DATCP2021-1

# Neonicotinoid contaminants in Wisconsin groundwater: relationships to landscape cropping systems

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# PROJECT SUMMARY

**Title**: Neonicotinoid contaminants in Wisconsin groundwater: relationships to landscape cropping system

**Project ID**: DATCP2021-1

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**Period of Contract**: July 1, 2020 – February 28, 2023

**Background/Need**: Neonicotinoids are a popular and widely used class of insecticides whose water-soluble nature and 20-year usage history has led to questions about their potential to accumulate in the environment and harm local ecosystems. Over 6.7 million pounds of neonicotinoid insecticides are now applied annually on 140 different crops in the United States, with the three most popular compounds, imidacloprid (IMD), clothianidin (CLO), and thiamethoxam (TMX), making up over 90% of agricultural usage nationally and generating over $4.6 billion in market activity in 2013. Neonicotinoid usage in the United States remained below 500,000 pounds per year until 2003, when the expansion of crop registrations and the introduction of additional active ingredients led to a rapid increase in total usage. Virtually all corn and soybean seeds planted in the United States are now treated with either IMD or TMX seed treatments intended to protect the developing seedlings from early-season pests. This heavy usage, combined with the water-soluble nature of neonicotinoids and their potential to harm beneficial wildlife, has brought their environmental fate (e.g., the life cycle of a chemical) into sharp focus. Contamination of surface and groundwater specifically, may occur from major agricultural sources such as spray drift during application, deposition of contaminated dusts released during drilling of treated seeds, surface runoff and leaching, greenhouse runoff, as well as human error or irresponsibility, sewer and storm water drainage, and residential usage. High water solubility and long environmental persistence times contribute to the potential for these compounds to migrate through the soil column and contaminate groundwater-fed streams, the consequences of which for aquatic invertebrates remains unknown.

**Objectives**: Objective 1: Utilize a calibrated groundwater flow model developed for the Central Sands Region to delineate groundwater-contributing areas to streams and relate local landscape compositions and associated detections of neonicotinoid insecticides. Objective 2: Refine our understanding of the spatial and temporal variations in groundwater and base flow along discrete sections of Fourteenmile Creek relating groundwater flow to surface water insecticide detection frequency and concentrations.

**Methods**: Objective 1: To evaluate the relationship between local landscape and neonicotinoid concentrations, groundwater contributing areas were delineated using the MODFLOW groundwater flow model by Kraft and Mechenich (2010) on the sample locations generated in ArcGIS. These contributing-area polygons were then intersected with Cropland Data Layer ("CDL", nassgeodata.gmu.edu/CropScape/) raster image files, where each pixel's color represents the crop or land use type detected within each pixel, as determined by remote-sensing technology. For each stream collection site, month and year, the proportion of each buffer occupied by one of these land cover classes was calculated by dividing the number of pixels belonging to a particular land cover class within each zone of influence buffer.

Objective 2: To refine our understanding of surface water-groundwater dynamics and variation in insecticide detection frequency and concentration, a variety of methods were used. Water-quality data from canoe floats and synoptic surface-water surveys was paired with measurements from streambed piezometers, that were well-connected to shallow groundwater. Methods involved collection of in-situ dissolved oxygen, water temperature, specific conductance, pH, and streamflow, as well as laboratory analysis for neonicotinoids (Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, and Thiamethoxam), nitrate-nitrite as N, metals, hardness, alkalinity, conductivity, pH, and chloride. Additional field methods included the evaluation of temperature-flux measurements between the stream and streambed, streambed sediment coring, continuous stream conductance measurements, and continuous water-quality monitoring using an ISCO autosampler. Surveys were also conducted to document ice on-off conditions of surface water during winter months.

**Results and Discussion:** Objective 1: Based on our NLCD regression analysis, we found a strong positive relationship between high fractions of cultivated crops in the landscape and higher average thiamethoxam detection. In the case of cultivated crops, the regression equation suggests a 31-times higher thiamethoxam concentration in a 100% cultivated crop landscape versus a 0% cultivated crop landscape. The analysis also reveals negative relationships between increasing landscape fractions of wetlands (woody or herbaceous) and forests (deciduous, mixed, evergreen). No relationship was found for pasture/hay, grassland/herbaceous, or developed land. One interpretation of these results is that higher fractions of wetlands and forests in the landscape can help to cleanse or detoxify surface and groundwater, resulting in lower concentrations in such landscapes, though perhaps a simpler explanation is that watersheds with more cultivated crops have less wetlands and forests, and vice versa, because of the finite nature of the landscape. Pastures and grasslands may not be subject to this same tradeoff, explaining the flat relationship. A similar set of regressions were also performed for imidacloprid, but no significant relationships (either positive or negative) were found between imidacloprid and any of the landcover types. In our sampling efforts, imidacloprid was detected less frequently than thiamethoxam and at lower concentrations. The different use patterns and lower water solubility of imidacloprid may help explain why these results differ from those for thiamethoxam.

