TABLE OF CONTENTS

MATERIALS AND METHODS .................................................................................................... 1
  Site Description .................................................................................................................. 1
  Sample Collection and Instrumentation at WF-1 .............................................................. 2
  Analysis of Herbicides in Water ...................................................................................... 3

RESULTS AND DISCUSSION ..................................................................................................... 4
  Precipitation and Hydrology .......................................................................................... 4
  Simazine Transport and Degradation ............................................................................. 6

REFERENCES ............................................................................................................................... 8
MATERIALS AND METHODS

Site Description

The primary soil series overlying the bedrock and surrounding the cave entrance is the Baxter gravelly silt loam, 12-20% slope (fine, mixed, semiactive, mesic Typic Paleudalfs), a clayey residuum formed on ridges of the karst uplands in this area (Figure S1). Within the sink, the soil is up to 3 m thick. Beneath the soil is an additional 15-20 m of rock to the cave ceiling. The Lost River Chert, a discontinuous interbedded silicified limestone, lies between the surface and cave ceiling. The climate is humid subtropical, and the site has a mean annual precipitation of 1,300 mm and air temperature of 14.7°C (Kentucky Climate Center,) based on five long-term weather observation stations located within 35 km of the cave. Soil temperature data at 5 and 100 cm depths were obtained by request from the Kentucky Mesonet for a site in Warren County, KY that is within 30 km of the cave (37.01 N; 86.11 W). Surface weather conditions adjacent to

Figure S1. Major soil series within the field and surrounding the Crumps Cave cave entrance.
the field and above the cave were recorded every 10 minutes with a Hobo model U30 Weather Station (Onset Computer Corporation, Bourne, MA; Figure 1). Measurements included rainfall, air temperature, relative humidity, solar radiation, wind speed and direction, and barometric pressure.

Major soil series within the cornfield and the proportion of the field area, in parentheses, are: Baxter gravelly silt loam, 6-12% slopes (30%); Crider silt loam, 2-6% slope (fine-silty, mixed, active, mesic Typic Paleudalfs) (40%); and Pembroke silt loam, 0-2% slope (fine-silty, mixed, active, mesic Mollic Paleudalfs) (21%) (Figure S1). The remaining 9% of the field was classified as Baxter silt loams of varying slopes. Soils extend to at least 2 m depth, have approximately 3% organic matter in the surface horizons, and are classified as well drained. Clay mineralogy is mixed and shrink-swell clays are generally absent or negligible. Saturated hydraulic conductivity ranges from 1.4-14.1 µm s⁻¹ and restrictive soil layers that impede root growth or vertical water movement are absent. These soils are not subject to flooding, but infrequent ponding can occur. All soils data were obtained from the SSURGO database for Warren County, KY (USDA-NRCS, 2014).

**Sample Collection and Instrumentation at WF-1**

Water samples were collected from WF-1 at varying intervals through the year either as grab samples or using Isco model 3700 automatic water samplers (Teledyne Isco, Lincoln, NE). The automated samplers were equipped with 24, 1-L polypropylene bottles, and the sip volume was 1 L. High-flow samples were collected from hours to days depending upon the magnitude of the event, but never longer than one week. The temperature in the cave at the samplers averaged about 12°C and ranged from 8°C to 16°C. Grab samples at WF-1 and WF-2 were collected into
1-L polypropylene sample containers. Samples were stored on ice during transport, then frozen until herbicide analyses could be conducted.

Discharge was measured at WF-1 by directing the flow into a large funnel-shaped tarp and then into a 20-L barrel shaped weir with four openings at different heights. Stage-discharge relationships were developed for each opening to accommodate a broad range of discharge. Water level in the barrel was measured with a Druck PDCR 1830 pressure transducer (General Electric Co., Fairfield, CT) every 10 minutes. Specific conductance (SpC) and water temperature were measured every 10 minutes with a model CS547-L probe (Campbell Scientific, Logan, UT) with all data recorded on a CR10x datalogger (Campbell Scientific, Logan, UT).

**Analysis of Herbicides in Water**

In addition to the triazines, all samples were analyzed for alachlor [2-chloro-N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide], acetochlor [2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide], metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methyl ethyl)acetamide], and metribuzin [4-amino-6-(1,1-dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one]. Prior to solid-phase extraction (SPE), 200 mL sample aliquots were spiked with the 100 ng of the surrogate terbutylazine (2-N-tert-butyl-6-chloro-4-N-ethyl-1,3,5-triazine-2,4-diamine). Terbutylazine is a structural analog of atrazine that was never registered for use on U.S. cropland and was, therefore, not present in the water samples. Samples were then extracted with Varian LRC 500-mg silica bonded C\textsubscript{18} SPE cartridges (Varian Inc., Harbor City, CA; now owned by Agilent Technologies, Inc. New Castle, DE), and the compounds were eluted from the SPE cartridge with ethyl acetate. Following elution, the ethyl acetate was evaporated to ~0.3 mL under a stream of ultra-pure N\textsubscript{2} at 30°C, and 100 ng of
phenanthrene-d$_{10}$ was then added as an internal standard for the purpose of determining the final solvent volume.

