included in MSQA study area: Illinois, Indiana, Iowa, Kansas, Kentucky, Minnesota, Missouri, Nebraska, Ohio, South Dakota, Wisconsin.
[d] Details and sources are provided in Appendix Table B.7.
[e] Carbendazim is both a fungicide and a major degradate of thiophanate-methyl (TM). The agricultural use estimate in the MSQA watersheds applies to TM. Both carbendazim and TM have nonagricultural applications nationally (U.S. Environmental Protection Agency, 2005).
[f] NA, negligible use in study area. No use was reported in the study area, i.e., for the crops and Crop Reporting Districts surveyed, and corresponding to the watershed boundaries of MSQA stream sites.
[g] Sum of dimethenamid and dimethenamid-P uses.
[h] Sum of metolachlor and *S*-metolachlor uses.

analyzed for glyphosate by LC-MS/MS at an MRL of 20 ng/L (Mahler et al., 2016), the glyphosate detection frequency was 87%.

Insecticides were detected more often and typically at higher concentrations at Urban than Agricultural-gradient sites (Fig. 2), as reported previously (Carpenter et al., 2016; Domagalski, 2000; Ensminger et al., 2013; Gilliom et al., 2006). Imidacloprid was the most frequently detected insecticide at all MSQA site types, followed at Urban sites by fipronil and its degradates. Imidacloprid was used on crops, including soybeans (Baker and Stone, 2015; Stone, 2013), as well as in forestry, on turf and ornamentals, and to control termites (U.S. Environmental Protection Agency, 2014a). At Urban sites, imidacloprid concentrations tended to peak during the last 3 weeks of the study period (late July-early August). Imidacloprid concentrations were significantly higher at Urban than Agricultural-gradient sites ($p < 0.001$) (Appendix Fig. E.1, Table E.3), and maximum concentrations were significantly related to the percentage of urban land in the basin ($p < 0.001$), but not to agricultural use intensity or percentage of cropland (Appendix Table E.4). Nonetheless, the agricultural use of imidacloprid probably contributed to detections in MSQA streams, as evidenced by the higher concentrations and detection frequencies at Med-High Ag sites (where it was detected in 43% of samples) compared to Low Ag sites (in 22% of samples). In a previous study of neonicotinoids in Iowa agricultural streams, imidacloprid was detected in 23% of water samples (Hladik et al., 2014), although this study had a lower MRL for imidacloprid (2 ng/L) than in the present study (11 ng/L).

Fipronil and several degradates were detected at Urban sites throughout the study period (Appendix Fig. E.2). Fipronil was not used in agriculture within the MSQA study area in 2013 (Baker and Stone, 2015), and 95th percentile concentrations of fipronil and degradates at Agricultural-gradient sites were <MRLs. Nonagricultural uses of fipronil include subterranean termite control and broadcast and bait treatments for ants (U.S. Environmental Protection Agency, 2007). Fipronil concentrations were significantly higher at Urban than Agricultural-gradient sites ($p < 0.001$) (Appendix Fig. E.1, Table E.3) and maximum concentrations were related to the percentage of urban land in the basin ($p < 0.001$) (Appendix Table E.4).

OP insecticides were detected sporadically throughout the study period at both Urban and Agricultural-gradient sites (Appendix Fig. E.5). Of the 49 OP compounds analyzed, 39 were detected—5 OPs and 3 degradates each were detected in 1–6% of samples, with the remainder observed in <1% of samples. OP compounds detected more often at Urban sites include 2-isopropyl-6-methyl-4-pyrimidinol (a degradate of diazinon), diazinon, acephate, and dichlorvos (which is both an insecticide registered for nonfood, greenhouse, and domestic indoor and outdoor uses, and a major degradate of naled). Other OPs used on corn and(or) soybeans (terbufos, tebupirimfos, chlorpyrifos; U.S. Geological Survey, 2016) and their degradates were detected rarely, but primarily at Agricultural-gradient sites (Appendix Tables E.1, E.2). The temporal distribution of OP detections varied by compound (Appendix Fig. E.5).

The majority of fungicides showed higher occurrence at Urban than Agricultural-gradient sites in the present study (Fig. 2; Appendix Tables E.1, E.2), which represents the first regional-scale characterization of fungicides in urban streams in the U.S. A notable exception was azoxystrobin, the most commonly detected fungicide overall; used on both turf and various crops, it was detected at comparable frequencies (Fig. 2) and concentrations (Appendix Fig. E.1, Table E.3) at Urban and

Agricultural-gradient sites. However, the maximum azoxystrobin concentration at all sites was significantly related to agricultural use intensity ($p < 0.01$) and to the percentage of cropland ($p < 0.05$) in the basin (Appendix Table E.4). Concentrations generally peaked during the last 3 weeks of the study period (late July-early August) (Appendix Fig. E.2).

Carbendazim was the most frequently detected fungicide at Urban sites, observed in between 83 and 95% of all urban samples (depending on how raised reporting levels were handled, as described in Sec. 2.3.1). Carbendazim is used as a fungicide in paints, adhesives, building materials, textiles, and other products, and it also is a major degradate of thiophanate-methyl (TM), which is used on turf and ornamentals (U.S. Environmental Protection Agency, 2005). TM had very low agricultural use in MSQA stream basins in 2013 (Baker and Stone, 2014). Carbendazim concentrations were significantly higher at Urban than Agricultural-gradient sites ($p < 0.001$) (Appendix Fig. E.1, Table E.3), and the maximum concentrations were related to the percentage of urban land in the basin ($p < 0.001$) (Appendix Table E.4). Carbendazim concentrations generally peaked during or after week 8 (late June) at Urban sites (Appendix Fig. E.2). Other fungicides detected more often at Urban sites also have nonagricultural applications (propiconazole, tebuconazole, and myclobutanil; Table 1) or are degradates of fungicides with nonagricultural applications (4-hydroxychlorothalonil).

3.2. Mixtures

Mixtures of 2 or more pesticide compounds were observed in all but 1 of the 1197 water samples (99.9%). The median number of pesticide compounds detected was 25 compounds per sample (range 1–62), and 54 compounds per site (range 24–79). Even the least developed (Low Ag) sites, which had <8% urban and <20% cropland in the basin, had a median of 15 pesticide compounds detected per sample (Appendix Fig. E.6). The Med-High Ag sites had the highest median number of herbicide compounds detected, whereas Urban sites had the highest median numbers of insecticide and fungicide compounds; the Low Ag sites had significantly fewer compounds detected than the other site types for herbicides, insecticides, and fungicides (Appendix Fig. E.6).

The mixtures detected in MSQA streams are more complex than previously reported for dissolved pesticides in discrete water samples. Past studies have reported a median of 10–13 or fewer pesticide compounds per sample in agricultural or mixed land-use streams in the U.S. (Belden et al., 2007a; Da Silva, 2016; Reilly et al., 2012; Smiley et al., 2014; Starner, 2011), Sweden (Gustavvson et al., 2017), Europe and Australia (Schäfer et al., 2013). Smiley et al. (2014) observed that mixtures of <10 pesticides tend to occur frequently in agricultural streams, regardless of the number of pesticides targeted.