Based on our CDL regression analysis, we found that potatoes, sweet corn, beans, and peas were all strongly associated with increased thiamethoxam concentrations, while field corn was moderately associated with thiamethoxam concentrations, and alfalfa had little to no relationship. The relationship strength (i.e., strong, moderate, none) for the CDL analysis was defined relative to the slope and R2 of the thiamethoxam concentration and cultivated crop land cover fraction. One noteworthy observation was the potential for “innocent bystander” crops, such as peas, that often rotate in sequence with neonicotinoid-treated crops such as potatoes, sweet corn and beans. In other words, as long as one crop type rotated onto a particular field parcel is treated with a neonicotinoid, that crop and all associated rotational crops are more likely to correlate with neonicotinoid detections within a groundwater contributing area. Considering the CDL regression analysis by watershed (Central Sands vs Fox River valley), the range of thiamethoxam detections and the range of land use fractions by land cover type differ greatly between the regions. For potatoes, sweet corn, dry beans, and peas, in particular, these crops make up a very small fraction of the landscape in the Fox valley rivers. Corn and alfalfa make up similar fractions of the landscape in each region, but while thiamethoxam concentrations were positively correlated with corn in the Central Sands, there was no relationship with corn in the Fox valley. A similar set of regressions were also performed for imidacloprid, but no significant relationships (either positive or negative) were found between imidacloprid and any of the crop types.

Objective 2: Hydrogeological field investigations along Leola Ditch, across multiple campaigns from fall 2020 to spring 2022, resulted in several consistent and repeatable observations regarding surface water-groundwater dynamics as well as surface water-groundwater quality and neonicotinoid concentrations. Canoe floats documented the presence of cooler surface water temperatures over the first two miles of Leola Ditch (two miles west of CTH D at STH 73), which markedly increased further to the west. These continuous temperature observations are largely attributed to groundwater inflows, which decrease with distance to the west. *(Note: mile-marker distances are relative to the intersection of Leola Ditch at CTH D and STH 73)*. Repeat synoptic streamgaging measurements, documented the doubling, or even tripling, of streamflow over the first three miles of Leola Ditch before levelling off or slightly decreasing further to the west. This suggests Leola Ditch transitions from a strongly gaining stream to a stable or losing stream from east to west. Similarly, heads in mini-piezometers changed from upward vertical head differences (positive head) to downward vertical head differences (negative head), providing evidence that Leola Ditch transitions from a gaining (groundwater inflow) to losing (stream-water outflow) system over this 5-6 mile section of stream. Lab analysis of major ions for groundwater samples collected from mini-piezometers and surface water samples provided another line of evidence of gaining/losing dynamics along Leola Ditch. A distinct major-ion signature of groundwater is observable in the shallow streambed mini-piezometers until mile-marker 3, when mini-piezometers become dominated by surface water, suggesting Leola Ditch is losing water to the surrounding aquifer.

Nitrate in surface water along Leola Ditch exhibits a pattern where relatively high concentrations (>10 mg/l) are present to the east (near STH 73) and gradually decline downstream to the west. By contrast, nitrate concentrations in groundwater displayed much more variability, ranging from equal or below surface water concentrations (10-12 mg/l) to as high 72 mg/l. Similar to our observations with major ions, nitrate concentrations in mini-piezometers west of mile-marker 3 resembled concentrations in surface water. High nitrate concentrations measured from streambed mini-piezometers in gaining sections of the stream, suggest that discrete "plumes" of groundwater nitrate discharge to the stream and contribute to the overall surface water nitrate concentration.

LC-MS analyses of water samples never detected acetamiprid and dinotefuran in groundwater (mini-piezometers) or surface water from Leola Ditch. Thiamethoxam, clothianidin and imidacloprid were commonly detected, with all three species prevalent in surface waters and groundwater from certain monitoring stations. Commonly, neonicotinoid concentrations at Leola Ditch follow a rule where thiamethoxam is present at roughly 10 times the abundance of clothianidin, with imidacloprid in turn about three times less abundant than clothianidin. Spatially, neonicotinoid concentrations in surface water were observed to decrease slightly downstream. In groundwater, neonicotinoids are universally low at the eastern-most mini-piezometer fence but increase to concentrations comparable to surface water within the losing section of Leola Ditch, consistent with trends observed for major ions and nitrate. Similar to nitrate, seasonality and stream stage was not observed to influence neonic concentrations with values remarkably consistent between sampling dates for surface water. By contrast, neonicotinoids in groundwater were inconsistent between sampling dates at the same time of year or same level of stream discharge. Neonicotinoid concentrations derived from the ELISA method were much noisier and more variable than concentrations obtained by LC-MS but are generally in agreement. Groundwater concentrations of thiamethoxam were frequently elevated and similar to counterpart surface water at stations in the losing section of Leola Ditch, with generally lower concentrations upstream. All three of the detected neonicotinoids show a distinct positive correlation with nitrate, with either surface water or groundwater impacted by nitrate concentrations ≥10 mg/L almost universally showing elevated concentrations of all three chemicals, while groundwater or surface water ≤10 mg/L nitrate have very low or no detectable neonicotinoids with only a few exceptions.

**Conclusions/Implications/Recommendations:** This study demonstrated how groundwater models can aid in the delineation of groundwater contributing areas to surface water features and provide an approach for evaluating relationships between land-use activities and water quality. In this study, evaluations were performed for a series of repeat stream-monitoring locations, with corresponding delineated groundwater contributing areas of anywhere from 1 to over 50 square miles across the Central Sands region. Similarly, this same approach could be applied by researchers and regional stakeholders as a means of interrogating groundwater and surface water dynamics, water quality conditions, and land-use activity at a variety of scales. Model output provides a means to 1) field validate the presence of gaining and losing stream sections with a watershed, 2) improve our conceptual models of how groundwater and surface water interact, and 3) help practitioners design better monitoring of streamflow, stream water quality, groundwater quality, and overall watershed health.