Two instruments were used for GC/MS quantification. Samples collected in 2011 were analyzed by a Varian 3400 with a Saturn 2000 ion-trap mass selective detector (Varian Inc., Harbor City, CA; Agilent Technologies, Inc. Wilmington, DE). Samples collected in 2012 were analyzed by an Agilent 7890A GC (Agilent Technologies Inc., New Castle, DE) equipped with a Varian 240 ion-trap mass selective detector. The same instrument configuration and detector settings were used for both instruments. An HP-1 (Agilent Technologies, Wilmington, DE) capillary fused-silica column (0.33 µm film thickness; 12 m X 0.2 mm i.d.) was used with He as the carrier gas at a flow rate of 1 mL min$^{-1}$. A multi-step temperature program with a total run time of 27 minutes was used to separate the analytes. The mass selective detector was operated in selected ion storage mode for quantification and in full scan mode to confirm compound identity. Final sample concentrations were corrected for terbutylazine and compound specific recoveries determined from field spikes. At least two samples from each set collected (30-40 samples per set) were spiked at environmentally relevant concentrations to determine compound recoveries. Analysis of duplicate and blank quality assurance samples were also performed with each sample set. The SPE and GC/MS procedures were described in further detail by Lerch et al. (2015).

RESULTS AND DISCUSSION

Precipitation and Hydrology

Daily precipitation during the study ranged from 0.0 to 72 mm, and 63% of all days had no measurable precipitation (Figure 2a). Monthly precipitation ranged from a low of 45.6 mm in Jan 2011 to a high of 264 mm in Apr 2011. The wettest period was from Feb to Jun 2011 when
every month had precipitation >100 mm. The other wet period was from Nov 2011 to Mar 2012; three months (Nov, Dec, and Jan) had >100 mm of precipitation and Mar 2012 had 97.1 mm. The 2011 total precipitation of 1492 mm was 15% greater than the long-term average.

Peak instantaneous discharge during the study was >11.0 L s\(^{-1}\) on three occasions, during events in Feb, Mar, and May 2011 (Figure S2). There were two periods of consistently high discharge during the study that coincided with the periods of high precipitation. One was from late Feb to mid-May 2011, a period in which 63% of all days with average discharge >0.50 L s\(^{-1}\) occurred. Another high flow period was from mid-Nov 2011 through March 2012 which accounted for the remainder of the days with average discharge >0.50 L s\(^{-1}\). Data for a series of high discharge events in Apr and May 2011 showed that discharge increased dramatically from

![Figure S2. Instantaneous precipitation measured at Crumps Cave (a), and instantaneous discharge, specific conductance, and water temperature measured at WF-1 (b) from Feb 15 to May 15, 2011. All measurements were made at 10-minute intervals.](image-url)
<0.3 L s\(^{-1}\) to >5 L s\(^{-1}\) within an hour following at least 5 mm of precipitation input during this time period (Figure S2b). Corresponding rapid decreases in SpC and increases in water temperature also occurred.

**Simazine Transport and Degradation**

Time series data for simazine showed similar trends at WF-1 and WF-2 (Figure S3). From late Jan to early May 2011, simazine was consistently detected at concentrations ranging from 0.008 to 0.025 µg L\(^{-1}\) at WF-1 and 0.015 to 0.043 µg L\(^{-1}\) at WF-2. This reflected the transport of simazine from previous application(s) within the recharge area. In addition, degradation of residual simazine in the soil would be expected to contribute DIA to the epikarst drains. The concentration ratio of DIA:DEA (D\(^2\)R) has been shown to distinguish the parent sources of metabolites transported through the vadose zone and to groundwater and streams (Mills and Thurman, 1994; Thurman et al., 1998; Meyer et al., 2001). Atrazine degradation in soils preferentially forms DEA, resulting in D\(^2\)Rs <0.5 in affected waters (Mills and Thurman,
Contributions of DIA from the degradation of other triazine parent compounds, such cyanazine and simazine, will increase $D^2Rs$ to >0.6 (Mills and Thurman, 1994; Thurman et al., 1998; Meyer et al., 2001). The $D^2R$ was computed for the two epikarst drains in Crumps Cave as,

$$D^2R = \frac{[DIA]}{[DEA]}$$  \hspace{1cm} (1)$$

where [DEA] and [DIA] are concentration in nmol L$^{-1}$. Results showed that the $D^2R$ was generally in the range of 0.6 to 1.0 from late Jan through Apr 2011, indicating a source of DIA in addition to that from atrazine degradation (Figure S4). Following the transport of recently applied atrazine in May 2011, $D^2Rs$ quickly declined to <0.5 by early summer and were generally in the range indicative of atrazine degradation and transport, with DEA concentrations consistently greater than DIA. Given the consistent low levels of simazine at both sites, the $D^2R$ data further support the conclusion that prior use of both simazine and atrazine within the recharge was impacting the water quality of the epikarst drains.
REFERENCES


Figure S4. Deisopropylatrazine (DIA) to deethylatrazine (DEA) ratios ($D^3R$) from Jan 2011 to May 2012.
Table S1. Detection frequency of herbicide and herbicide metabolites in water samples collected from WF-1 and WF-2 sites from January 2011 to May 2012.

<table>
<thead>
<tr>
<th>Site</th>
<th>Atrazine</th>
<th>DEA</th>
<th>DIA</th>
<th>Simazine</th>
<th>Acetochlor</th>
<th>Alachlor</th>
<th>Metolachlor</th>
<th>Metribuzin</th>
</tr>
</thead>
<tbody>
<tr>
<td>WF-1†</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>99.5</td>
<td>6.5</td>
<td>16.8</td>
<td>61.6</td>
<td>25.4</td>
</tr>
<tr>
<td>WF-2</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>98.0</td>
<td>9.8</td>
<td>19.6</td>
<td>72.5</td>
<td>13.7</td>
</tr>
</tbody>
</table>

†n = 185 for WF-1 and 51 for WF-2.