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Assessing microplastics, per- and polyfluoroalkyl substances (PFAS), and other contaminants of global concern in wadable agricultural streams in Iowa†

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Microplastics, per- and polyfluoroalkyl substances (PFAS), antibiotic resistance genes (ARGs), pharmaceuticals and personal care products (PPCPs), and pesticides may lead to unintended environmental contamination through many pathways in multiple matrices. This statewide, multi-matrix study of contaminants of global concern (CGCs) in agricultural streams across Iowa (United States) is the first to examine multiple CGCs in water, bed sediment, and fish to understand their occurrence in small streams located in regions of intense agriculture activity. Iowa plays a pivotal role in agriculture, with more than 85% of Iowa's landscape devoted to agriculture, making it an ideal location for determining the prevalence of CGCs to provide critical baseline exposure data. Fifteen sites were sampled across a range of predominant land uses (*e.g.*, poultry, swine); all sites had detections of microplastics in all matrices. Concentrations of PFAS varied but were detected in water and sediment; all fish had detections of perfluorooctanesulfonate (PFOS), a type of PFAS. More than 50% of water and bed sediment samples had detections of ARGs. The most frequently detected PPCP was metformin. No sites had a cumulative exposure activity ratio greater than 1.0 for chemical exposures; 13 sites were above the 0.001 precautionary threshold. Toxicity quotients calculated using Aquatic Life Benchmarks were below the 0.1 moderate risk threshold for chemical exposures for all but one site. For fish, all sites exceeded the moderate and high-risk thresholds proposed for microplastic particles for food dilution (both chronic and acute exposures) and all sites exceeded the microplastic moderate threshold proposed for chronic tissue translocation, and two sites exceeded the threshold for acute tissue translocation.

Environmental significance

This statewide, multi-matrix study of contaminants of global concern (CGCs) in agricultural streams across Iowa (USA) is the first to examine multiple CGCs in water, bed sediment, and fish to understand their occurrence in small streams located in regions of intense agriculture activity. The CGCs microplastics, per- and polyfluoroalkyl substances, antibiotic resistance genes, pharmaceuticals and personal care products, and pesticides cause unintentional environmental contamination through many pathways in multiple matrices. Iowa plays a pivotal role in agriculture, with more than 85% of Iowa's landscape devoted to agriculture, making it an ideal location for determining the prevalence of CGCs to provide critical baseline exposure data.

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4em00753k

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Environ. Sci.: Processes Impacts, 2025, **27**, 1401–1422 | **1401**

Introduction

Mounting evidence indicates that anthropogenic actions and products intended to improve the quality of life are causing unintentional environmental contamination on a global scale.[1,2] The persistence, mobility, and bioaccumulation potential of these materials has translated to their presence in all physical and biological environmental compartments. Such contaminants of global concern (CGCs) include microplastics, per- and polyfluoroalkyl substances (PFAS), antibiotic resistance genes (ARGs), pharmaceuticals and personal care products (PPCPs), and pesticides. Previous research has documented that the simultaneous presence of a complex mixture of a wide range of contaminants often occurs.[3,4] Contaminants can contribute synergistically to toxicity even if they are present below their own individual effect threshold.[5] In addition, such contaminant mixtures can cause surface waters to be biologically active (e.g., estrogen, androgen, glucocorticoid activity).[6,7]

Plastics play an important role in many aspects of modern human life, and their transformation products are often problematic. As produced plastics become microplastics (less than [<] 5 mm in size)[8] via physical or mechanical weathering, they enter the "natural" environment, with a nebulous fate. Microplastics are ubiquitous in the environment, including in oceans,[8] rivers,[9] lakes,[10] sediments,[11,12] biota,[13,14] humans,[15,16] atmosphere,[17,18] and even remote areas far-removed from anthropogenic sources.[19–21] By 2050, it is projected that 12 000 million metric tons of total plastic waste will have accumulated in landfills or the environment.[22] Although much microplastic research to date has been conducted in marine settings, growing evidence indicates lotic systems are instrumental in transporting microplastics,[23,24] with particles prevalent in both stream water and bed sediment.[25–27] Although the bulk of freshwater microplastic research is focused on urban activity, agricultural production also contributes microplastics to the environment[28–30] through various management practices, including municipal biosolid applications to farmland.[31,32]

As with plastics, PFAS play a decades-long role in modern society with innumerable uses including firefighting foams, water and stain repellents, and PPCPs. Optimization for consumer and industrial applications also allows them to enter the environment and be transported through a variety of pathways[33] and accumulate in plant, animal, and human tissues.[34–38] PFAS are linked to a range of deleterious health effects including low birth weights, hormone interference, reduced immune response, and various cancers.[39–42]

Concern regarding antimicrobial resistance (AMR) has increased due to the extensive use of antimicrobials for both human and agricultural purposes. Approximately 80% of the antibiotics used in the United States are for food animal production.[43] Antibiotic overuse has led to AMR now being recognized as a global threat to human health with the serious potential of a post-antibiotic era where antibiotics are rendered obsolete.[44] It is projected that resistant bacterial infections could become the leading cause of global deaths by 2050.[44]

Antibiotic resistant bacteria (ARB) and associated antibiotic resistance genes (ARGs) are now recognized as environmental contaminants.[45] Numerous mechanisms and pathways for environmental transfer of ARB and ARGs have been identified, with water considered an important transmission pathway.[46,47] Recent investigations found that antibiotic resistant enterococci and staphylococci were common in Iowa streams, with stream bed sediment an important reservoir for ARGs.[48]

PPCPs are organic chemicals that have been developed to enhance the quality of human life; however, evidence indicates that environmental exposure to PPCPs and their associated transformation products such as guanylurea can have deleterious effects on ecosystems.[49,50] PPCP consumption worldwide is rising due to an increasing global population with a rising median age.[51] As the demand for PPCPs increases, corresponding discharge through wastewater treatment plants (WWTPs) and manufacturing facilities into the environment also increases.[52–54] PPCPs have both urban and agricultural inputs to streams[55–61] and have been found in streams globally on all seven continents.[1]

Pesticides are exceedingly common, particularly in agricultural production in the United States, for which more than 226 million kilograms of pesticides[62] were land applied annually between 2013 and 2017.[63] This intensive use and related exposure to nontarget organisms is of increasing global concern.[64] Although pesticides have commonly been used in agriculture since the 1950s, conventional agriculture has increasingly adopted prophylactic technologies using pesticides at or before planting[65] that is concomitant with a rise in the global use of pesticides.[66] Mixtures of pesticides[62] and pesticide transformation products[67] are increasingly common in urban and agricultural streams across the United States, often with deleterious effects to aquatic and terrestrial organisms.[68–72]

Rural streams have a long history of research on contaminants traditionally associated with agriculture. These contaminants, often from land-applications, waste lagoons, and confined animal feeding operations, include nutrients, pesticides, and bacteria that move into streams and groundwater through a variety of mechanisms, such as overland flow.[73–75] The ecosystem services of rural lotic environments are often overlooked, despite the critical value of the habitat.[76] Much research exists on the presence of non-traditional contaminants such as pharmaceuticals in streams in agricultural areas.[77] Conversely, there is a paucity of information on contaminant exposures in non-aqueous matrices and biota. To address the research gap, we conducted a statewide, multi-matrix study of CGCs in wadable, agricultural streams across Iowa (United States). Iowa plays a pivotal role in agricultural in the United States,[78] with more than 85% of Iowa's landscape devoted to agriculture, leading the nation in corn, soybean, swine, and egg production,[79] and consequently is ideal for determining baseline exposure data regarding the prevalence of CGCs in agricultural streams. To our knowledge, this is the first study to simultaneously examine multiple classes of CGCs in multiple compartments (e.g., water, sediment, and fish) to better understand their occurrence in small streams located in regions of intense agriculture activity.

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Materials and methods

Site selection & sample collection

Fifteen stream sites were selected across Iowa based on basin characteristics, previous water quality data, and proximity to established U.S. Geological Survey (USGS) gaging stations (Table S1†). Of the 15 sites sampled, 6 were in basins dominated by a specific livestock type (2 each for cattle, poultry, and swine), 3 sites had mixed livestock (livestock polyculture), 3 sites had substantial municipal biosolids applied to farmland in the basin, 2 sites were affected by WWTPs discharging directly upstream from sampling points, and 1 site was considered to be a reference site (dominated by forested land) with minimal agriculture in the basin (Fig. 1). The streams sampled had a wide range of characteristics including developed land use[80] (1.9–62%), cultivated land[80] (14.4–93.3%), animal units[81] (AU; 0–643 AU per square kilometer [km^2]), human population[82] (2–739 people per km^2), and WWTPs[83] (0–10).