Observations made along Leola Ditch were collected during dry, wet, and snowmelt conditions and suggest that nitrate and neonicotinoid concentrations in surface water may represent time-weighted, spatially averaged concentrations of groundwater from across the entire contributing area, which gradually discharge to surface water over time. The stream, in this case, integrates groundwater quality across the contributing area and may provide a convenient way to track groundwater quality over time. If true, long-term synoptic surveys of stream water quality (e.g., annual surveys over decades) could provide an approach for tracking changes in the overall quality of groundwater within the corresponding contributing areas. Taken together these techniques could help watershed managers looking to monitor long-term stream and groundwater quality and water resource technicians looking to optimize the design of watershed-scale sampling and monitoring programs.

**Related Publications:** None

**Key Words**: neonicotinoids, Wisconsin Central Sands Region, surface water, groundwater, modeling

**Funding**: Wisconsin Department of Agriculture, Trade, and Consumer Protection

**Final Report**: A final report containing detailed information on this project is included in the Groundwater Project Repository of the Wisconsin Groundwater Research and Monitoring Program (WGRMP).

# INTRODUCTION

Neonicotinoids are a popular and widely used class of insecticides whose water-soluble nature and 20-year usage history has led to questions about their potential to accumulate in the environment and harm local ecosystems (Goulson 2013, Huseth and Groves 2014, Bradford et al 2018, van Lexmond et al 2014, Bonmatin et al 2015, Main et al 2014, Morrissey et al 2015). Over 6.7 million pounds of neonicotinoid insecticides are now applied annually on 140 different crops in the United States, with the three most popular compounds, imidacloprid (IMD), clothianidin (CLO), and thiamethoxam (TMX), making up over 90% of agricultural usage nationally (Jeschke et al 2011, USGS 2016) and generating over $4.6 billion in market activity in 2013. Neonicotinoid usage in the United States remained below 500,000 pounds per year until 2003, when the expansion of crop registrations and the introduction of additional active ingredients led to a rapid increase in total usage. Virtually all corn and soybean seeds planted in the United States are now treated with either IMD or TMX seed treatments intended to protect the developing seedlings from early-season pests. This heavy usage, combined with the water-soluble nature of neonicotinoids and their potential to harm beneficial wildlife, has brought their environmental fate (e.g., the life cycle of a chemical) into sharp focus (Goulson 2013, Hopwood et al 2013). Contamination of surface and groundwater specifically, may occur from major agricultural sources such as spray drift during application, deposition of contaminated dusts released during drilling of treated seeds, surface runoff and leaching, greenhouse runoff, as well as human error or irresponsibility, sewer and storm water drainage, and residential usage (Gerecke et al 2002). High water solubility and long environmental persistence times contribute to the potential for these compounds to migrate through the soil column and contaminate groundwater-fed streams, the consequences of which for aquatic invertebrates remain unknown. Previous work by Devita and McGinley (2021) within the Central Sands region has documented the presence of elevated neonicotinoid detections in surface water and the variability of neonicotinoid concentrations within the hyporheic zone of streams as sampled using shallow streambed piezometers.

Neonicotinoids are registered for a variety of application methods to include seed treatments, foliar sprays, and in-furrow soil applications; however seed treatments and soil applications constitute 60% of agricultural neonicotinoid usage (Jeschke et al 2013). Seed and soil application methods are of particular environmental concern because uptake rates of applied active ingredients have been reported as low as 2-5% in cotton, eggplant, potato, and rice, and up to 20% in maize, meaning that in excess of 80% of applied active ingredients remain in field soils potentially resulting in off-site movement and environmental contamination (Bonmatin et al 2011). Neonicotinoid compounds have reported soil half-lives measured in months to years depending on conditions such as temperature, depth, and microbial activity (IMD: 100-1230 days; CLO: 148-7000 days; TMX: 3.4-1000 days) (Bonmatin et al 2015). Once in the soil environment, the risk that these long field persistence times will translate into off-site movement of neonicotinoid compounds is further increased by the high-water solubility of the major neonicotinoid compounds: IMD = 610 mg/L; CLO = 340 mg/L; and TMX = 4100 mg/L (Bonmatin et al, 2015). Indeed, laboratory and field studies have demonstrated a high risk of leaching associated with soil and seed applications of neonicotinoid insecticides (Huseth and Groves 2014, Wettstein et al 2016, Gupta et al 2008, Kurwadkar et al 2014).

Emerging concern about neonicotinoid contamination has motivated the development of ecosystem- and regional-scale water quality surveys (Main et al 2014, Main et al 2016, Starner and Goh, 2012, Hladik et al 2014, 2017, 2018). Conservation groups have also raised calls for neonicotinoids to be banned or phased out due to the substantial ecological risks their continued use may pose (Carnemark et al 2015, Code et al 2016). Neonicotinoid residues have now been documented in a variety of locations around agricultural fields including in dusts exhausted during drilling of treated seed (68-15,000 mg/Kg) (Tapparo et al 2012), pollen (up to 51 µg/L) and nectar (up to 8.6 µg/L) of treated plants (Goulson 2013, Krupke 2012), plant guttation fluid (10-200 mg/L) (Sur and Stork 2003, Girolami et al 2009), soil (1-100 mg/Kg) (Goulson 2013), puddles on the ground surface (up to 63 µg/L) (Samson-Robert et al 2014), and surface water (river and stream) systems (up to 225 µg/L) (Morrissey et al 2015, Starner and Goh 2012, Hladik et al 2018). Recent reports of neonicotinoid detections in surface waters across the United States have been reviewed (Starner and Goh 2012, Krupke and Long 2015): maximum IMD detections were reported as 3.29 µg/L in California (Wettstein et al 2016), 6.90 µg/L in Massachusetts Winja et al 2014), 9.00 µg/L in South Carolina (Delorenzo et al 2012), and 25 µg/L in Maryland (Johson and Pettis 2014). One study reported CLO detections up to 0.257 µg/L in Iowa (Kurwadkar et al 2014). Detections of TMX have been reported up to 2.49 µg/L in South Dakota (Starner and Goh 2012), 8.93 µg/L in Wisconsin (Huseth and Groves 2014, Bradford et al 2018), and 225 µg/L in the playa wetlands of the southern high plains region of the United States (Anderson et al 2013). Recent surveys in the US Midwest have also indicated that neonicotinoid contaminants can be found year-round in 10 different tributaries of the Great Lakes spanning six states (Gupta et al 2008).