The large number of pesticide compounds detected and the resulting mixture complexity in MSQA stream samples result from a combination of the intensive use of pesticides in the Midwest; the large number of compounds targeted in the present study, including degradates; and the sensitivity of the analytical method used. A total of 135 pesticide analytes (48 active ingredients and 87 degradates) targeted in MSQA were not included in previous national NAWQA assessments (Gilliom et al., 2006; Stone et al., 2014b), as designated in Appendix Tables E.1 and E.2. Targeting degradates also increased the complexity of the detected mixtures. MSQA streams had a median of 11 parent pesticides and 14 degradates detected per sample, and a median of 27 parents and 26 degradates detected per site during the study period. Many more degradates were analyzed in MSQA discrete water samples (116 degradates) than in most past pesticide monitoring studies. For example, the number of degradates out of the total number of pesticide analytes targeted was 8 of 83 analytes in Gilliom et al. (2006); 1 of 13 analytes in Smiley et al. (2014); 7 of 44 in Starner (2011); 10 of 141 in Gustavvson et al. (2017); and at least 7 of the 103 pesticides and industrial compounds detected by Schäfer et al. (2013). Herbicide degradates observed here, and in past studies of Midwestern streams, include the sulfonic and oxanilic acid degradates of acetanilide herbicides and atrazine degradates CAAT, CIAT, OIET, and CEAT (Battaglin et al., 2005; Kalkhoff et al., 2003; Thurman et al., 1992). The atrazine degradates OIAT, OEAT, OIET, and 21 acetanilide or amide herbicide degradates are new analytes in the NAWQA project as of 2013 (Appendix Tables E.1, E.2). Many of these herbicide degradates, the fungicide/degradate carbendazim, and several degradates of fipronil were among the most frequently detected pesticide compounds at some MSQA site types (Fig. 2).

Analytical sensitivity is critical because detection frequencies, and therefore perceived mixture complexity, tend to increase as MRLs are lowered. For example, the median number of unique compounds detected per sample (25) and per site (54), when all detections were counted, decreased to 17 and 37, respectively, when only compounds detected at concentrations >10 ng/L were considered.

Detection frequencies and mixture complexity are expected to be higher in composite samples that integrate pesticide exposure over multiple weeks than in discrete samples. For example, complex mixtures of 30–50 pesticide compounds per sample were previously observed in two-week, time-proportional composite samples collected from five Swiss streams using automated samplers (Moschet et al., 2014); these authors screened for 249 pesticides and 134 degradates. Similar results were seen in a study of pesticides in Polar Organic Chemical Integrative Sampler (POCIS) extracts from MSQA streams (Van Metre et al., 2016a), which was conducted concurrently with the present study and used the same LC-MS/MS analytical method (targeting 111 pesticides and 116 degradates); a median of 62 pesticide compounds (range of 28–85) were detected in composite samples that integrated exposure over a 5-week period, compared to a median of 46 compounds detected per site in the weekly discrete water samples bracketing the 5-week sampler deployment.

3.3. Potential toxicity screening

3.3.1. Aquatic-life benchmark comparisons

Concentrations of at least one compound exceeded one or more aquatic-life benchmarks at 81% of sites. The most frequently exceeded benchmarks were Acute-Nonvascular Plant benchmarks (exceeded in one or more samples at 74% of sites), followed by Chronic-Invert benchmarks (exceeded by 21-d average concentrations at 46% of sites). Exceedance rates by site for the other benchmarks were much lower: Acute-Fish (0% of sites), Acute-Invertebrate (Acute-Invert) (8%), Acute-Vascular Plants (11%), Chronic-Fish (5%) benchmarks, and the chronic plant community threshold for atrazine (9%). One or more Acute-Plant benchmarks (either Acute-Vascular or Acute-Nonvascular) were exceeded at 75% of sites.

The temporal distribution of aquatic-life benchmark exceedances by pesticides in individual samples varied by land use and taxonomic group, reflecting the different pesticides responsible. Predicted toxicity to plants was highest during late May-late June at both Agricultural-gradient and Urban sites (Fig. 3). Although potential plant toxicity was much higher in magnitude at Agricultural-gradient than Urban sites, the timing was similar because the spring-flush herbicides were largely responsible at both site types (Fig. 4). Specifically, the compounds responsible for Acute-Plant benchmark exceedances were triazine (at 73% of sites), acetanilide (19% of sites), acid (5%), and sulfonylurea (3%) herbicides.

Chronic-Invert benchmark exceedances for individual samples tended to occur later in the study period at Urban sites (weeks 12–14, late July-early August) compared to Agricultural-gradient sites (weeks 3–8, late May-late June; Fig. 3). At Urban sites, the compounds responsible for Chronic-Invert exceedances were largely insecticides, especially imidacloprid, OPs, and fipronil (Fig. 4B). Concentrations of imidacloprid were higher during the latter half of the study period (July–August); high OP and fipronil concentrations occurred throughout the study period (Appendix Figs. E.2, E.5), although more than half of OP benchmark exceedances occurred during the last 3 weeks (late

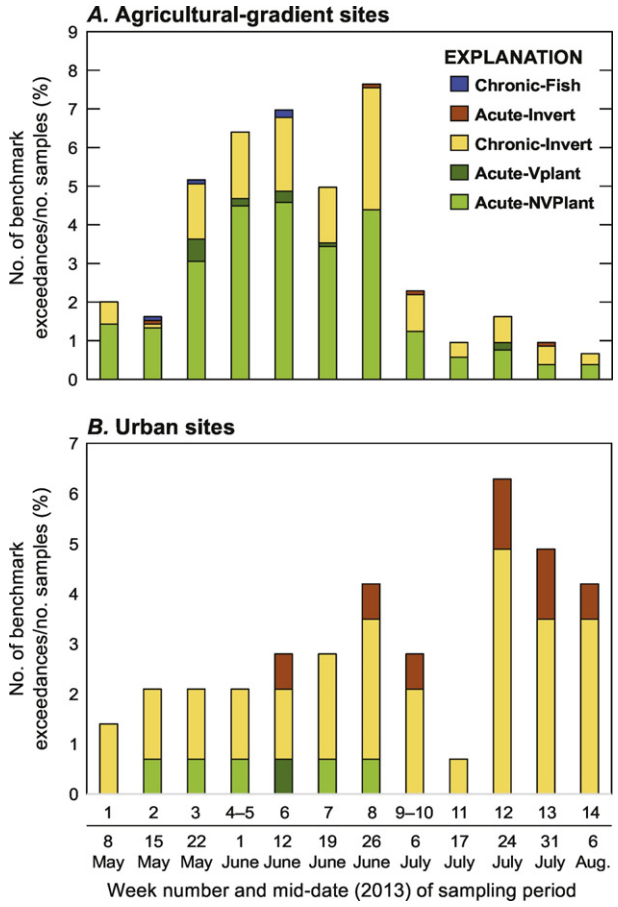

Fig. 3. The temporal distribution of aquatic-life benchmark exceedances by pesticides in individual samples at (A) Agricultural-gradient sites and (B) Urban sites. Invert, invertebrate; NVPlant, nonvascular plant; VPlant, vascular plant.

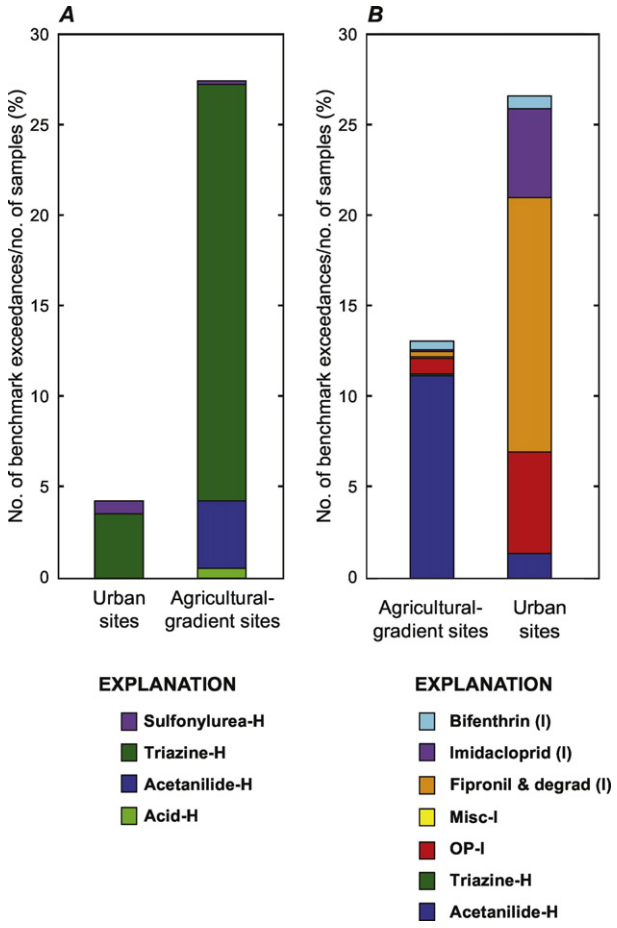

Fig. 4. The distribution by land use of (A) acute plant exceedances and (B) chronic invertebrate exceedances by pesticides in individual samples. Degrad, degradates; H, herbicide; I, insecticide; Misc, miscellaneous; OP, organophosphate. Atrazine makes up the largest share of the Triazine-H contribution, and metolachlor the largest share of the acetanilide-H contribution.