Water, bed sediment, and fish samples were collected April through June 2021 from sites during base-flow conditions. Water samples were always collected first, sediment was then collected from areas of deposition upstream from the water sample, and then netted microplastics samples were collected in the center of flow. Fish sample collection was opportunistic, and fish were collected using a seine net with a mesh size of 6.4 millimeters (mm)[84] within the sampling reach (within 100 feet of discrete sampling). Fish sampling was not meant to be an ecological assessment; rather, samples were meant to be part of the overall snapshot of the chemical burden of the stream during the few hours the crew was on-site. Lastly, *in situ* field properties and discharge measurements were made. Water samples were collected and analyzed for microplastics, PFAS, bacteria concentrations and ARG presence, pesticides, PPCPs, total estrogenicity, nutrients, and suspended sediment. Bed sediment samples were collected and analyzed for microplastics, PFAS, and bacteria concentrations and ARGs. Fish, typically Cyprinidae minnows, were collected at 13 sites; 2 sites were netted but did not yield fish. All 13 sites had fish analyzed for PFAS while only 5 of the 13 were able to be analyzed for microplastics. Whole fish samples were collected and placed in jars and/or bags as required by individual laboratories and analyzed for microplastics, PFAS, three pharmaceuticals, and one pesticide. The number and size of fish varied by site, with some sites having as few as two minnows per jar; jars were immersed in ice in coolers immediately post-collection[85] and all fish sampling was in accordance with the State of Iowa scientific collector permitting procedures. All samples were stored on ice at 4 °C in the field and shipped to arrive within 24 hours of collection to the various laboratories, as necessary.

Whole water samples were collected as single verticals/center-of-flow (VCF) samples.[86] Filtered inorganic water samples were processed streamside from water collected in a high-density polyethylene (HDPE) bottle using a 60-cc disposable Luer lock syringe and an Aquaprep 0.45 μm disk filter. Filtered organic water samples were processed by collecting water in the center of flow using a 30 milliliter (mL) polypropylene Luer lock syringe and were filtered using a Whatman 0.7 μm, 25 mm glass fiber syringe tip filter for
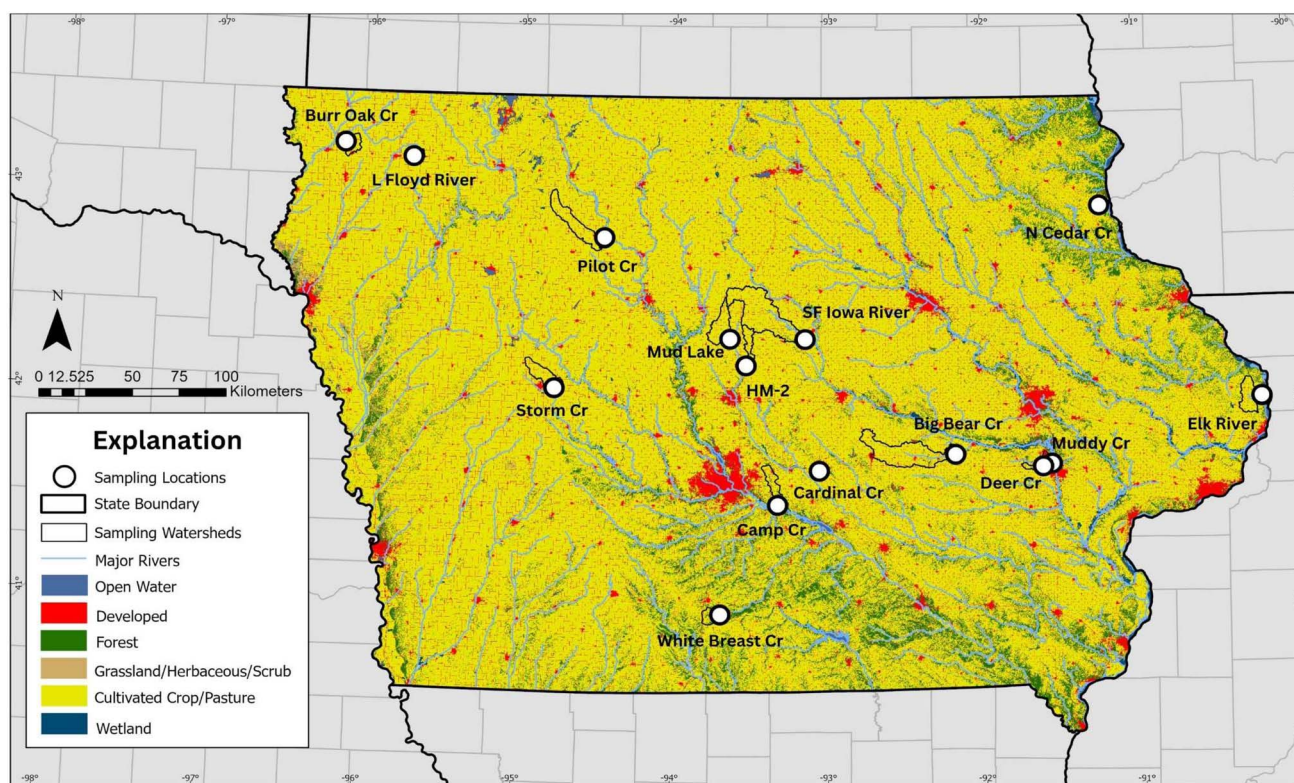

Fig. 1 Site locations for 15 Iowa streams, 2021.

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analysis of 82 pesticides by the USGS. A 1 liter (L) amber glass bottle was used to collect water for pharmaceutical analysis, and a 500 mL amber glass bottle was used to collect water for pesticide analysis at the University of Iowa in Iowa City, Iowa.

Microplastic water samples were collected in duplicate in the center of flow. Microplastic samples were collected both as netted and whole water samples as a comparison between the two methods. Netted samples were collected using a Hydro-Bios 300 μm net with a 125 μm cod end. Nets were held in flow for 15 minutes or until the net had too much debris (whichever came first). Nets were rinsed with deionized water (DI) into the cod end, which was decanted into a quart-sized mason jar that had been triple rinsed with DI water, fired at 450 °C for 4 hours, and then wrapped in foil. Whole water samples were collected in the center of flow in a quart-sized mason jar, prepared as previously mentioned. Subsequent analysis documented that the net used for microplastic collection shed substantial amounts of microplastics when using a laboratory method with a lower-than-standard size detection limit (detailed in the Laboratory Methods section). Thus, only whole water samples were analyzed and are presented here. PFAS water samples were collected in three 2 mL microcentrifuge tubes and submitted to the USGS National Water Quality Laboratory (NWQL) in Denver, Colorado, for analysis.

In situ field properties (water temperature, specific conductance, pH, dissolved oxygen, and turbidity) were collected in the center of flow using a Yellow Springs Incorporated (YSI) EXO2 multiparameter sonde. For ungaged sites, discharge measurements were made immediately following sample collection using a SonTek FlowTracker Acoustic Doppler Velocimeter.

Bed sediment samples were collected with stainless-steel spoons from three to five areas of deposition within the sampling reach with only the top 3 centimeters (cm) of bed sediment being sampled. Microplastic and PFAS bed sediment samples were composited in one glass jar each; microbial samples were composited in sterile plastic specimen cups. All reuseable field equipment used for sampling (HDPE bottle and stainless-steel spoons) was cleaned by dilute phosphate-free detergent soak, tap water rinse, and DI rinse.

Laboratory methods

Water and bed sediment samples for microplastic analysis were stored at room temperature, and fish were stored in a freezer; all samples were delivered to the University of Illinois, Illinois Sustainable Technology Center in Urbana, Illinois, after all sampling was complete. Full microplastic analytical methods are published in Scott and Green;[87] the method is a Modified NOAA Method[88] wherein water samples are concentrated by wet sieving (5 μm stainless steel sieve), then undergo wet peroxide oxidation (Fenton reagent), density separation, filtration to 0.45 μm, and then examined with a Zeiss SteREO Discovery V20 microscope for sizing, as well as notation of particle type (round, fiber, or fragment) and color. To measure the area of the particles, the x and y axes were measured with the Zeiss microscope and analyzed with the Zeiss Zen Blue software package. The z-axis was assumed to be 1 μm, a conservative

value; detailed sizing methods are available in Prada.[89] Microplastic analysis of bed sediment samples was similar to water, but started with density separation, wet sieving, wet peroxide oxidation, density separation, filtration to 0.45 μm, and then microscope examination as with water samples. Whole fish sample processing started with wet peroxide oxidation, wet sieving, and then oxidized and sieved again until free of visual biological and organic matter. Samples were then sorted by density, filtered to 0.45 μm, and then examined by microscope as with water samples. This modified method yields a lower detection limit (20 μm × 20 μm) and larger densities (greater than [>] 1.7 grams per cubic centimeter), which allow for more polymer types than the standard analytical method (Table S2†).