Neonicotinoid detections in surface water systems at these concentrations are cause for alarm as aquatic invertebrates are key members of many freshwater ecosystems and some species are extremely sensitive to neonicotinoid insecticides, with acute toxicity endpoints reported down to 1 µg/L and chronic toxicity endpoints reported down to 0.1 µg/L (Morrissey et al 2015). In a recent review, Morrissey et al. (2015) suggest an ecological threshold for neonicotinoids be established at 0.2 µg/L for long-term acute and 0.035 µg/L for long-term chronic exposure limits. Similar aquatic invertebrate benchmarks of 0.385 µg/L acute exposure and 0.01 µg/L chronic exposure have been established by the US Environmental Protection Agency (EPA) as part of their ongoing re-registration eligibility review of IMD (US EPA 2016). Proposed EPA benchmarks also exist for the neonicotinoids CLO (11 µg/L acute, 0.05 µg/L chronic), TMX (17.5 µg/L acute, 0.74 µg/L chronic), acetamiprid (10.5 µg/L acute, 2.1 µg/L chronic), and thiacloprid (18.9 µg/L acute, 0.97 µg/L chronic) (US EPA: [https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk](https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk)). Currently these benchmarks are only advisory and any detections exceeding these benchmarks will not result in regulatory action at the present time but are helpful in evaluating the potential ecological effects of any environmental neonicotinoid detections in surface and groundwater.

In Wisconsin, groundwater monitoring efforts have been conducted by the state, Department of Agriculture, Trade and Consumer Protection, Environmental Quality Section (DATCP). DATCP regularly tests for select contaminants in private potable wells and groundwater monitoring wells as part of its mission of monitoring and protecting water quality in the state. These samples encompass several ongoing survey efforts, including new private potable wells pending certification, private potable wells flagged for resampling due to past detections of certain chemicals exceeding enforcement standards (such as nitrate and the herbicide atrazine), and static groundwater monitoring wells established to monitor shallow groundwater for agricultural contaminants in locations deemed at elevated risk of such contamination. In 2008, DATCP added tests for select neonicotinoids (initially only TMX, later IMD and CLO) as a part of this groundwater monitoring effort in response to significant public concern among rural communities about the rapidly expanding use of this new class of insecticides and their potential for accumulation in groundwater resources (WI DATCP 2011, WI DATCP 2014). These ongoing surveys have revealed concentrations of one or more neonicotinoid compounds in dozens of test wells, with most detections occurring in the Central Sands and Lower Wisconsin River Valley (LWRV) agroecosystems. In addition, similar concentrations of neonicotinoid active ingredients were also detected in water drawn from a small number of high-capacity, overhead center-pivot irrigation systems (defined by the Wisconsin

Department of Natural Resources as capable of pumping more than 380,000 L of water per day) used to water potatoes and processing vegetables (Huseth and Groves 2014, Bradford et al 2018).

The frequency of neonicotinoid detections specifically in the Central Sands and LWRV agroecosystems suggested that further study of this area was warranted. A significant fraction of irrigated potato and processing vegetable production in Wisconsin occurs in the Central Sands and the LWRV, and neonicotinoid insecticides are frequently employed as crop protectants by commercial producers. In addition, the hydrology of these regions is characterized by sandy, fast-draining soils, and shallow, unconfined aquifers that have been identified as at an elevated risk of contamination according to the Wisconsin Groundwater Contamination Model (Ecological Landscapes of Wisconsin - Map S16, Wisconsin Department of Natural Resources, available: http://dnr.wi.gov/topic/landscapes/).

The current study was funded to assess the magnitude, spatial extent, and temporal dynamics of neonicotinoid and nitrate contamination in groundwater-fed streams in these regions at a higher spatial and temporal resolution than existed within the monitoring data available from state agencies. The study also had a focus on a discrete range of the Fourteenmile Creek complex (e.g., Leola Ditch), where direct comparisons of stream water to local groundwater entering the stream were made using shallow piezometers set at different points along the confluence. This work is well aligned with the USDA NIFAs, Research, Education and Economics mission areas and priorities, including Goal 3, '*Sustainable Use of Natural Resources*', with particular emphasis on '*managing agricultural watersheds and landscapes to improve the delivery of ecosystem services while sustaining or enhancing agricultural production*'. The specific actionable items described under the strategies section include the development of indicators for 1) spatial connectivity of landscape elements; 2) quality of ecosystem services derived from agricultural landscapes; and 3) values of ecosystem services.

# PROCEDURES AND METHODS

## Objective 1

The first objective of this study was to utilize a calibrated groundwater flow model developed for the Central Sands Region to delineate groundwater-contributing areas to streams and relate local landscape compositions and associated detections of neonicotinoid insecticides. This objective leveraged prior neonicotinoid sampling performed by the Groves lab from 2015-2020, where neonicotinoids had been analyzed at 87 locations across the Wisconsin River Watershed in the Central Sands and the Fox River watershed to the southeast. By developing model-derived groundwater contributing areas for each surface water sampling location, local landscape compositions could be interrogated for their relationship to neonicotinoid concentrations detected in stream water.