July–early Aug). In contrast, the majority of Chronic-Invert exceedances at Agricultural-gradient sites were due to the acetanilide herbicide metolachlor (Fig. 4B), for which concentrations peaked during weeks 4–8 (late May–June; Appendix Fig. E.2) as part of the spring flush.

Acute-Invert benchmarks were exceeded by OP insecticides at 4 Urban and 3 Med-High Ag sites and by the insecticide diflubenzuron at 1 Med-High Ag site. Atrazine exceeded the Chronic-Fish benchmark (for fish reproduction) at 5% of sites, and the chronic plant community threshold at 9% of sites, all Med-High Ag sites.

3.3.2. Pesticide Toxicity Index

The PTI was used to assess potential acute toxicity of pesticide mixtures to fish and invertebrates, and to identify key individual compounds with the largest TU values. As an illustration, the 12 weekly samples from West Papillion Creek, an Urban site, each contained a complex mixture of 21–43 compounds (Fig. 5A). After concentrations were normalized by relative toxicity to benthic invertebrates in computing the Benthic Invertebrate-PTI (Fig. 5B), two compounds in these complex mixtures (imidacloprid and the OP insecticide/degradate dichlorvos) dominated the Benthic Invertebrate-PTI, illustrating that the major contributors to potential invertebrate toxicity were not necessarily the compounds that occurred most frequently or at the highest concentrations (typically herbicides).

The maximum Fish-PTI score was <0.1 at 97 of the 100 sites, indicating low potential toxicity to fish at the majority of sites. At the remaining 3 sites, one sample at each site contained either acetochlor (at 2 Med-High Ag sites) or carbendazim (at 1 Urban site) with TU values >0.1. Although the Fish-PTI was low relative to potential toxicity thresholds, it tended to be higher at Urban sites (Fig. 6). Temporal patterns in the Fish-PTI differed by land use and reflected the temporal occurrence of the major pesticides contributing to the PTI. At Agricultural-gradient sites, the pesticides responsible for the Fish-TUmax were usually herbicides (77% of samples), especially acetochlor (34% of samples) and atrazine (20%); the Fish-PTI was highest in late May–June, corresponding to high spring-flush herbicide concentrations. At Urban sites, the fungicide/degradate carbendazim was responsible for the TUmax in 83% of samples, and both Fish-PTI scores (Fig. 6) and carbendazim concentrations (Appendix Fig. E.2) tended to be highest in late July–August.

For invertebrates (either cladocerans, benthic invertebrates, or both), the maximum PTI score exceeded the acute threshold (PTI > 1) at 11 of the 100 sites at some point during the study period. At 2 of these 11 sites, the PTI score exceeded the >1 threshold only after summing individual TU values for pesticides in the sample mixture, whereas 9 sites had a TUmax >1 for one or more pesticides, indicating acutely toxic levels of these individual pesticides. Proportionally more Urban sites (33%) had PTI > 1 than did Agricultural-gradient sites (8%). An additional 24 sites had one or more pesticides with a maximum 21-day average concentration exceeding the PTI-TU threshold of 0.1 for benthic invertebrates and(or) cladocerans, indicating potential chronic effects at these sites. The temporal distributions of both Cladoceran- and Benthic Invertebrate-PTI scores showed similar relations with land use, with PTI scores higher during weeks 4–8 (late May–June) at Agricultural-gradient sites and during weeks 12–14 (late July–early August) at Urban sites (Fig. 6).

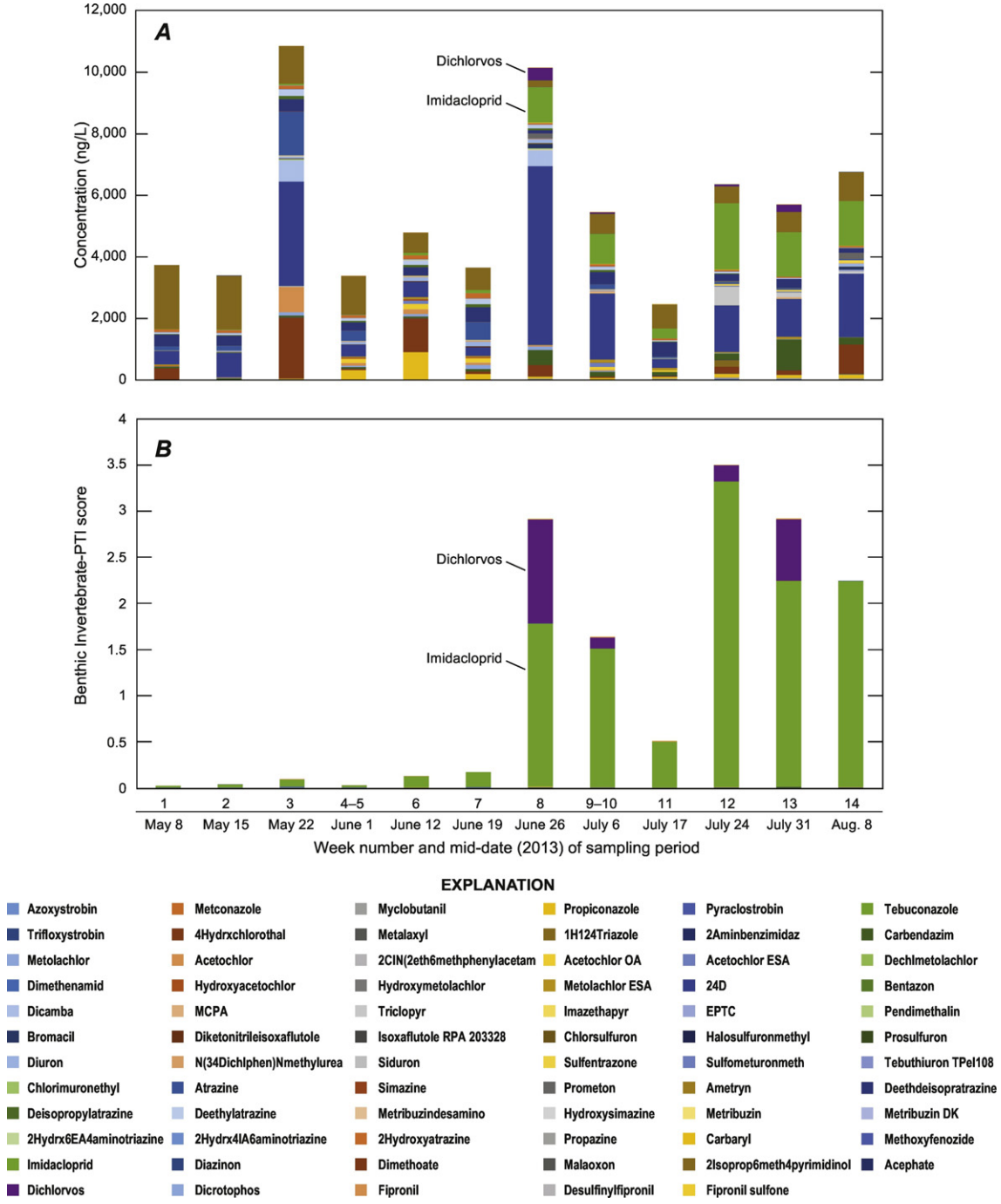

Fig. 5. Pesticide mixtures in 12 weekly water samples at an example Urban site, West Papillion Creek. Each stacked bar represents, for one weekly water sample: (A) the summed concentrations (ng/L) of all pesticide compounds detected and (B) the Benthic Invertebrate-PTI, in which each concentration has been normalized by that pesticide's acute toxicity to benthic invertebrates. Each segment represents the contribution of an individual pesticide compound.