Water samples were analyzed for 34 PFAS at the NWQL using liquid chromatography-tandem mass spectrometry (LC/MS-MS).[90] Sediment and whole fish samples were analyzed for 28 PFAS using a modified version of EPA Method 533 by SGS Axys in British Columbia, Canada.[91]

Water and bed sediment samples were enumerated for heterotrophic bacteria counts (HPC), total coliforms, *Escherichia coli* (*E. coli*), and enterococci at the USGS Michigan Bacteriological Research Laboratory (MI-BaRL) in Lansing, Michigan, using the IDEXX Quanti-Tray/2000 System (Westbrook, Maine) with either HPC, Colilert, or Enterolert tests (IDEXX; Westbrook, Maine). Growth from IDEXX HPC incubations was aseptically extracted from the tray system and plated on Reasoner's 2A (R2A) agar and extracted DNA from subsequent growth used to determine the presence of ARGs (Tables S3 and S4†). Polymerase chain reaction (PCR) was used to detect the presence of 25 bacterial gene markers (BGMs), including a Bacteria 16S rRNA gene, 20 ARGs, 3 virulence genes (*fem*A, *inv*A, and *spv*C), and 1 integrase gene (*int*) as described in Givens.[48]

Water samples were analyzed for pesticides at the NWQL and UIowa, and water and whole fish samples were analyzed for PPCPs at UIowa. Water samples submitted to the NWQL were analyzed for 82 pesticides and transformation products using direct aqueous injection-LC/MS/MS-(DAI LC-MS/MS).[92] Water samples submitted to UIowa were analyzed for 14 PPCPs and 6 pesticides. The samples were vacuum filtered (Whatman glass microfiber filters [GF/F], nominal 0.7 μm) and frozen at −20 °C until extraction. Water samples were processed with solid-phase extraction (SPE) to increase detection limits and then analyzed using an Agilent LC-MS/MS. Whole fish samples were analyzed for three PPCPs and one pesticide. The fish samples were solvent extracted using a modified method from LeFevre[93] and analyzed with the same Agilent LC-MS/MS.

Water samples were prepped for total estrogenicity analysis at the USGS Functional and Molecular Bioassay Laboratory in Kearneysville, West Virginia, by the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas. Samples were extracted within 36 hours of arrival, 1 L samples were filtered with a 0.7 μm glass-fiber, solid phase extracted to give 100 μL of extract, and stored at −20 °C until analysis. Sample extracts were screened for total estrogenicity using the bioluminescent yeast estrogen screen (BLYES) as previously described by Ciparis[94] and Sanseverino.[95] In brief, 20 μL of sample extracts were added in triplicate to the wells of white, solid-bottom 96-well

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microtiter plates and evaporated at room temperature. Following evaporation, 200 μL of a 48 hour culture of strain BLYES adjusted to 0.4 (OD600) in Yeast Minimal Media (YMM leu-, ura-)[96] was added to each well. A 12-point standard curve of 17β-estradiol (E2; Sigma-Aldrich Co.) was included on each plate, and plates were covered and incubated in the dark at 30 °C for 4 hours. Luminescence was quantified using a SpectraMax M4 microplate reader (Molecular Devices) in luminescence mode, and estrogen equivalents (E2Eq) of each sample were determined *via* interpolation to a 4-parameter curve using SoftMax Pro 7.1.0 (Molecular Devices). The detection limit for this assay was 0.244 ng L^{-1} estradiol equivalents (E2Eq) BLYES.[7,97]

Quality assurance/quality control

Two field blanks were processed on-site using Fisher Scientific organic-free blank water for organic constituents and sterile DI water for microbial samples. Multiple blanks were processed for microplastics: a DI machine source blank was processed in the USGS Iowa City Water Quality Laboratory, a DI blank from a glass jug used to transport DI water and used in the field was processed in the Iowa City laboratory; a field blank was processed using DI water at a sampling site; water and sediment travel blanks provided by the University of Illinois Prairie Research Institute were analyzed. As necessary, blank water was run through the same equipment used to collect the environmental samples. All blank samples had low-level detections of microplastics (DI instrument: 11 counts per liter (CPL); DI blank: 3 CPL; field equipment blank: 10 CPL; travel blank, water: 9 CPL; travel blank, sediment: 3 counts per gram [CPG]). All other blanks were free of the target constituents. In addition to field quality control samples, three PFAS samples were randomly chosen and run as duplicates; there were no PFAS detections in these samples. Microbial laboratory blanks consisting of sterile DI water were processed along with the workflow of IDEXX enumeration, DNA extraction, and PCR. Aside from blanks, microplastic positive control spikes were run on three DI spike samples analyzed with each matrix batch with the recovery percentages ranging from 88–119.

Statistical analysis and risk assessment

All data analysis was done using RStudio statistical software, version 4.4.0.[98] Initially, data were evaluated using Spearman's rank correlation coefficient (rho) to evaluate relations between laboratory results and basin characteristics including land use, basin size, and animal units within the watershed. This nonparametric statistical test was used because the analytical results contained left-censored data (*i.e.*, non-detections). Results were considered significant when $p < 0.05$ and rho values were >0.50. The assumptions of normality and constant variance for the particle size of detected microplastics were evaluated by examining individual histograms for each site and sample median. Additionally, the Shapiro–Wilk test was applied to statistically assess normality, and the Fligner-Killeen test was used to evaluate homogeneity of variances.[99] Because the normality and constant variance assumptions were not met,

a one-factor permutation test, which does not rely on these assumptions, was used to assess differences in mean particle size across sites and primary land use types (based on land use in the basin) for each sample median. Results were considered significant when $p < 0.05$. A pairwise comparison of particle size by site and primary land use type between surface water and bed sediment samples was conducted using the Wilcoxon rank-sum test, with a Benjamini–Hochberg correction applied to control the family-wise error rate.[100]

Cumulative-exposure effects of potential human-health interest were screened using two bioactivity-weighted approaches: the cumulative Exposure-Activity Ratios (ΣEAR), and the cumulative toxicity quotient (ΣTQ). Both approaches are (1) constrained by the analytical scope of this study, which greatly underestimate the organic chemicals in commercial use and, by extension, in the environment;[101] (2) limited to the available weighting-factors of ToxCast activity concentration at cutoff (ACC) and human-health benchmarks;[102] and (3) assume cumulative effects are reasonably approximated by concentration addition.[103–105] The ΣEAR were computed using chemical analytical results for pesticides, pharmaceuticals, and PFAS water samples by site using toxEval version 1.3.0 [106] to determine potential biological effects of contaminants found in each sample. The ΣEARs were of potential concern when results were greater than or equal to (≥) 0.001. Lastly, toxicity quotients (TQ) were calculated using chemical results for pesticides as well as the physical results for microplastics for the stream water samples. Toxicity quotients were calculated in RStudio[98] using Aquatic Life Benchmarks (ALB) established by the U.S. Environmental Protection Agency's Office of Pesticide Program to determine risk to freshwater fish from acute and chronic exposure to pesticides and their transformation products.[107] Benchmarks do not currently exist for microplastics data, so thresholds proposed in Mehinto[108] were adjusted for the purposes of this study. Mehinto[108] proposed four thresholds for two fish health outcomes (food dilution and tissue translocation); this study used thresholds 1 and 2 as the benchmark criteria for chronic health issues and thresholds 3 and 4 for acute health issues for each health outcome. For food dilution, this study followed Mehinto[108] using a benchmark of 0.3 microplastic particles per liter (PPL) for chronic health issues and 5 microplastic PPL for acute health issues with a particle size threshold of less than 25 000 000 square micrometers (μm^2), to be referred to as 0.25 centimeters squared (cm^2) in this study. Still following Mehinto,[108] this study used a tissue translocation benchmark of 60 microplastic PPL for chronic health issues and 890 PPL for acute health issues with a particle size threshold of 83 μm, which is <6,889 μm^2 when converting to area. Toxicity quotient results of ≥1 were considered significant. For the purposes of this study and these data, all estimated data values were included in all analyses.

Results and discussion

Contaminants ubiquitously present in stream water

Complex mixtures of chemical and microbial contaminants were present in the rural agricultural streams sampled in this

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Table 1 Number of contaminant detections, percent detections, and minimum, median, and maximum concentrations by sample medium for 15 Iowa streams, 2021. Water sample concentration units are nanograms per liter (ng L⁻¹); bed sediment and fish sample concentrations are in nanograms per kilogram (ng kg⁻¹)[a]