### *Applied flow modeling: MODPATH and the Endpoint Analysis Approach*

A steady-state groundwater flow model developed for the Central Sands Region by Kraft and Mechenich (2010), referred to as the 2010 Central Sands Model, was used to simulate groundwater flow and analyze groundwater-contributing areas to streams. The native model files were obtained from Dr. Kraft at UW-Stevens Point in August 2020 and run using Groundwater Vistas 8 (GWV8). GWV8 is a software program published by Environmental Simulations, Inc. that provides a graphical interface for building, running, and analyzing groundwater flow models that are based around the U.S. Geological Survey (USGS) modular finite-difference model MODFLOW (Harbaugh et al 2000).

**Figure 1**, reprinted from Figure VI-1 of Kraft and Mechenich (2010), illustrates the extent of the Central Sands model as well as the MODFLOW model boundaries that were incorporated into the model, including the River and Drain package for rivers, streams, and ditches and both the No-Flow and Constant Head packages for the model's perimeter. The inset graphic depicts the 200 m x 200 m (656 x 656 ft) grid cell size for the Little Plover River near Plover, WI. This steady-state model was well suited to this project because all 87 surface-water sampling locations evaluated by the Groves Lab from 2015-2020 were located along surface water features represented explicitly in the model by MODPATH River (RIV) or Drain (DRN) packages.

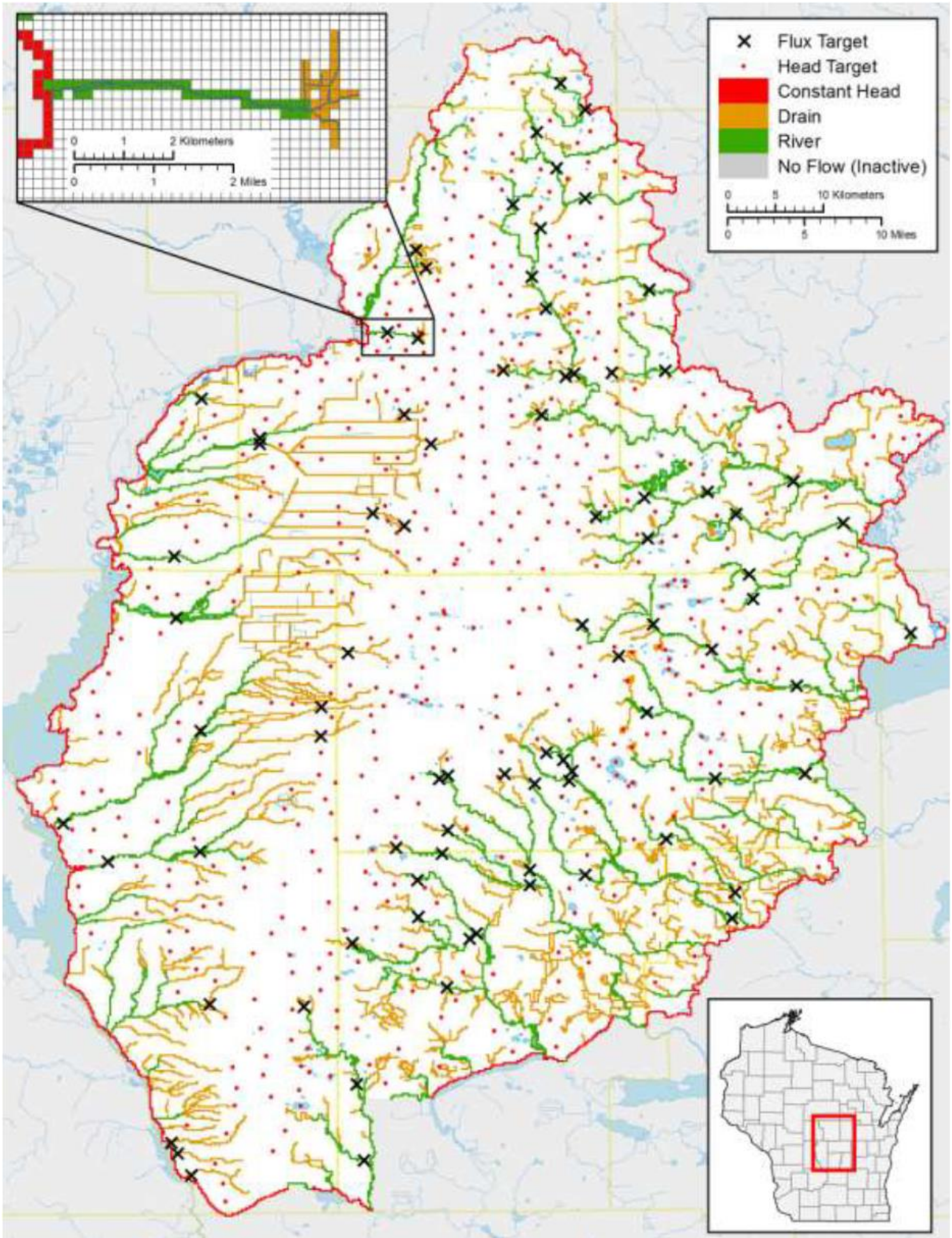



***Figure 1.*** *Central Sands groundwater model as presented in Figure VI-1 of Kraft and Mechenich (2010) showing the model domain, the spatial extent of modeled surface water features, the boundary conditions and an inset graphic of the model grid.*