The PTI scores for all taxonomic groups were dominated by a single compound in most individual samples with potential toxicity (i.e., samples with PTI scores >0.1). For both the Fish-PTI and Cladoceran-PTI, the TUmax comprised a smaller proportion of the PTI score when the PTI score was low (<0.001)—that is, multiple compounds contributed to the PTI but no single compound dominated. However, that proportion increased to >90% for the majority of samples with PTI scores >0.1 (Appendix Fig. F.4A, F.4B). Only 3 samples had Fish-PTI >0.1, and the compounds responsible were acetochlor (n = 2) and carbendazim (n = 1). For 27 of the 28 samples (96%) with a Cladoceran-PTI score > 0.1, the compound responsible for the TUmax was an OP insecticide. Bifenthrin in one sample was the sole exception. For the Benthic Invertebrate-PTI, the TUmax was consistently 80–100% of the PTI score for the majority of samples, regardless of the magnitude of the PTI score (Appendix Fig. F.4C). For the 175 samples with Benthic Invertebrate-PTI > 0.1, the compounds responsible for the TUmax were imidacloprid (n = 152 samples, 87%), OP insecticides (n = 10, 6%), atrazine (n = 10, 6%), or bifenthrin (n = 3, 2%).

The high TUmax proportions observed here for invertebrates are consistent with sporadic detection of insecticides at acutely toxic concentrations, which overwhelm other pesticides in the mixture after concentrations are toxicity-weighted—a pattern that

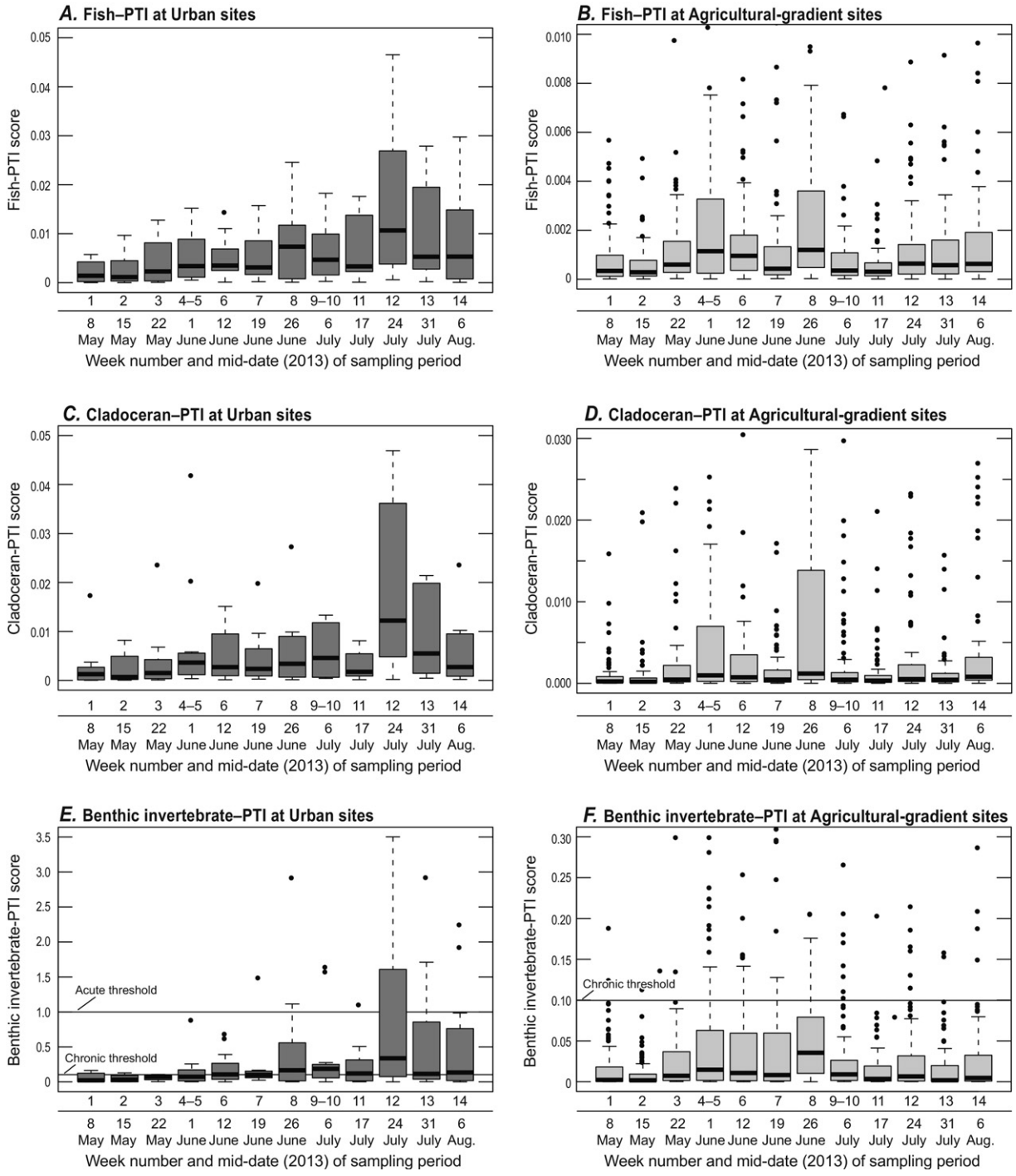

Fig. 6. Temporal variability of the PTI at Urban and Agricultural-gradient sites to (A–B) Fish, (C–D) Cladocerans and (E–F) Benthic invertebrates in relation to acute (PTI = 1) and chronic (PTI = 0.1) thresholds. The Y-axis scale varies in plots A–F.

may be exacerbated by the weekly sampling design, which underestimates acute exposures (Sec. 3.3.3). This is similar to results from Schäfer et al. (2013), who reported that the TUmax (based on acute toxicity data for cladocerans) performed as well as summed TUs for pesticides (i.e., a CA model) in explaining macroinvertebrate community abundance metrics in agricultural streams in Europe and Australia, although a hybrid CA-RA model performed better if other organic contaminants besides pesticides occurred. Similarly, three or fewer pesticides were responsible for >80% of summed potential toxicity towards fish, *Daphnia* and algae in drift and runoff from a small agricultural river in Italy (Verro et al., 2009).