Contaminant	Type	Water samples					Bed sediment samples					Fish samples				
		Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum
bla_{ACC}	ARG	9/15	60	—	—	—	7/14	50	—	—	—	—	—	—	—	—
bla_{FOX}	ARG	9/15	60	—	—	—	12/14	86	—	—	—	—	—	—	—	—
bla_{CIT}	ARG	5/15	33	—	—	—	6/14	43	—	—	—	—	—	—	—	—
bla_{CTX-M}	ARG	4/15	27	—	—	—	7/14	50	—	—	—	—	—	—	—	—
bla_{DHA}	ARG	4/15	27	—	—	—	6/14	43	—	—	—	—	—	—	—	—
bla_{EBC}	ARG	4/15	27	—	—	—	5/14	36	—	—	—	—	—	—	—	—
flo_{ST}	ARG	4/15	27	—	—	—	9/14	64	—	—	—	—	—	—	—	—
imv A	ARG	4/15	27	—	—	—	2/14	14	—	—	—	—	—	—	—	—
van A	ARG	4/15	27	—	—	—	6/14	43	—	—	—	—	—	—	—	—
bla_{MOX}	ARG	3/15	20	—	—	—	3/14	21	—	—	—	—	—	—	—	—
qnr A	ARG	3/15	20	—	—	—	1/14	7	—	—	—	—	—	—	—	—
qnr B	ARG	3/15	20	—	—	—	10/14	71	—	—	—	—	—	—	—	—
qnr S	ARG	2/15	13	—	—	—	3/14	21	—	—	—	—	—	—	—	—
spv C	ARG	2/15	13	—	—	—	5/14	36	—	—	—	—	—	—	—	—
tet M	ARG	2/15	13	—	—	—	4/14	29	—	—	—	—	—	—	—	—
int	ARG	13/15	87	—	—	—	12/14	86	—	—	—	—	—	—	—	—
bla_{CMY-2}	ARG	1/15	7	—	—	—	9/14	64	—	—	—	—	—	—	—	—
erm B	ARG	1/15	7	—	—	—	1/14	7	—	—	—	—	—	—	—	—
van B	ARG	1/15	7	—	—	—	0/15	0	nd	nd	nd	—	—	—	—	—
$bla_{CTX-M-32}$	ARG	0/15	0	—	—	—	3/14	21	—	—	—	—	—	—	—	—
bla_{TEM}	ARG	0/15	0	—	—	—	3/14	21	—	—	—	—	—	—	—	—
fem A	ARG	0/15	0	—	—	—	1/14	7	—	—	—	—	—	—	—	—
BLYES	Estrogenicity	5/15	33	0.34	0.72	2.34	—	—	—	—	—	—	—	—	—	—
Escherichia coli	Microbial	15/15	100	260	2450	18 270	12/14	86	10.0	103	512	—	—	—	—	—
Enterococci	Microbial	15/15	100	50.0	2600	11 235	—	—	—	—	—	—	—	—	—	—
Heterotrophic bacteria	Microbial	15/15	100	9090	77 100	>2419 600	14/14	100	5475	>241 960	>241 960	—	—	—	—	—
Total coliforms	Microbial	15/15	100	4430	77 100	120 980	14/14	100	10.0	1821	129 970	—	—	—	—	—
Microplastics	Microplastics	15/15	100	112	220	17 033	15/15	100	3782	43 668	109 435	5/5	100	199	301	1168
Atrazine (UI)	Pesticide	13/15	87	7.87	41.82	321.64	—	—	—	—	—	4/13	31	0.10	1.20	1.20
2,4-D	Pesticide	9/15	60	39.33	78.27	305.90	—	—	—	—	—	—	—	—	—	—
Sulfentrazone	Pesticide	9/15	60	19.66	50.97	185.13	—	—	—	—	—	—	—	—	—	—
OIAT	Pesticide	8/15	53	1.09	8.70	15.61	—	—	—	—	—	—	—	—	—	—
Azoxystrobin	Pesticide	7/15	47	1.20	2.17	6.17	—	—	—	—	—	—	—	—	—	—
Bentazon	Pesticide	6/15	40	4.87	6.41	20.65	—	—	—	—	—	—	—	—	—	—
CEAT	Pesticide	6/15	40	10.51	27.56	74.74	—	—	—	—	—	—	—	—	—	—
Dimethenamid	Pesticide	6/15	40	1.54	18.12	187.32	—	—	—	—	—	—	—	—	—	—
Prometon	Pesticide	6/15	40	1.81	2.99	17.61	—	—	—	—	—	—	—	—	—	—
Acetochlor SA	Pesticide	5/15	33	124.7	170.67	1329.53	—	—	—	—	—	—	—	—	—	—
Metalaxyl	Pesticide	5/15	33	5.48	7.47	10.04	—	—	—	—	—	—	—	—	—	—
Propazine	Pesticide	5/15	33	0.97	4.75	13.63	—	—	—	—	—	—	—	—	—	—

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Table 1 (Contd.)

Contaminant	Type	Water samples					Bed sediment samples					Fish samples				
		Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum
Diketonitrile isoxaflutole	Pesticide	4/15	27	4.24	9.86	37.42	—	—	—	—	—	—	—	—	—	—
Propiconazole	Pesticide	4/15	27	2.67	5.30	52.55	—	—	—	—	—	—	—	—	—	—
Tebuconazole	Pesticide	4/15	27	4.55	7.96	11.51	—	—	—	—	—	—	—	—	—	—
Bromacil	Pesticide	3/15	20	5.09	8.72	20.28	—	—	—	—	—	—	—	—	—	—
Carbendazim	Pesticide	3/15	20	2.92	10.79	18.54	—	—	—	—	—	—	—	—	—	—
Imidacloprid	Pesticide	3/15	20	17.89	36.86	70.00	—	—	—	—	—	—	—	—	—	—
Metribuzin	Pesticide	3/15	20	7.95	20.63	20.13	—	—	—	—	—	—	—	—	—	—
Fipronil	Pesticide	2/15	13	8.10	—	10.27	—	—	—	—	—	—	—	—	—	—
Phenylpyrazole	Pesticide	2/15	13	8.10	—	10.28	—	—	—	—	—	—	—	—	—	—
Simazine	Pesticide	2/15	13	10.01	—	17.61	—	—	—	—	—	—	—	—	—	—
Atrazine	Pesticide	15/15	100	9.43	67.77	1386.29	—	—	—	—	—	—	—	—	—	—
Metolachlor SA	Pesticide	15/15	100	197.09	595.26	1596.23	—	—	—	—	—	—	—	—	—	—
OIET	Pesticide	15/15	100	30.82	60.97	152.93	—	—	—	—	—	—	—	—	—	—
CAAT	Pesticide	14/15	93	26.12	100.45	316.37	—	—	—	—	—	—	—	—	—	—
CIAT	Pesticide	14/15	93	8.27	42.11	199.68	—	—	—	—	—	—	—	—	—	—
Metolachlor	Pesticide	14/15	93	3.78	77.91	612.07	—	—	—	—	—	—	—	—	—	—
Acetochlor	Pesticide	12/15	80	3.32	65.52	608.56	—	—	—	—	—	—	—	—	—	—
Dechlorometolachlor	Pesticide	12/15	80	2.27	7.34	41.09	—	—	—	—	—	—	—	—	—	—
Acetochlor OA	Pesticide	11/15	73	36.22	203.51	1251.32	—	—	—	—	—	—	—	—	—	—
Clothianidin	Pesticide	10/15	67	6.77	10.99	25.28	—	—	—	—	—	—	—	—	—	—
Hydroxymetolachlor	Pesticide	10/15	67	3.18	12.70	38.65	—	—	—	—	—	—	—	—	—	—
Imazethapyr	Pesticide	10/15	67	4.14	18.81	69.97	—	—	—	—	—	—	—	—	—	—
Metolachlor OA	Pesticide	10/15	67	113.28	266.09	688.61	—	—	—	—	—	—	—	—	—	—
Desulfinylfipronil	Pesticide	1/15	7	0.75	—	—	—	—	—	—	—	—	—	—	—	—
Dinotefuran	Pesticide	1/15	7	16.05	—	—	—	—	—	—	—	—	—	—	—	—
Fipronil amide	Pesticide	1/15	7	7.00	—	—	—	—	—	—	—	—	—	—	—	—
Sulfometuron methyl	Pesticide	1/15	7	3.48	—	—	—	—	—	—	—	—	—	—	—	—
Tebupirimfos	Pesticide	1/15	7	0.47	—	—	—	—	—	—	—	—	—	—	—	—
Tetraconazole	Pesticide	1/15	7	3.03	—	—	—	—	—	—	—	—	—	—	—	—
Thiamethoxam	Pesticide	1/15	7	17.50	—	—	—	—	—	—	—	—	—	—	—	—
Triclopyr	Pesticide	1/15	7	49.19	—	—	—	—	—	—	—	—	—	—	—	—
PFPeA	PFAS	3/15	20	7.00	E15.0	36.8	0/15	0	nd	nd	nd	0/13	0	nd	nd	nd
PFHpA	PFAS	0/15	0	—	—	—	0/15	0	nd	nd	nd	0/13	0	nd	nd	nd
PFBA	PFAS	6/15	40	6.70	12.9	E25.2	2/15	13	220	—	380	0/13	0	nd	nd	nd
HFPO DA	PFAS	0/15	0	—	—	—	0/15	0	nd	nd	nd	0/13	0	nd	nd	nd
6:2-FTS	PFAS	1/15	7	9.10	—	—	2/15	13	206	—	2600	1/13	8	2430	—	—
PFBS	PFAS	1/15	7	—	—	—	1/15	7	40.5	—	—	1/13	8	164	—	—
N-EtFOSAA	PFAS	0/15	0	—	—	—	3/15	20	46.6	114	314	1/13	8	102	—	—
PFOA	PFAS	2/15	13	2.00	—	36.7	2/15	13	50.3	—	50.3	2/13	15	119	—	451
PFHxS	PFAS	0/15	0	—	—	—	1/15	7	197	—	—	2/13	15	106	—	227
N-MeFOSAA	PFAS	0/15	0	—	—	—	2/15	13	58.0	—	495	2/13	15	121	—	145
PFOSA	PFAS	0/15	0	—	—	—	1/15	7	65.2	—	—	3/13	23	149	313	355

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Table 1 (Contd.)