Once the 2010 Central Sands Model was run and the baseline model solution was generated in GWV8, the particle-tracking code MODPATH was used to simulate advective transport of groundwater across the model domain. For this analysis four particles were placed in each model cell at the top of model layer 1, representing the top of the water table. An example showing the distribution of particles within a portion of the Fourteenmile Creek watershed is presented below in **Figure 2.**

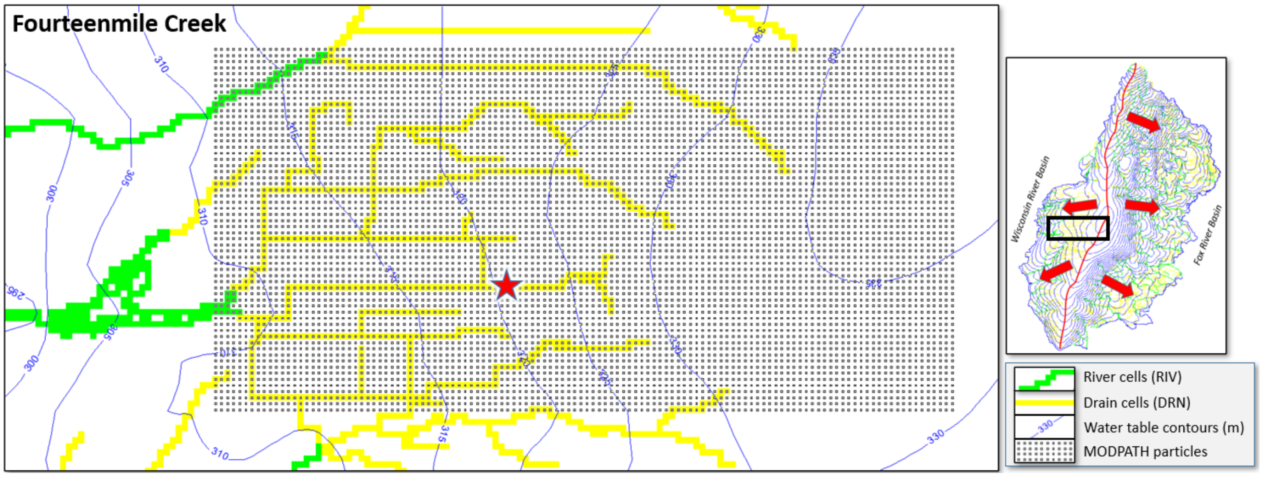



***Figure 2.*** *Example of MODPATH particles (black dots) centered over the headwaters of the Fourteenmile Creek watershed. In this example the red star represents a surface water sampling location for which we wanted to delineate the corresponding groundwater-contributing area. MODFLOW River (RIV) package cells are depicted in yellow and Drain (DRN) package cells are shown in green.*

MODPATH was then run in forward-tracking mode until all particles exited the groundwater system to either a surface water feature or model boundary. The simulated forward-particle traces from the example presented in **Figure 2** are illustrated in **Figure 3**. Each red line represents the entire forward trace of the MODPATH particle from its origin location in a model cell to its destination in the groundwater flow model. As can be seen in this example, most particles terminate at surface water features while some exit the image area towards other surface water features or model boundaries.

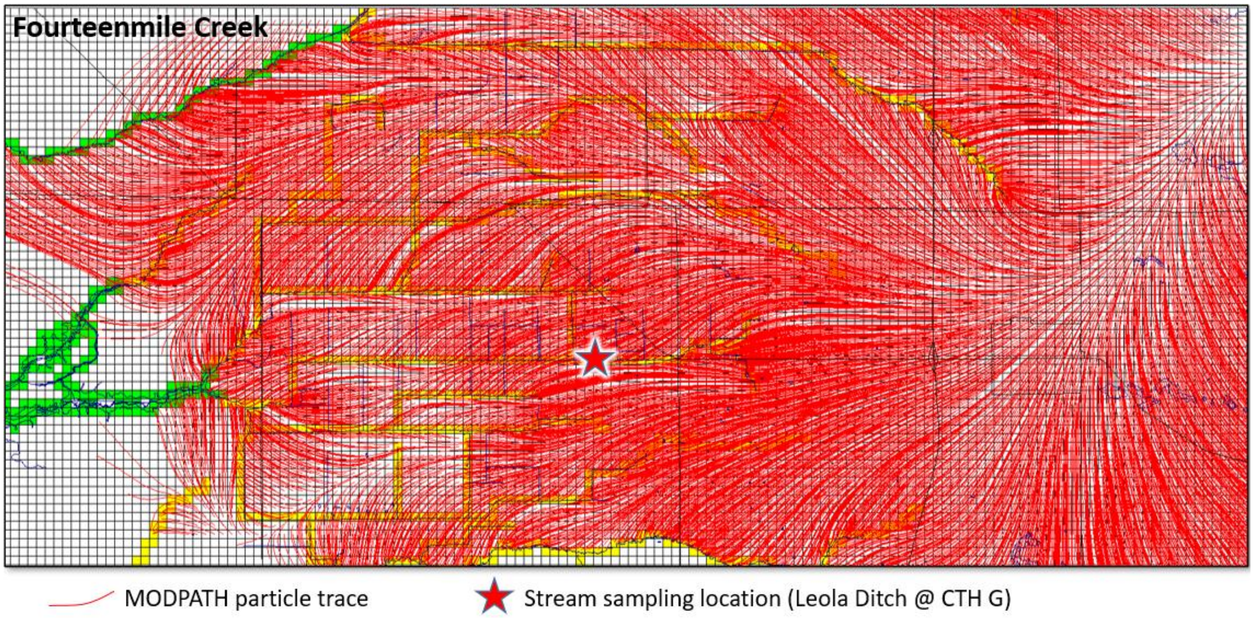



***Figure 3.*** *Results of MODPATH particle tracking in forward-tracking mode from the example run for the Fourteenmile Creek watershed. Each particle delineates a complete flow path from its starting location (presented in Figure 2) to where it exited the model at a surface water feature or boundary condition.*