3.3.3. Predicted toxicity and major contributors

Predicted toxicity, which was evaluated by combining results from screening with OPP benchmarks and the PTI, varied greatly by taxonomic group (Fig. 7). Potential acute toxicity was predicted for a stream if either (1) one or more pesticides exceeded acute benchmarks for fish, invertebrates, or aquatic plants; and(or) (2) summed PTI scores were >1 for fish, cladocerans or benthic invertebrates at any time during

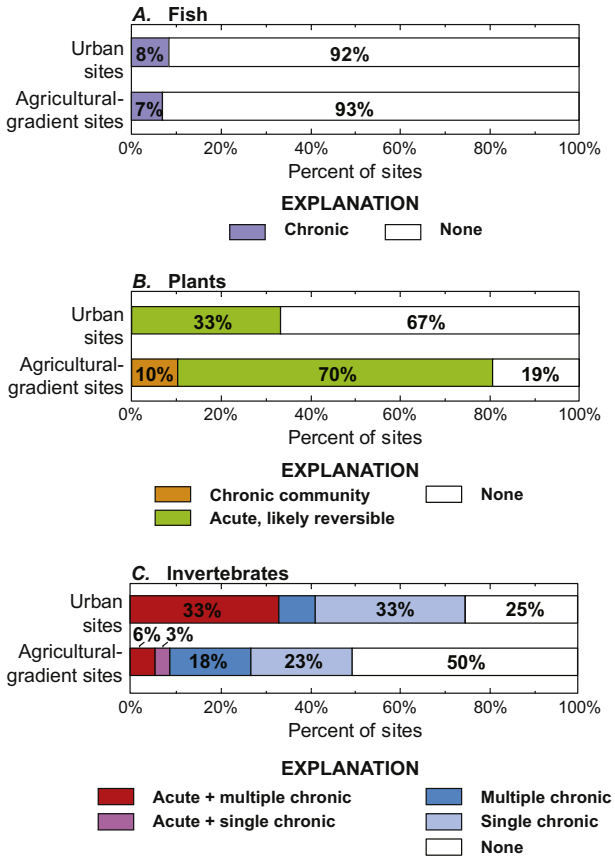

Fig. 7. Proportion of streams predicted to have potential acute and chronic toxicity to (A) fish, (B) plants, and (C) invertebrates, at Agricultural-gradient and Urban sites. Acute, exceeded acute benchmark or (for fish and invertebrates) the PTI was >1 in a sample. Chronic, maximum time-averaged concentration exceeded chronic benchmark or the maximum time-averaged PTI-TU was >0.1. Multiple, criteria are met by multiple pesticides. None, no benchmarks or TU thresholds were exceeded. Single, criteria are met by only 1 pesticide.

the study period. Potential chronic toxicity was predicted for a stream if the maximum time-averaged concentration during the study period (see Sec. 2.5) of one or more pesticides either (1) exceeded its chronic benchmark or (2) after toxicity-weighting, exceeded the PTI-TU threshold of 0.1.

Potential effects on fish were unlikely in 93% of MSQA streams overall during the index period (Fig. 7A), with potential chronic effects at 6 Med-High sites due to atrazine and(or) to acetochlor and at 1 Urban site due to carbendazim. The geographic distribution of these sites is shown in Appendix Fig. F.1. Potential effects of atrazine were ascertained from comparison of 60-day average atrazine concentrations to the Chronic-Fish benchmark, which is based on fish reproduction. Although 60-day average concentrations of all remaining pesticides were below chronic thresholds, the Fish-PTI TUmax was >0.1 in one individual sample for acetochlor (at 2 sites) and for carbendazim (at 1 site). Because exposure levels were below most chronic thresholds and because a single compound dominated the Fish-PTI, we see no evidence that mixtures were compounding potential toxicity to fish.

Potential effects on plants were evaluated on the basis of Acute-Plant benchmarks for herbicides (exceeded at 75% of sites), and the chronic plant community threshold for atrazine (exceeded at 9% of sites) (Fig. 7B). Herbicides temporarily suppress growth of non-target algae and macrophytes, but populations tend to recover once exposure is reduced (Fairchild, 2011). In multispecies microcosm and field mesocosm studies, impacts on aquatic communities required higher atrazine concentrations than were predicted from single-species laboratory bioassays (Huber, 1993; Solomon et al., 1996), which typically form the basis of OPP Acute-Plant benchmarks. Inhibition of photosynthesis in plants by atrazine is reversible, although there is uncertainty about the duration and timing of exposure relative to the ability of primary producers to recover their photosynthetic capacity after exposure ends (Solomon et al., 1996). Therefore, Acute-Plant benchmark exceedance probably indicates potential for acute, but short-term, effects on aquatic plant growth. In contrast, USEPA's chronic plant community threshold for atrazine is compared to 60-day average concentrations, and its exceedance indicates potential for sustained effects of atrazine on algal plant community structure and function. In MSQA streams, potential reversibility may limit the adverse effects on plants from triazine and acetanilide herbicide exposures, which tended to be concentrated in May and June (Fig. E.2, parts A–F) following planting of corn and soybeans. Overall, benchmark screening predicted reduction of aquatic plant growth at 75% of MSQA sites at some point during the 14-week study period from exposure to herbicides; these effects may be short-term at most sites, with potential for longer-term effects of atrazine on algal community structure and function at 9 Med-High Ag streams, where high mean concentrations were sustained over 60 days. Predicted effects on plants were more frequent in Agricultural-gradient than Urban streams (Fig. 7B), especially in the southern part of the study area (Appendix Fig. F.2).

Potential toxicity in the study area was more pronounced for invertebrates (Fig. 7C). This was evaluated by combining screening results for invertebrate benchmark comparisons and the Cladoceran- and Benthic Invertebrate-PTI, and is represented graphically in detail in Fig. 8 (also see Appendix C.2 for limitations). Overall, chronic invertebrate thresholds were exceeded by 21-day average concentrations of one or more pesticides at 53% of streams overall, with 26% of sites exceeding chronic thresholds for multiple (2–4) pesticides. At 12% of sites, insecticide concentrations exceeded acute thresholds at least once during the study period. Acute threshold exceedances were due to imidacloprid, OPs, and (at one site) diflubenzuron. Potential acute toxicity of mixtures—in the absence of any individual pesticide exceedance of acute thresholds—was predicted for three samples in which the summed Benthic-Invertebrate PTI >1, but the TUmax <1. The acute exceedances for these samples, which all occurred at Med-High Ag sites, are shown in Fig. 8 as due to $\sum$PTI. Although 39 OP insecticides or degradates were detected at least once during the study, chlorpyrifos, diazinon, dichlorvos, methamidophos, or tebupirimfos typically had the highest TU values.

Potential invertebrate toxicity was higher at Urban than Agricultural-gradient sites, as was noted separately for invertebrate benchmark exceedances (Sec. 3.3.1) and the PTI (Sec. 3.3.2). Fig. 8 shows proportionally more acute and chronic threshold excursions at Urban sites (33% and 75% of sites, respectively) compared to Med-High Ag sites (10% and 52%) and Low Ag sites (0% and 36%). Acute excursions occurred in 1% of samples from Med-High Ag streams, and 10% of samples from Urban streams; however (discussed in Sec. 3.3.4), acute exposures are underestimated here because of the weekly sampling design. All acute excursions were due to insecticides (OPs, imidacloprid, diflubenzuron) or the summed Benthic-Invertebrate-PTI. Chronic excursions at Urban sites were caused by insecticides (imidacloprid, OPs, and to a lesser extent fipronil), and at Agricultural-gradient sites by both herbicides (metolachlor, atrazine, acetochlor) and insecticides (imidacloprid, OPs, fipronil). Metolachlor frequently exceeded the Chronic-Invert benchmark at Agricultural-gradient sites. However, this analysis may overestimate potential invertebrate toxicity for metolachlor because (1) the racemic and S-forms of metolachlor have different benchmarks (U.S. Environmental Protection Agency, 2017a), and we used the more sensitive benchmark (for racemic metolachlor) because the LC-MS/MS analytical method used here cannot distinguish between enantiomers and therefore measures total metolachlor concentrations; and (2) more *S*-metolachlor was applied than racemic metolachlor in the Midwest during 2013 (U.S. Geological Survey, 2016). There is relatively little data on the relative toxicity of these forms of metolachlor (U.S. Environmental Protection Agency, 2014b), but the benchmarks are

based on the most sensitive toxicity endpoints available in the risk assessment. The present study may underestimate potential toxicity of neonicotinoids, as imidacloprid was the only neonicotinoid analyzed here. Hladik et al. (2014) reported that both clothianidin and thiamethoxam were detected more frequently than imidacloprid in 9 Midwestern agricultural streams in 2013. Agricultural-gradient sites with the highest potential invertebrate toxicity tended to be located in the southern (MO, IL, IN) or western (IA, NE) parts of the study area. Urban sites with the highest potential invertebrate toxicity were in NE, KS, and IL (Appendix Fig. F.3).