Contaminant	Type	Water samples					Bed sediment samples					Fish samples				
		Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum	Detect	Percent detection	Minimum	Median	Maximum
PFDS	PFAS	0/15	0	—	—	—	1/15	7	190	—	—	4/13	31	160	171.5	203
PFTrDA	PFAS	0/15	0	—	—	—	1/15	7	766	—	—	4/13	31	111	168	265
PFTDeDA	PFAS	0/15	0	—	—	—	1/15	7	834	—	—	5/13	38	101	168	292
PFHxA	PFAS	0/15	0	—	—	—	5/15	33	47.3	71.40	102	6/13	46	220	371	1740
PFNA	PFAS	0/15	0	—	—	—	1/15	7	61.4	—	—	6/13	46	113	189	431
PFDoDA	PFAS	0/15	0	—	—	—	2/15	13	57.2	—	585	6/13	46	103	229	803
PFDA	PFAS	0/15	0	—	—	—	2/15	13	78.7	—	186	8/13	61	110	533	9620
PFUnDA	PFAS	0/15	0	—	—	—	3/15	20	46.9	81.8	330	10/13	77	103	241	620
PFOS	PFAS	0/15	0	—	—	—	8/15	53	45.3	55.25	413	13/13	100	413	3040	8580
Citalopram	PPCP	0/15	0	—	—	—	—	—	—	—	—	1/13	8	1	—	—
Venlafaxine	PPCP	3/15	20	6.63	141.47	143.21	—	—	—	—	—	2/13	15	0.80	—	2.8
Bupropion	PPCP	2/15	13	3.49	—	17.71	—	—	—	—	—	2/13	15	1.1	—	5.7
Sucralose	PPCP	8/15	53	133.68	271.28	782.42	—	—	—	—	—	—	—	—	—	—
1H-Benzotriazole	PPCP	6/15	40	1.69	33.74	233.62	—	—	—	—	—	—	—	—	—	—
4/5-Methyl-1H-benzotriazole	PPCP	6/15	40	4.88	37.17	230.72	—	—	—	—	—	—	—	—	—	—
Carbamazepine	PPCP	4/15	27	9.22	24.47	90.98	—	—	—	—	—	—	—	—	—	—
Desvenlafaxine	PPCP	4/15	27	13.66	217.40	594.47	—	—	—	—	—	—	—	—	—	—
Guanylurea	PPCP	4/15	27	40.33	1046.72	2038.24	—	—	—	—	—	—	—	—	—	—
Sulfamethoxazole	PPCP	4/15	27	10.26	30.17	90.85	—	—	—	—	—	—	—	—	—	—
Fexofenadine	PPCP	3/15	20	86.45	105.94	570.36	—	—	—	—	—	—	—	—	—	—
Fluconazole	PPCP	3/15	20	10.08	15.44	18.73	—	—	—	—	—	—	—	—	—	—
Methocarbamol	PPCP	3/15	20	9.60	17.80	74.33	—	—	—	—	—	—	—	—	—	—
Lidocaine	PPCP	2/15	13	16.22	—	85.68	—	—	—	—	—	—	—	—	—	—
Metformin	PPCP	13/15	87	113.33	822.50	8990	—	—	—	—	—	—	—	—	—	—

[a] ARG, antibiotic resistance genes; PFAS, per- and polyfluoroalkyl substances; PPCP, pharmaceutical and personal care products; nd, not detected; —, not applicable; UI, University of Iowa.

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study, which indicate unexpected anthropogenic sources. Microplastics, PFAS, ARGs, PPCPs, and pesticides were found in all streams sampled with 86 of 164 (about 52%) target contaminants being detected in water from at least one stream.[109] Additionally, 28 target contaminants were detected in at least 50% of water samples, and 8 contaminants were detected in water from every stream sampled. Contaminants detected in every stream were *E. coli*, enterococci, heterotrophic bacteria, total coliforms, microplastics, atrazine, 2-hydroxyatrazine (OIET), and metolachlor sulfonic acid (SA). The number of contaminants in individual stream water samples ranged from 17 to 54 (Tables 1, S5 and S6†).[109] Although the presence of pesticides (including pesticide transformation products), nitrate, and bacteria and ARGs was expected based on known agricultural inputs[48,67,73,75] and previous research indicates that transport of PFAS and ARGs to streams does occur,[48,90] such ubiquity for microplastics, PFAS, ARGs, and PPCPs in these small agricultural streams was unexpected (Fig. 2).

The PPCPs metformin and sucralose have previously been associated with urban wastewater;[61,110] thus, their presence in agriculturally dominated streams is particularly notable. For example, metformin, detected in 13 of 15 sites (Table S5†), had detections in the Little Floyd and Storm Creek at estimated values of 9,000 ng L^{-1} and 5500 ng L^{-1}, respectively.[109] In WWTPs, roughly 90% of metformin is microbially transformed to guanylurea;[111] the Little Floyd has a single WWTP in its watershed, and Storm Creek has five. Neither site had noteworthy guanylurea detections (40 ng L^{-1} at Little Floyd Creek and no measurable concentrations at Storm Creek[109]). This indicates that metformin (and presumably detected PPCPs) is not likely derived from the WWTPs in the watershed, but instead may be from substandard septic systems in these areas.[112,113] Consistent with this hypothesis, two sites that were sampled directly downstream from WWTPs had substantial concentrations of both metformin and guanylurea of 2435 ng L^{-1} and 2038 ng L^{-1}, respectively (Muddy Creek), and 4820 ng L^{-1} and 2009 ng L^{-1}, respectively (Cardinal Creek).[109] Sucralose was detected at several sites (8 of 15), with detections ranging from about 134 ng L^{-1} to about 782 ng L^{-1}. Although the presence of sucralose does not generally confirm the presence of untreated sewage (such as through a septic system or other point source) as with metformin, sucralose may be used as an indicator of human waste.[114] These detections may point towards leaking or ineffective/substandard septic systems[115] as sources of PPCPs detected at sites other than those directly associated with WWTPs in this study.

The presence of complex mixtures of contaminants in water may drive biological endpoints, including total estrogenicity. One-third of the stream water samples (5 of 15) for this study had measurable estrogenicity with 20% (3 water samples, one each from South Fork Iowa River, Muddy Creek, and Cardinal Creek) having estrogenicity within the range of potential environmental concern (exceeding 0.5 ng E2Eq/L).[109] Estrogenicity in surface water may be influenced by various organic contaminants including but not limited to steroidal estrogens (*e.g.*, estrone), phytoestrogens, and mycotoxins.[6,116] These estrogenic compounds are known to be present in streams in this area through land application of animal manure and multiple mechanisms of transport such as overland flow and tile drainage, among others.[117–119] Additionally, the presence of
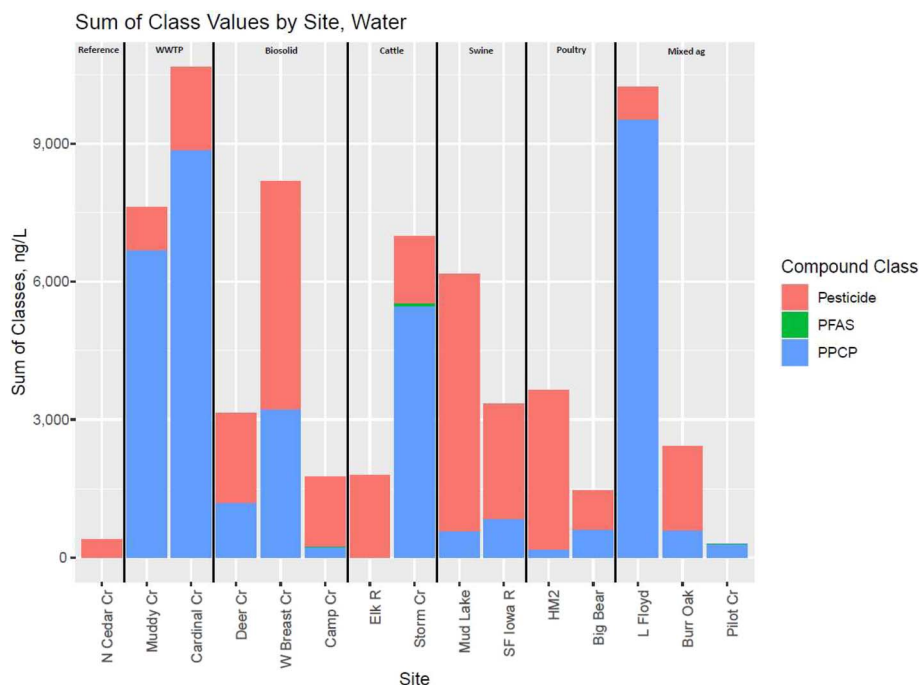
Fig. 2 Sum of compound class values in nanograms per liter by site for 15 Iowa streams, 2021 [ng L^{-1}, nanograms per liter; PFAS, per- and polyfluoroalkyl substances; PPCP, pharmaceuticals and personal care products; refer to Table S1† for full site names and information; Cr, Creek; R, River].