### *Landscape analysis: Neonicotinoid detection dataset*

The response data for our planned landscape analysis was a set of neonicotinoid detections (thiamethoxam and imidacloprid) in surface water grab samples that the Groves lab had performed over the preceding several years (2015-2020). A series of 87 locations had been identified across the Wisconsin River watershed in the Central Sands and the Fox River watershed to the southeast. Sampling locations had been selected with an emphasis on ease of access (bridges, culverts) and broad spatial coverage of select river systems from headwaters to discharge into the Wisconsin or Fox rivers. In the Wisconsin River watershed, sites were located (from N to S) in Fourmile, Tenmile, and Fourteenmile creeks, and the Big and Little Roche-a-Cri rivers. In the Fox River watershed, sites were located in the White, Mecan, and Montello rivers. These two major drainage basins were expected to provide a distinct contrast to one another, with the rivers in the Central Sands representing a higher level of agricultural activity, in particular cultivated vegetable production, as well as sandier soils with strong connections between groundwater and surface water.

Samples were collected by plunging 250 ml brown HDPE plastic bottles either by hand or suspended from a rope into the stream and placing collected samples on ice for transport back to UW-Madison. Thiamethoxam concentrations were estimated using commercially available, 96-well competitive enzyme-linked immunosorbent assay (ELISA) kits (Thiamethoxam HS Plate Kit, Cat. # 20-0102, Beacon Analytical Systems, Saco, ME). Imidacloprid concentrations were estimated using a similar ELISA test kit produced by Envirologix Inc. (Portland, ME). Completed plates were quantified using an optical plate reader (VersaMax tunable microplate reader, Molecular Devices, Sunnyvale, CA) and the accompanying Softmax Pro software. Each plate was standardized against a four-parameter logistic curve generated from the four provided standards (0, 0.05, 0.30 and 2.00 µg/L) run alongside well water samples. All standards and samples were run in triplicate and results were averaged to reduce kit error. The limit of quantification of these kits (below which precision and accuracy are reduced) is 0.05 µg/L according to the manufacturer.

Over our 5-year sample collection campaign, we collected 490 water samples, and generated thiamethoxam and imidacloprid concentrations for each. Our previous efforts at analyzing the resultant dataset from this campaign were focused on seasonal, annual, and broad spatial differences in mean neonicotinoid concentrations (**Figure 4**, below). However, we lacked the ability to generate accurate upstream contributing area delineations for each grab sample (effectively, the reach of the river system upstream of the sampling point, plus a surface or groundwater contributing area from which stream water would have flowed). A major objective of the present study was to use a calibrated groundwater flow model to identify specific contributing areas to each sampling location, given a range of flow times (1, 5, 20 years, e.g.), and intersect those contributing areas with landscape raster datasets to determine the relationship between surface water neonicotinoid grab sample detections, groundwater contributing areas, and landscape cropping systems.

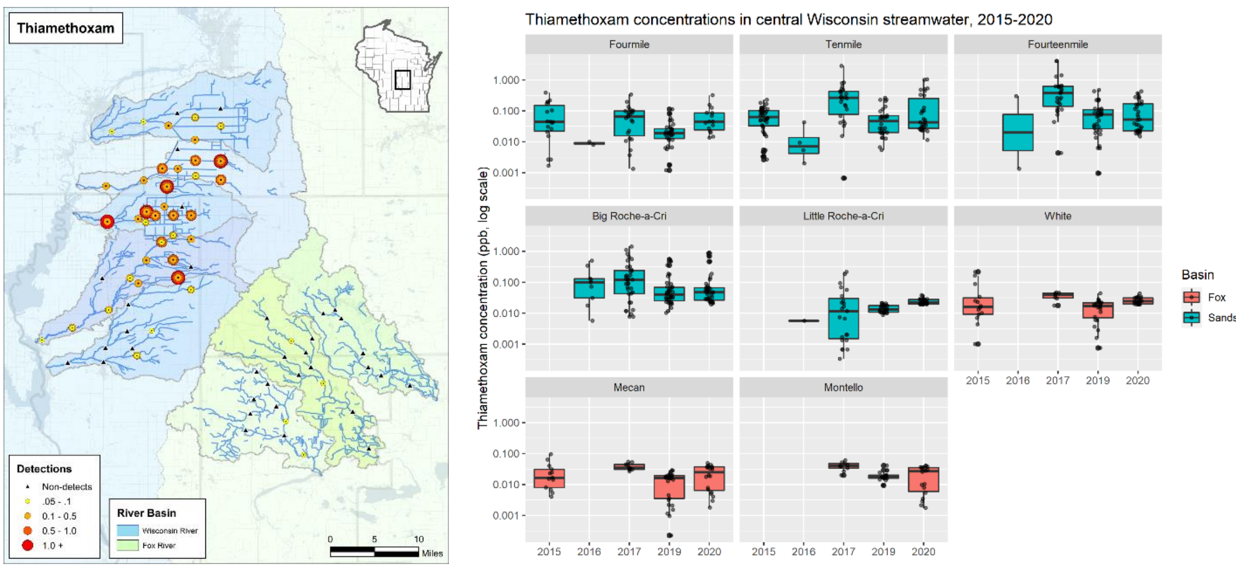



***Figure 4.*** *Summary of past regional neonicotinoid sampling efforts. On left, a map showing sampling locations with surface watersheds highlighted (blue shades, Wisconsin River/Mississippi, green shades, Fox River/Great Lakes). Positive thiamethoxam detections at sampling locations are highlighted by colored bulls-eyes surrounding points. On right, the range of thiamethoxam detections in each watershed are illustrated across years.*