Overall, individual pesticide compounds dominated potential acute toxicity to invertebrates in MSQA streams—subject to the caveat that the weekly sampling design likely underestimates acute exposures to pesticides that occur sporadically, such as insecticides (Sec. 3.3.4).

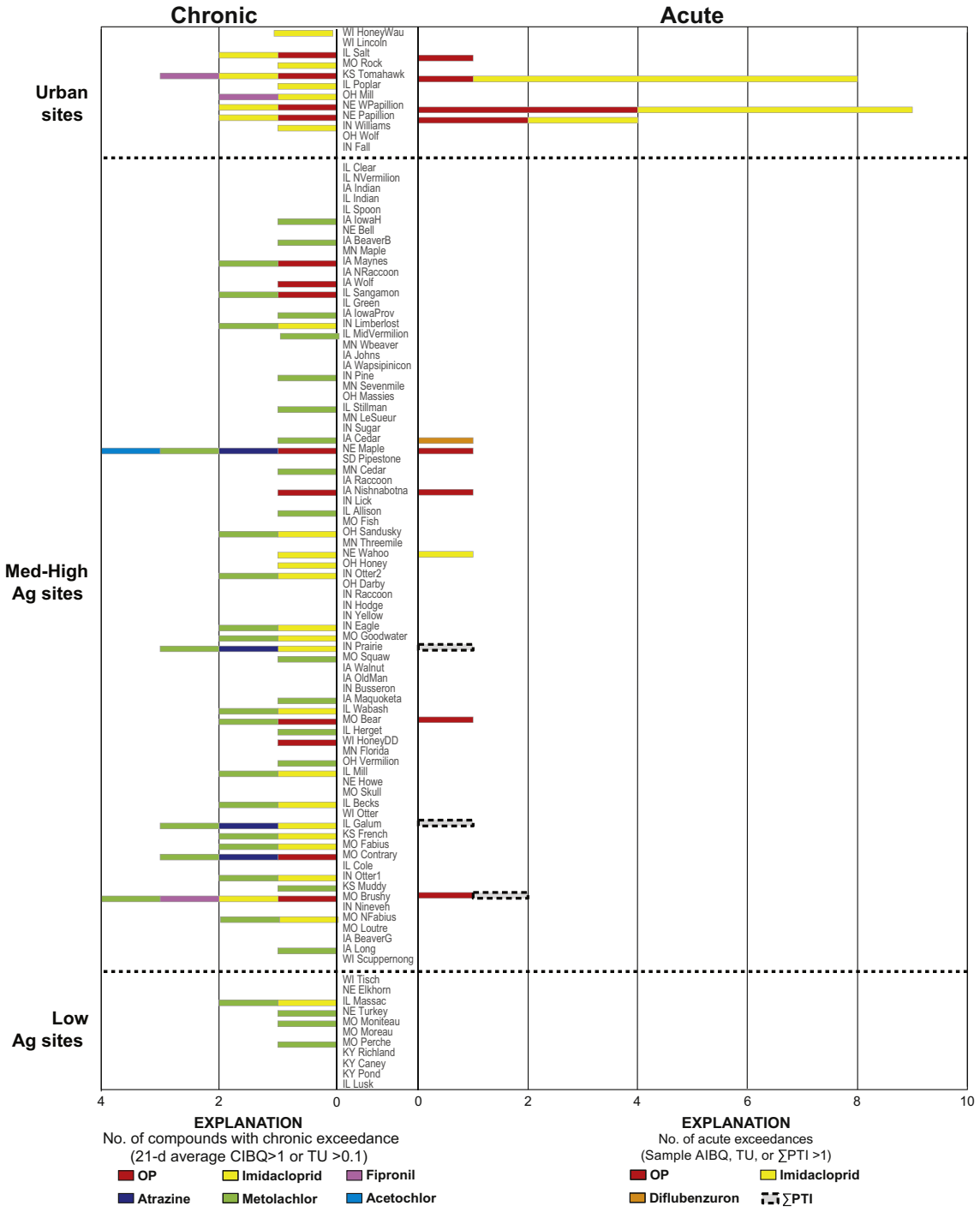

Fig. 8. Potential for invertebrate toxicity in each MSQA stream during the study period, expressed as the number of compounds with chronic threshold excursions (to the left of the central axis) and the number of acute threshold excursions (to the right of the central axis). Left of axis: Bar length is the number of pesticides that exceeded chronic threshold(s) during the study, defined as either (i) exceedance of the chronic benchmark by the maximum 21-day average concentration and(or) (ii) the 21-day average TU was >0.1 for cladocerans and(or) benthic invertebrates. Right of axis: Bar length is the number of times each pesticide exceeded an acute threshold, defined as either (i) exceedance of the acute benchmark and(or) TU > 1 for any individual pesticide in any single sample, or (ii) the summed PTI was >1 in any single sample (but with all individual pesticide TUs <1). Bar segments are color-coded by the pesticide responsible for the threshold excursion. Sites are listed by Short name (Appendix Table A.1) and ordered by % cropland (for Low and Med-High Ag sites) or % urban land (for Urban sites) in the basin, increasing from bottom to top. OP, cumulative toxicity of organophosphate insecticide compounds.

However, all streams had complex pesticide mixtures, the majority (53%) of streams exceeded chronic invertebrate thresholds for at least one pesticide for 21 days or longer, and 26% of streams exceeded chronic thresholds for multiple (2–4) pesticides for 21 days or longer. The impacts of concurrent exposure to multiple pesticides at chronic effect levels are unknown. Of the 100 streams in the present study, 17 exceeded chronic thresholds for multiple compounds without exceeding any acute thresholds in weekly discrete samples—suggesting the possibility that organisms in these streams may be adversely affected by chronic exposure to pesticide mixtures.

3.3.4. Screening caveats and limitations

Potential toxicity estimation, made here on the basis of PTI scores and benchmark exceedances, is intended to be a screening-level assessment. OPP aquatic-life benchmarks are based on the most sensitive toxicity test, and Sensitive-PTI scores represent either the 5th percentile or minimum LC50 (or immobilization EC50) from available toxicity tests, so for pesticides with a substantial number of toxicity values available, both screening tools tend to be conservative (protective). The uncertainty in the underlying toxicity concentrations is high for those pesticides with limited toxicity bioassays. As noted in Sec. 3.3.3, the benchmark comparison for metolachlor has additional uncertainty because the composition of individual enantiomers present in environmental residues is unknown, and different enantiomers may have different toxicities.

Generally, several factors combine to underestimate potential toxicity during screening. Available toxicity data are typically limited to standard test taxa, and may not represent more sensitive taxa that may be present in streams. PTI scores can be considered minimum values because the screening assessment is limited to pesticide compounds analyzed in this study, nondetections (reported as concentrations <MRL) are assumed equal to zero, and toxicity data are not available for all pesticide compounds analyzed in this study. PTI toxicity concentrations were available for 73–98% of parent pesticides and 9–28% of degradates (depending on the taxonomic group). Similarly, OPP benchmarks for fish, plants and invertebrates were available for 75–91% of parent pesticides but only 11–18% of degradates (again depending on the taxonomic group).