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Table 2 Modified National Oceanic and Atmospheric Administration (NOAA) microplastics analysis method counts as compared to NOAA microplastics analysis method (90 000 µm²/0.3 mm threshold) by site and sample medium for 15 Iowa streams, 2021. Per size distribution charts, most particles were under 5000 µm²,[a]

Sites	Water samples 90 000 µm cutoff Count	%	Mass	5000 µm cutoff Count	% <5000 µm	Bed sediment samples 90 000 µm cutoff Count	%	5000 µm cutoff Count	% <5000 µm	Fish samples 90 000 µm cutoff Counts	%	5000 µm cutoff Count	% <5000 µm
Big Bear Creek at Ladora, Iowa	E4124	—	—	E4124	—	3/109 435	0.003	108 185/109 435	0.989	0/301	0.000	239/301	0.794
Burr Oak Creek near Perkins, Iowa	1/159	0.629	0.118	85/159	0.535	0/18 633	0.000	18 610/18 633	0.999	—	—	—	—
Camp Creek near Runnells, Iowa	E17033	—	—	E17 033	—	3/27 951	0.011	27 558/27 951	0.986	—	—	—	—
Deer Creek near Coralville, Iowa	0/246	0.000	0.000	148/246	0.602	119/35 589	0.334	30 686/35 589	0.862	0/1168	0.000	981/1168	0.840
Elk River near Almont, Iowa	3/143	2.098	0.433	78/143	0.545	7/62 362	0.011	61 680/62 362	0.989	—	—	—	—
HM-2	1/396	0.253	0.114	347/396	0.876	2/43 668	0.005	43 279/43 668	0.991	—	—	—	—
Little Floyd River near Sanborn, Iowa	5/577	0.867	0.672	508/577	0.880	93/51 216	0.182	39 860/51 216	0.778	—	—	—	—
Mud Lake near Jewell, Iowa	0/358	0.000	0.000	304/358	0.849	1/3782	0.026	3746/3782	0.990	—	—	—	—
Muddy Creek near Coralville, Iowa	2/201	0.995	0.205	162/201	0.806	9/9551	0.094	8758/9551	0.917	3/199	1.508	120/199	0.603
North Cedar Creek near Clayton, Iowa	0/112	0.000	0.000	55/112	0.491	3/53 437	0.006	51 788/53 437	0.969	—	—	—	—
Pilot Creek at County Highway P15 near Rolfe, Iowa	3/171	1.754	0.390	121/171	0.708	4/61 799	0.006	59 229/61 799	0.958	0/295	0.000	201/295	0.681
Cardinal Creek below Newton, Iowa	0/207	0.000	0.000	149/207	0.720	6/88 664	0.007	87 389/88 664	0.986	—	—	—	—
South Fork Iowa River near New Providence, Iowa	4/220	1.818	0.959	143/220	0.650	4/17 193	0.023	16 419/17 193	0.955	0/877	0.000	840/877	0.958
Storm Creek near Glidden, Iowa	0/468	0.000	0.000	396/468	0.846	32/10 974	0.292	9632/10 974	0.878	—	—	—	—
South White Breast Creek near Osceola, Iowa	1/592	0.169	0.091	540/592	0.912	2/43 668	0.005	43 279/43 668	0.991	—	—	—	—

[a] E, estimated; #, number; —, not applicable; %, percent; <less than; µm, micrometer.

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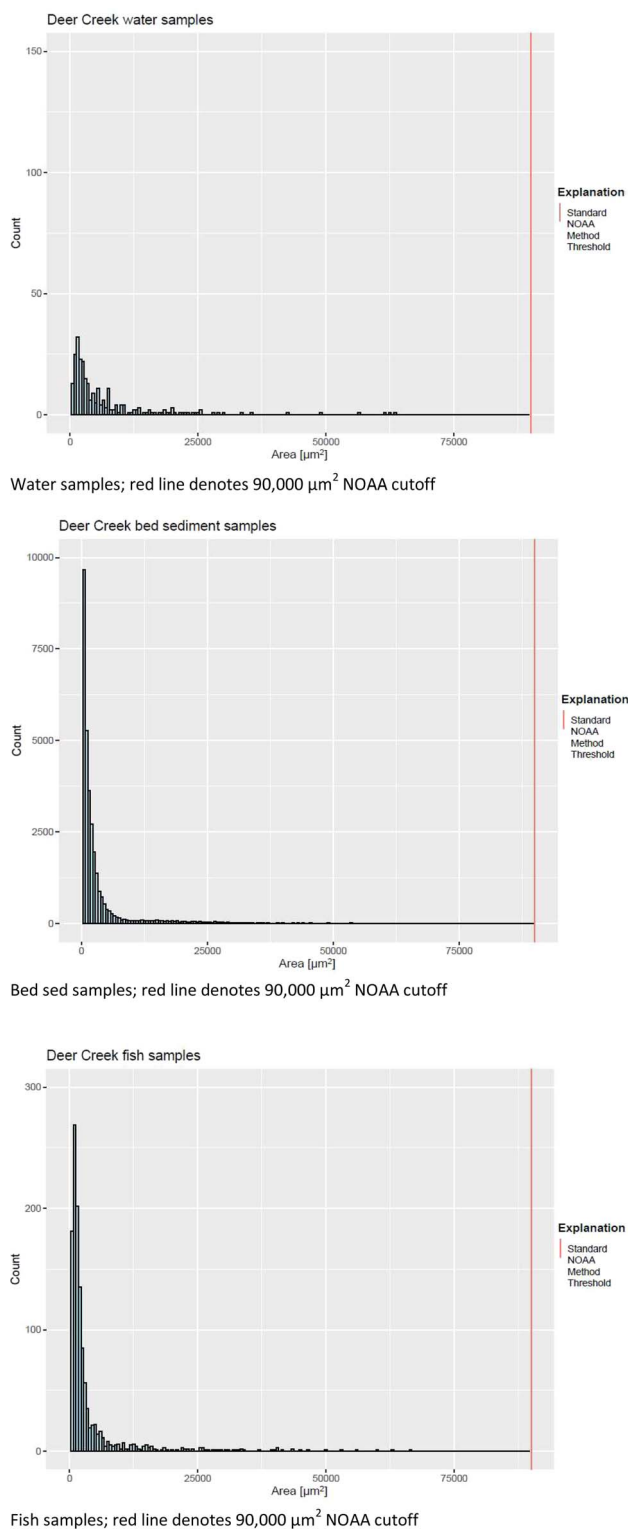
Fig. 3 Size distribution of microplastic particles by medium at Deer Creek, Iowa, 2021 [μm^2, square micrometers].

plastics in streams may influence estrogenicity, as phthalates are known to leach from plastics.[120] Phthalates may also be present in other personal care products and are known to accumulate in the environment.[121]

The number of ARGs present in individual water samples (2–10) and bed sediment samples (2–13) (Table S6†) was highly variable by site. Storm Creek ($n = 9$) and Camp Creek ($n = 10$) had the highest number of ARGs detected in the water, whereas the highest number of ARGs detected in the bed sediment ($n = 13$) were from the Little Floyd River, Big Bear Creek, and Burr Oak Creek. As in Givens,[48] a higher number of ARGs were detected in the bed sediment than the water, indicating that bed sediment may enhance the environmental persistence of antibiotics, ARB, and ARGs in the environment.[48] The integrase (*int*) gene was detected in bacteria growth cultivated from either bed sediment or water from all samples except one site (HM-2). The prevalence of this ARG occurrence is notable as this gene indicates a potential for horizontal gene transfer between environmental bacteria and consequently the spread of environmental antibiotic resistance.[122–124]

The presence of PFAS in water samples was not unexpected and found in 9 of 15 sites, with concentrations ranging from 6.70 to 36.8 ng L^{-1} (Table 1).[109] Additionally, PFAS were detected in 12 of 15 sediment samples (40.5–2600 nanograms per kilogram [ng kg^{-1}]) and all fish samples collected at 13 sites (101–9620 ng kg^{-1}). PFAS detections in water were found to be significantly positively correlated to detections of an insecticide, imidacloprid, specifically, as well cumulative insecticide detections (Table S7†). Both inert ingredients in pesticide formulations and leaching from containers can contribute to associations between pesticide and PFAS concentrations.[125–127]

Microplastics prevalent in all physical and biological compartments

Microplastics were detected at all sites in water and bed sediment samples and in fish samples from five sites (Table S8†). Statistically significant relations were observed between water clarity (field turbidity), suspended sediment mass, and suspended sediment concentration and microplastic counts in water samples (Table S7†). While counts and sizes of particles varied by site and medium, the bulk of the particles were smaller than 5000 μm^2 (Table 2); the percentage of water sample microplastic particles <5000 μm^2 ranged from about 49 to 91 by site (median 72; Table 2). Microplastics were generally smaller in sediment samples, with about 78 to 99.9% (median 98.6%) <5000 μm^2 (Table 2). The water sample with the lowest number of particles <5000 μm^2 was North Cedar Creek at about 49% (55 of 112 particles), but the associated sediment sample had about 97% of particles <5000 μm^2 (51 788 of 53 437 particles; Table 2). For all five fish samples analyzed for microplastics, the percentages of particles <5000 μm^2 was more comparable to the water samples with a range of about 60 to 95% (median 79.4; Table 2).

Had the microplastic analysis been conducted using the standard NOAA method,[88] the particle counts by sample would have been fundamentally different. For all sites and results across matrices, the standard NOAA method would have only detected 2% of particles compared to the modified methods. Similarly, the standard NOAA method would have only accounted for up to 0.4% of particle mass (only quantified for

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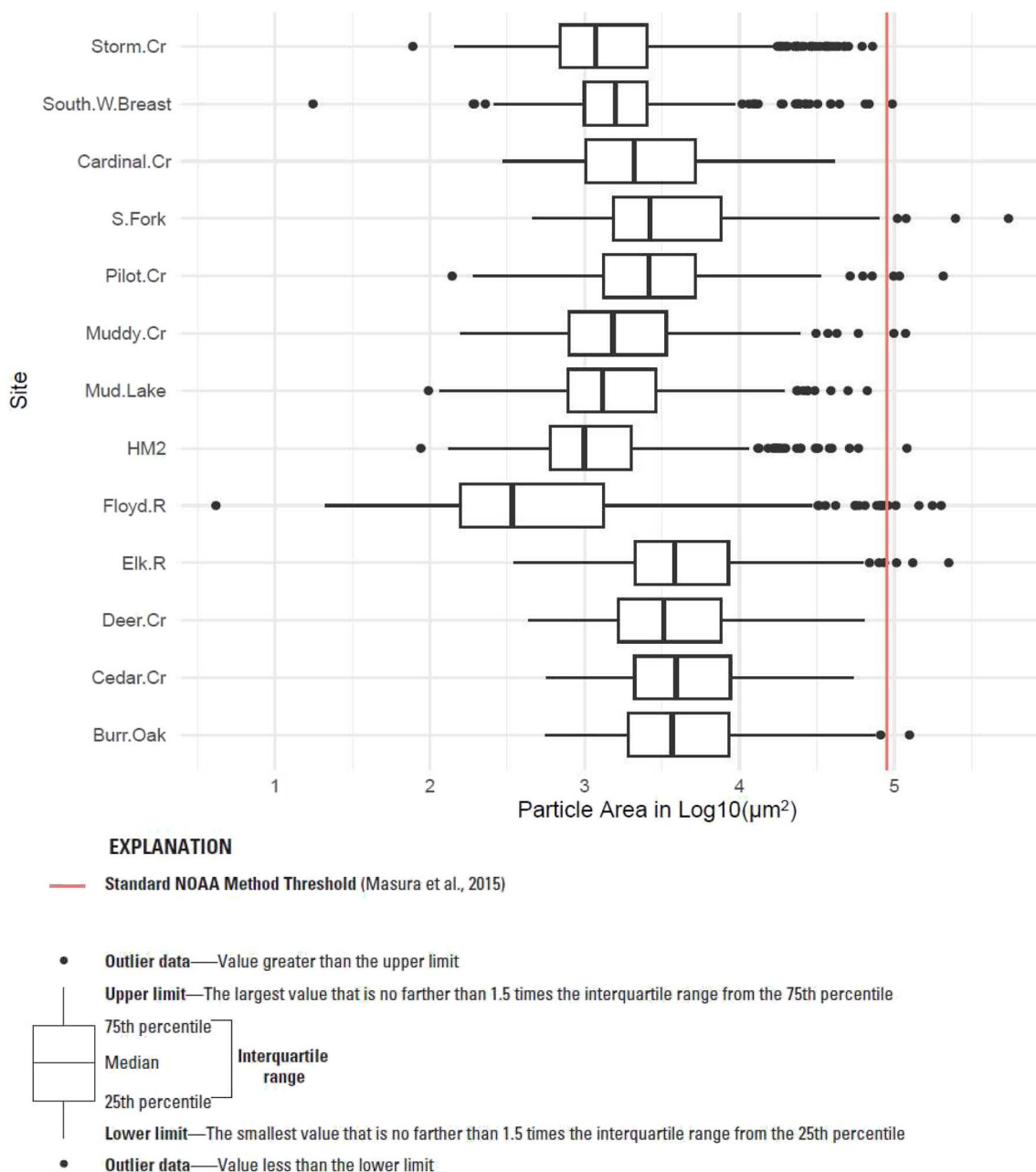
Fig. 4 Log transformed areas of microplastic particles by site for water samples for 15 Iowa streams, 2021. Red line denotes NOAA method threshold (90 000 μm^2).

water samples). For example, the water sample for Deer Creek had a total count of 246 with particle sizes ranging from 433–63 700 μm^2 (Table S7†).[109] The size threshold for the standard NOAA method is 0.3 mm (90 000 μm^2), which would have missed all 246 particles at this site. Likewise, the bed sediment sample from Deer Creek had particle sizes ranging from 41.9–556 000 μm^2, and all but 119 of 35 589 particles would have been missed by the standard NOAA method (Table 2). Fish samples from the same site had all 1,168 particles below the standard NOAA method threshold. The standard NOAA method would have resulted in a gross underestimation of the influence of

microplastics at Deer Creek (Fig. 3 and S9†).[88] These numbers are similar for all sites (Table 2) and matrices (water 2%, sediment 0.33%, fish 1.5%). Notably, recent research has documented that microplastics below the standard NOAA threshold can have significant environmental effects.[128] Using a microscope, all sample particles were evaluated for their shape and identified as either a fiber, fragment, or round. When evaluating by shape, most sites were dominated by plastic fragments, with fibers as secondary (ranging from 2–31%). The exception to this was the Little Floyd River, which had only fragments (64%) and particle rounds (35.7%) identified.[109] For perspective, the

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Table 3 Sum of exposure activity ratios ($\sum$EAR) for water samples and toxicity quotients ($\sum$TQ) for chronic and acute chemical exposures using the Aquatic Life Benchmarks (U.S. Environmental Protection Agency, 2023) by site (chemical exposures only) for 15 Iowa streams, 2021

Site	$\sum$EAR water	$\sum$TQ fish chronic	$\sum$TQ fish acute
Big Bear Creek at Ladora, Iowa	0.001	0.010	0.010
Burr Oak Creek near Perkins, Iowa	0.002	0.022	0.010
Camp Creek near Runnells, Iowa	0.001	0.015	0.022
Deer Creek near Coralville, Iowa	0.004	0.018	0.015
Elk River near Almont, Iowa	0.000*	0.000	0.018
HM-2	0.003	0.029	0.000
Little Floyd River near Sanborn, Iowa	0.005	0.007	0.029
Mud Lake near Jewell, Iowa	0.006	0.054	0.007
Muddy Creek near Coralville, Iowa	0.004	0.006	0.054
North Cedar Creek near Clayton, Iowa	0.000*	0.002	0.006
Pilot Creek at County Highway P15 near Rolfe, Iowa	0.020	0.095	0.002
Cardinal Creek below Newton, Iowa	0.007	0.053	0.095
South Fork Iowa River near New Providence, Iowa	0.007	0.050	0.053
Storm Creek near Glidden, Iowa	0.005	0.015	0.050
South White Breast Creek near Osceola, Iowa	0.009	0.299	0.015
EAR	$\geq$0.001 potential concern	1.0 high risk	
TQ	0.1 moderate risk	1.0 high risk	
	* Rounded values		

highest percentage of rounds for other sites was 0.8% at South White Breast Creek and 0.4% at Storm Creek.

All sites, in all sample mediums, had right-skewed distributions in the particle size of detected microplastics (Fig. S10, Table S11†). Results from Fligner-Killeen tests also found statistically significant differences in the variance across sites, with $p < 0.05$. Using a one-factor permutation test,[99] statistically significant differences in the mean particle size were found in water samples for both site and primary land use type. Similar results were found for bed sediment samples by both site and primary land use type. The mean microplastic particle size in surface water samples was significantly larger than that of the bed sediment samples for all primary land use types and all but two sites, with p values <0.05. There were not statistically significant differences in water *versus* bed sediment samples at the Little Floyd River and Storm Creek. Further, the size of particles found in water samples from the Little Floyd River were smaller than all other samples (Fig. 4).

Potential biological effects using exposure activity ratios (EAR)

As an assessment of the potential for biological effects from pesticide, pharmaceutical, and PFAS concentrations for water samples, EAR values were computed by site (Tables 3 and S12†). No sites exceeded the cumulative EAR ($\sum$EAR) (sum of EAR for each contaminant) threshold of 1.0, a level at which biological effects may be expected. Thirteen sites did have $\sum$EAR of $\geq$0.001, a conservative threshold used to document samples of potential concern,[129] with $\sum$EAR values ranging from 0.001–0.020. Pilot Creek had the highest $\sum$EAR value (0.020) and Elk River and North Cedar Creek had the lowest $\sum$EAR values (0.000003 and 0.00003, respectively). Individual EAR values were >0.001 for acetochlor (9 sites), metformin (3 sites), fipronil (2 sites each), and atrazine and metolachlor (1 site each) (Table S12†). Notably, of the 128 chemicals detected for this study, only

40 had available toxicity data necessary to calculate EAR. Thus, this assessment is likely conservative as it does not account for potential biological effects of chemicals without relevant toxicity data or for chemicals not in our suite of targeted analysis.