On the other hand, the PTI assumes additive toxicity, which may overestimate toxicity for pesticides with different modes of action, typically by a factor of <2–3 (Belden et al., 2007b; Deneer, 2000; Faust et al., 2003; Warne, 2003). In the present study, this degree of overestimation is not likely because—although complex mixtures of pesticides were common and pesticides with multiple modes of action were represented—PTI scores were dominated by a single compound, with TUmax values comprising >90% of the total PTI for the majority of samples predicted to be toxic (i.e., for samples with PTI scores >0.1) for all three taxonomic groups, fish, cladocerans and benthic invertebrates (Appendix Fig. F.4). The PTI also assumes that co-occurring pesticides do not have synergistic or antagonistic effects. The degree of interactive toxicity for the complex mixtures of pesticides observed in MSQA streams cannot be evaluated because of limited data. As an example, however, the maximum concentrations of propiconazole, a known azole synergist, detected in MSQA streams (0.9 μg/L) was well below the threshold (50 μg/L) reported for synergistic effects on pyrethroid toxicity towards *Daphnia magna* (Bjergager et al., 2017).

Although the weekly sampling schedule adopted by the MSQA study is considered high frequency fixed-interval sampling (Gilliom et al., 1995), compared to most synoptic studies (Kimball, 1997), weekly-distributed discrete sampling provides only snapshots of pesticide occurrence over the 14-week exposure period. A weekly sampling design substantially underestimates acute exposure to pesticides in small streams, as demonstrated by MonteCarlo simulations based on pesticide concentration data from four Midwestern streams that were sampled 16–20 times per month during the high pesticide-use season (Crawford, 2004); by a recent comparison of 2-day discrete and weekly discrete sample results for glyphosate in MSQA streams (Mahler et al., 2016); and by comparisons of concentrations in grab vs. flow-triggered sampling (Bundschuh et al., 2014; Xing et al., 2013). Fixed-interval sampling is unbiased with respect to time, but is biased low with respect to risk when highly transient but toxic concentrations (e.g., of insecticides) are being evaluated (Stehle et al., 2013). Overall, these factors suggest that potential toxicity in MSQA streams—especially potential acute toxicity—may be higher than predicted from the screening analyses in the present study.

3.4. Invertebrate community condition

3.4.1. Quantile regression of benthic macroinvertebrates

Comparison of various pesticide stressors compared to several macroinvertebrate metrics revealed an inverse wedge shaped response that was evaluated with quantile regression (e.g., Fig. 9). The pesticide stressors that best predicted the 70th to 95th quantiles of the invertebrate community metrics evaluated were the Benthic Invertebrate-PTI and imidacloprid concentrations (Table 2). (Results for additional stressors evaluated are provided in Appendix Table G.1.) The results for the Benthic Invertebrate-PTI and imidacloprid were similar to one another, which is not unexpected because Benthic Invertebrate-PTI scores in MSQA streams often were dominated by imidacloprid (Sec. 3.3.2). The distributions of pesticide stressors were skewed, with relatively few high values, so in some cases individual data points ("outliers") were influential in the regressions. In such cases, quantile regressions were repeated after removing outliers or after square-root transformation to test the robustness of the relation.

Of the other stressors tested, the Cladoceran-PTI scores (which tended to be dominated by OP insecticides) rarely predicted a majority of quantiles for any invertebrate metrics and the few significant relations were influenced by one or more high values. The Chronic-Invert BQ was significantly and inversely related to all six quantiles for EPT richness, EPT abundance, and Ephemeroptera abundance (Appendix Table G.1); however, these regressions were influenced by outliers and the relations were no longer significant when outliers were removed, suggesting there were too few data points with moderate-to-high Chronic-Invert BQ values to evaluate these relations. For the other pesticides evaluated, few or no quantiles of the invertebrate metrics were related to OP insecticide concentrations and only occasionally were 3 or more quantiles of an invertebrate metric related to fipronil or total fipronil concentrations (Table 2; Appendix Table G.1); however, these comparisons also were limited by the small number of data points at high stressor values. In general, the relations to pesticide stressors were similar for EPT- and Ephemeroptera-based metrics, which tended to show more significant relations than did the MMI. Generally, there were more significant, inverse relations (at $p < 0.05$) between pesticide stressors that focused on the last 7 weeks of the sampling period, which were closer in time to the invertebrate sampling date, than with stressors representing pesticide concentrations over the entire study period. However, measures from the last week alone of the sampling window were not useful in predicting the invertebrate responses (data not shown).

Screening predictions of adverse effects on invertebrates from pesticides in MSQA streams are supported by these quantile regression results, which indicate significant relations for multiple upper quantiles of benthic invertebrate community metrics with the Benthic Invertebrate-PTI and imidacloprid. These findings show a statistically significant association between pesticides and invertebrate community condition. In a previous study of Colorado streams, the influence of other recognized drivers of ecological community health, such as instream habitat, elevation, drainage area, and temperature were detected on lower quantiles (e.g., quantiles below the median), whereas poor water quality limited quantiles above the median (Schmidt et al., 2012). The significant association reported here for pesticides occurred for the upper quantiles of invertebrate community condition—suggesting the hypothesis that pesticides, especially imidacloprid, may limit the ecological potential of

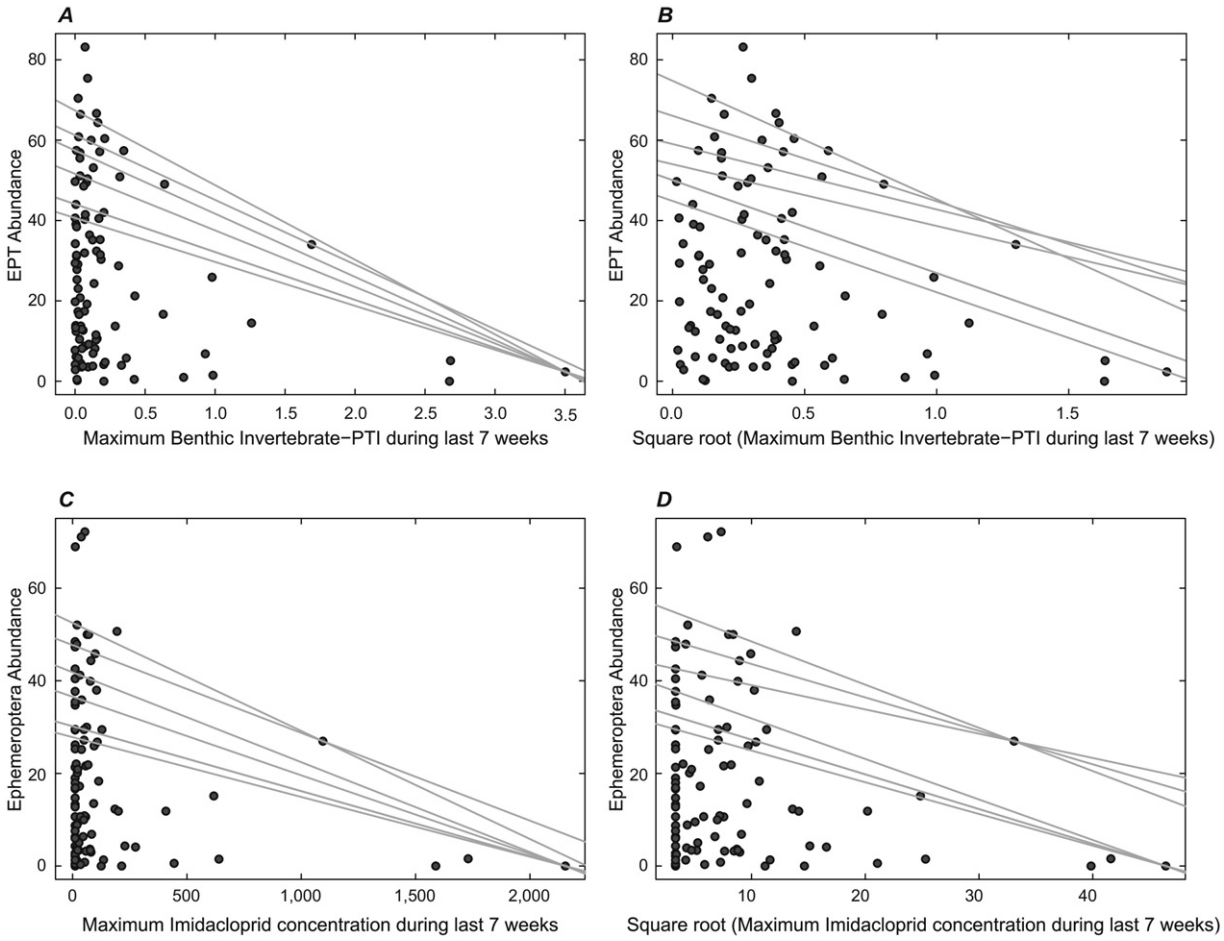

Fig. 9. Example quantile regression plots of invertebrate community metrics in response to pesticide stressors: percent (relative to total abundance) EPT abundance quantiles vs. Benthic Invertebrate-PTI during the last 7 weeks of the study period, without (A) and with (B) square root-transformation; Ephemeroptera abundance quantiles vs. imidacloprid concentrations during the last 7 weeks of the study period, without (C) and with (D) square root-transformation. The 70th to 95th quantiles are shown.

stream ecosystems independent of other limiting factors. This association does not establish cause and effect.

3.4.2. Mesocosm toxicity test

In the 10-day (acute exposure) mesocosm test with imidacloprid, declines in total abundance and in EPT taxa were observed at 1000–3300 ng/L concentrations. Because the controls were not analyzed until day 30 (i.e., 20 days after the treatment communities were analyzed), acute effects relative to controls in this range-finding test may be underestimated. EPT taxa abundance, a metric considered sensitive to chemical stressors, precipitously declined between imidacloprid exposures of 1000 and 3300 ng/L. This change was driven by the orders Ephemeroptera (mayflies) and Plecoptera (stoneflies), with individual genera and species within these two taxa reduced by upwards of 50% in this exposure range. The mayfly *Baetis tricaudatus* was notably more sensitive, and its numbers were greatly reduced at nearly every exposure concentration (as low as 60 ng/L). EC50s were not calculated because this range-finding test lacked replication. Nonetheless, the mesocosm results indicate that imidacloprid was toxic to EPT taxa exposed for 10 days to concentrations in the 1000–3300 ng/L range, and to one mayfly taxon (*Baetis tricaudatus*) at concentrations as low as 60 ng/L.

These concentrations are similar to the 1050 ng/L OPP Chronic-Invert benchmark for imidacloprid, which is based on the NOAEC for growth and movement in *Chironomus dilutus* (U.S. Environmental Protection Agency, 2008) and to the acute (96-hour) mayfly LC50 for imidacloprid of 650 ng/L used in PTI calculations for benthic invertebrates (Nowell et al., 2014). Thus, the mesocosm results also support an inference of potential acute effects on invertebrates from imidacloprid, at the upper end of the imidacloprid concentration range detected in MSQA streams (up to 2200 ng/L). The mesocosm test strengthens the link between pesticide monitoring data and invertebrate community impairment observed in MSQA streams, by demonstrating that similar adverse effects on invertebrate communities occurred in mesocosms under controlled conditions at environmentally relevant imidacloprid concentrations.

4. Conclusions

This study provides a spatially intensive, short-term temporal assessment of currently used pesticides and their potential aquatic toxicity in agricultural and urban streams in the Midwestern U.S. during May–August 2013. Using a sensitive, broad-spectrum LC-MS/MS method to determine pesticides and degradates, we detected more complex pesticide mixtures than previously reported for discrete water samples—with a median of 25 pesticide compounds detected per sample and 54 compounds per site. When mixtures were screened using aquatic-life benchmarks and the PTI, a small number of pesticides were predicted to be the dominant contributors to potential toxicity—the herbicides metolachlor, acetochlor, and atrazine; the insecticides imidacloprid, fipronil, and OPs; and the fungicide/degradate carbendazim.

Potential toxicity to fish was predicted to be low in MSQA streams. Acute (but likely reversible) effects on plant biomass were predicted for 75% of streams, with 9% of streams having atrazine concentrations high and sustained enough to have longer-term effects predicted on

Table 2
Quantile regression scores of the Pesticide Toxicity Index or dissolved concentrations of selected pesticides in predicting upper quantiles of four types of invertebrate community metrics. Each explanatory variable represents one pesticide stressor type, computed as a specified stressor value over a specified time period. Dark shade indicates 4 or more of 6 quantiles were statistically significant ($p < 0.05$) and inversely related; light shade indicates 3 of 6 quantiles were significant. ( ) values indicate number significant after square root transformation (*) or outlier removal (+).

Pesticide stressor (explanatory) variable			Ecological (response) variable			
			MMI	EPT richness	EPT abundance	Ephemeroptera abundance
Stressor type	Statistical value	Time period	No. of significant quantiles (out of 6)			
Benthic Invert-PTI	Max	All 14 weeks	1	2	0	0
Benthic Invert-PTI	Max	Last 7 weeks	2	0	6 (4*, 5+)	6 (5*)
Benthic Invert-PTI	Max 21-d MVA	All 14 weeks	3	6 (4*)	1	0
Benthic Invert-PTI	Max 21-d MVA	Last 7 weeks	5 (2*,3+)	5 (2*)	6 (5*,6+)	3
Cladoceran-PTI	Max 21-d MVA	All 14 weeks	2	4	1	0
Cladoceran-PTI	Max 21-d MVA	Last 7 weeks	1	0	2	0
Imidacloprid	Max	All 14 weeks	4	0	0	0
Imidacloprid	Max	Last 7 weeks	0	0(0*,4+)	5 (3*,4+)	5 (3*,4+)
Imidacloprid	Max 21-d MVA	All 14 weeks	4	6	6 (4*,3+)	5 (6*,1)
Imidacloprid	Median	Last 7 weeks	5(1*,1+)	5(4*,4+)	5	2
Fipronil	Max 21-d MVA	All 14 weeks	1	0	0	1
Fipronil	Median	Last 7 weeks	3 (3+)	4 (2*,1+)	0	1(5+)

plant community structure and function. Potential chronic invertebrate toxicity was predicted for one or more pesticides at 53% of sites, punctuated at 12% of sites by an acutely toxic insecticide pulse. Potential for acute invertebrate toxicity was dominated by a single pesticide per sample—usually imidacloprid or OP insecticides—subject to the caveat that acute exposures were underestimated by the MSQA weekly sampling design, especially for sporadically occurring compounds such as insecticides. The role of mixtures in chronic and sublethal effects is unknown, but warrants investigation. Organisms clearly were exposed to complex mixtures (a "soup") of dissolved pesticides, with multiple (2–4) pesticides exceeding invertebrate chronic-effect thresholds over a 21-day period in 26% of streams.

Potential toxicity-screening predictions were supported by quantile regression showing an association between measured pesticide stressors—the Benthic-Invertebrate PTI and imidacloprid concentrations—and upper quantiles of invertebrate community condition in MSQA streams. Furthermore, mesocosm toxicity testing demonstrated that acute effects on mesocosm benthic invertebrate communities occurred in response to imidacloprid under controlled conditions at the upper end of imidacloprid concentrations observed in MSQA streams, consistent with the hypothesis that imidacloprid may have adversely affected invertebrate community condition in some of the sampled Midwestern streams.

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Appendix A. Supplementary data

All data used in analyses presented here are available in machine-readable format (Nowell et al., 2017; Waite and Van Metre, 2017). Supplementary methods information is provided on study design and sampling sites (Appendix A), chemical analysis and quality control (Appendix B), aquatic-toxicity screening (Appendix C), and benthic invertebrate sample collection and processing (Appendix D).

Supplementary results are provided on pesticide occurrence in relation to land use (Appendix E), potential toxicity (Appendix F), and quantile regression (Appendix G). Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.scitotenv.2017.06.156.

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