Potential biological effects using toxicity quotients (TQs)

To assess potential cumulative-exposure effects from organic contaminants, ΣTQs were calculated using ALBs.[107] A single site, White Breast Creek, exceeded the moderate risk TQ threshold of 0.1 for chronic and acute chemical exposures using the ALB analysis of pesticides and their transformation products in water samples. The highest $\sum$TQ using pesticide data was 0.299 at White Breast Creek, well below the established criteria (Tables 3 and S13†). When computing $\sum$TQs using microplastics data in water, however, the results are very different. Food dilution or false satiation may result from fish ingesting microplastic particles, which then may modify foraging efficiency. Insufficient nutrition including physical issues and depressed immune function can also result from the ingestion of non-food items.[130] As the analysis of microplastic samples for this study allows for detection of very small particles, all particles were included in the analysis of TQs for food dilution (all particles under the method detection limit were considered estimated values; no particles were censored). All particle detections were under the threshold criteria for size and PPL for each site. As such, the TQs for both chronic exposure (37.3–5680, median 82) and acute exposure (3.29–501, median 7.24) are very high, all above the 1.0 threshold for high risk (Table 4). Tissue translocation is a scenario where particles can potentially cross organ boundaries, or create physical stress such as inflammation, which can lead to longer term health issues.[131] When assessing tissue translocation, only the smallest fraction of particle

Table 4 Toxicity quotients (TQs) for proposed microplastic benchmarks for chronic and acute exposures for two health outcomes. Results presented by site for 15 Iowa streams, 2021

Site	Primary landuse	Percentage less than 6889 μm²	Food dilution (FD)			Tissue translocation (TT)		
			FD MP counts[c]	Chronic TQ[d]	Acute TQ[d]	TT MP counts[b]	Chronic TQ[d]	Acute TQ[d]
Big Bear Creek at Ladora, Iowa	Poultry	Unable to quantify	4120	**1370**	**121**	E3050[a]	**E210**	**14.2**
Burr Oak Creek near Perkins, Iowa	Mixed Ag	75.4	159	53	4.68	120	2	0.135
Camp Creek near Runnells, Iowa	Biosolid	Unable to quantify	17 000	**5680**	**501**	E12 600[a]	**E50.9**	**3.43**
Deer Creek near Coralville, Iowa	Biosolid	75.6	246	82	7.24	186	3.1	0.209
Elk River near Almont, Iowa	Cattle	77.6	143	47.7	4.21	111	1.85	0.125
HM-2	Poultry	93.2	396	132	11.7	369	6.15	0.415
Little Floyd River near Sanborn, Iowa	Mixed Ag	92.7	577	192	17	535	8.92	0.601
Mud Lake near Jewell, Iowa	Hog	95.3	358	119	10.5	341	5.68	0.383
Muddy Creek near Coralville, Iowa	WWTP	92.0	201	46.3	4.09	185	3.08	0.208
North Cedar Creek near Clayton, Iowa	Reference	74.1	112	37.3	3.29	83	1.38	0.093
Pilot Creek at County Highway P15 near Rolfe, Iowa	Mixed Ag	87.7	171	57	5.03	150	2.5	0.169
Cardinal Creek below Newton, Iowa	WWTP	86.0	207	69	6.09	178	2.97	0.2
South Fork Iowa River near New Providence, Iowa	Hog	79.1	220	73.3	6.47	174	2.9	0.196
Storm Creek near Glidden, Iowa	Cattle	90.4	468	156	13.8	423	7.05	0.475
South White Breast Creek near Osceola, Iowa	Biosolid	96.1	592	197	17.4	569	9.48	0.639
				Benchmark 0.3	Benchmark 5		Benchmark 60	Benchmark 890

[a] Estimated based on lowest percentage of particles within threshold criteria for all sites (74%); benchmarks are in microplastics particles per liter (PPL); #E, estimated; μm², micrometers squared. [b] Limited to microplastic particles <6889 μm². [c] No size cutoff. [d] Italic = 0.1, moderate risk and bold = 1.0, high risk.

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sizes was included. In this instance, all particles larger than 6889 μm^2 were censored out of the analysis. Two sites, Big Bear Creek and Camp Creek, had extremely high microplastics counts in water samples (4120 and about 17 000, respectively, Table 2), which did not allow for the computation of particle sizes. To compute TQs for these two sites, we made inferences on the percentage of particle sizes that would fit the size criteria to arrive at estimated (E) values. Of the 13 sites that did have particle size information, the percentage of particles in the smaller fraction ranged from 74 to 96% (Table 4). To remain conservative and allow for error, the lowest percentage (74%) was used to estimate the number of particles within the size criteria at Big Bear Creek and Camp Creek. All tissue translocation TQs for chronic exposure and two tissue translocation TQs for acute exposure exceed the high-risk threshold of 1.0. For chronic exposure, this range was 1.38–E210, median 3.1; for acute exposure, those values were 14.2 and 3.43. All other tissue translocation TQs for acute exposure exceed the moderate risk threshold of 0.1, with values ranging from 0.09–14.2, median 0.21 (Table 4).

As this study collected fish opportunistically, there was some variation between species, trophic levels, and feeding zones. All fish were small, with the largest fish not exceeding about 127 mm in length. Most fish were Cyprinid minnows, but white sucker (*Catostomus commersonii*) were also collected. Whole fish were analyzed, so information on where in or on the body the microplastics particles had accumulated is not available. All five fish samples analyzed had microplastics present, with particle counts ranging from 199 to 1168 per individual.[109] Without information on the health of the fish analyzed (*e.g.*, inflammation presence/absence, location of the particles, abnormalities) and if we extend the proposed benchmarks to apply to these fish, 100% of all particles found would be within the proposed benchmark cutoff for food dilution (<0.25 cm^2). The tissue translocation results would be similar to the food dilution results for fish; using the particle size benchmark for tissue translocation of <6889 μm^2, 74 to 97% of the particles found within the individual fish would fall within the benchmark.

Potential future work

As the target analytes were not identical between water, bed sediment, and fish samples due to method and funding availability, it would be beneficial for future work to include as much analysis cross-over as possible. For this study, microplastics and PFAS were measured in all three matrices (*i.e.*, stream water, stream bed sediment, fish). Water sample results show that microplastics and PFAS were prevalent in wadable Iowa streams influenced by diverse agricultural activities and human wastewater contributions. A more complete understanding of environmental exposures, however, is obtained when additional environmental matrices are included. For example, the trends between sample types were substantially different for microplastics (bed sediment > water > fish) compared to that observed for total PFAS (fish > bed sediment > water). Although these two classes of CGCs are very different—microplastics are physical

particles, PFAS are chemicals—they both were found to be ubiquitous in environmental compartments in wadable streams across Iowa. In future research, the collection of additional aquatic and terrestrial species to better understand their trophic transfer through the food web is warranted.[35,132,133] Additionally, the present study has limitations based on the duration, scope, and resources available that may make future work valuable to build on the findings herein. For example, although this work focused on wadable streams throughout the agriculturally intensive state of Iowa, consideration of other land uses across different land use types and other regions, as well as other aquatic species to consider for biological endpoints is beneficial. For example, including ecological assessment of other aquatic species relevant to the trophic structure of wadable streams may expand the understanding of effects in agroecosystems. Specifically, the inclusion of additional biological endpoints, such as assessing the health of fish (*e.g.*, inflammation or immune function), may enhance our understanding of the ecological impacts of these contaminants.[134] Finally, more detailed assessments of potential sources of CGC and exposure routes in wadable streams in agroecosystems, including potential for eventual human exposure through water, food, and contact may also be beneficial. We frame this current work as the first concurrent, multi-matrix study of wadable streams in Iowa as a representative agriculturally intensive water system, but future efforts as described may improve the detailed understanding, implications, and generalizability of the findings.

Data availability

All data are available in the ESI,† a data release,[109] and/or the U.S. Geological Survey National Water Information System (NWIS) database.[135] All data from analyses for water done at the NWQL (pesticides, PFAS, and nutrients) and PFAS analyses for bed sediment and fish done at SGS Axys are available in NWIS using the site numbers in Table S1.† Data are presented as rounded to 3 significant figures in this publication.

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Author contributions

Shannon Meppelink: project conceptualization, data collection/analysis, writing – original draft preparation. Dana Kolpin: project conceptualization, data analysis, writing – original draft preparation, reviewing and editing. Gregory LeFevre: project conceptualization, laboratory analysis, original draft preparation, reviewing and editing. David Cwiertny: project conceptualization, writing – reviewing and editing, funding acquisition. Carrie Givens: project conceptualization, laboratory analysis, writing – reviewing and editing. Lee Ann Green: laboratory analysis, writing – reviewing and editing. Laura Hubbard:

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Conflicts of interest

All authors declare that they have no conflicts of interest.

Acknowledgements

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