### *Landscape analysis: Groundwater contributing areas*

The generation of groundwater contributing areas relied on MODPATH particle tracking approach, explained earlier in the **Objective 1** methods section. The datasets produced by that effort are effectively spatial point data, in the form of a 100m grid covering the entire model domain (bounded by the Wisconsin River to the west, and the Fox River to the east). The groundwater flow model itself is constructed of 200m grid cells, but for the particle-tracking analysis four particles were assigned to each model cell. During particle-tracking analysis, a series of travel times were selected (i.e., 1, 2, 5, 10, and 20 years, for this objective) for the MODPATH to run in forward-tracking mode, and a final location was determined for each MODPATH particle. MODPATH particles discharging to modeled surface water features representing ditches, creeks or rivers (i.e., DRN or RIV cells in the MODFLOW model), effectively delineated the groundwater contributing area for that modeled surface water feature for the specified travel time.

Joining our neonicotinoid grab sample data to the groundwater flow model required manually identifying, for each grab sample location, all of the upstream modeled surface water cells, which could have collected groundwater and contributed to the surface water collected at that sample location. Once the set of modeled surface water cells was assigned for each grab sample location, groundwater contributing areas could be generated for each travel time. This was done by identifying all of the MODPATH particles that terminated in one of the modeled surface water cells during each respective travel time. An example contributing area is shown in **Figure 5**, below, for regional sampling Site 17 (red dot lower left) in Fourmile Creek. Similar shapefiles were generated for each of the 87 grab sample sites. This methodology is broadly applicable, and most of the work was automated through R scripts, but the selection of upstream drain cells for each site was performed manually in QGIS, a mapping program.

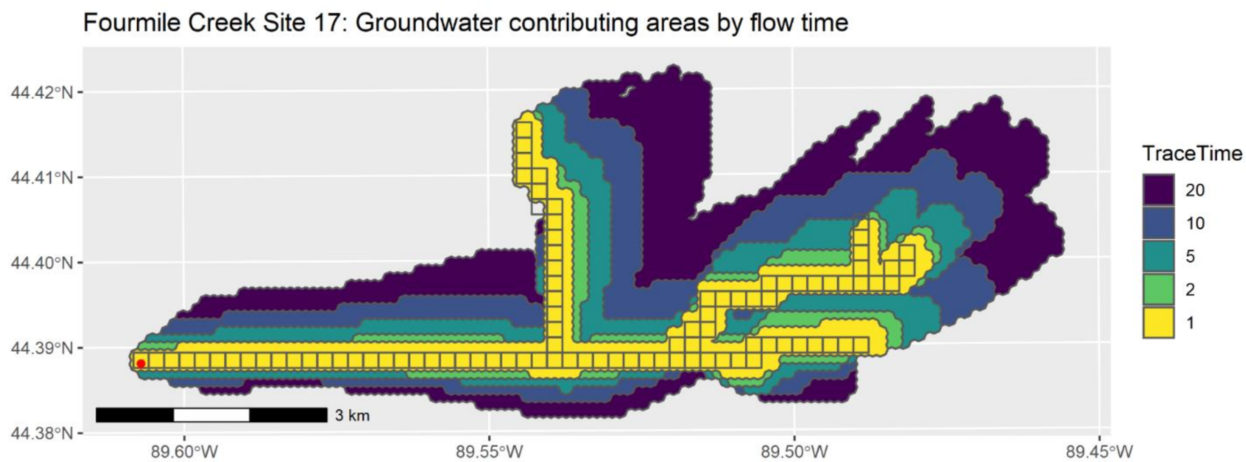



***Figure 5.*** *Groundwater contributing areas generated for a sampling site in Fourmile Creek. TraceTime (aka: modeled travel time) indicates the time in years that the model was propagated, and the shapes on the map reflect the size of the groundwater contributing area to model drain cells (surface water systems). Longer trace times always result in larger contributing areas.*

### *Landscape analysis: Relationships to cropping systems*

Two raster datasets, the National Land Cover Dataset (NLCD, produced by the US Geological Survey) and the Cropland Data Layer (CDL, produced by the USDA National Agricultural Statistics Service), were selected for joining to the groundwater contributing areas described above. Both datasets have similar attributes but differ in the level of detail associated with agricultural production. The NLCD raster is valid across all stream sampling years, and contains 16 different land use classes, two of which are related to agriculture: cultivated crops, and pasture/hay. The CDL, by contrast, uses the same broad landcover classes as the NLCD, but expands the agricultural category into 112 individual crop classifications. Because crops are rotated across a landscape, the CDL is generated each year and agricultural classes are valid only within that year. However, though an individual field changes crop classification each year, land use across the region more broadly is considerably more stable. For our analysis, we extracted land use statistics from CDL rasters from 2010-2021 and averaged the land use proportion by land use class, whereas the same NLCD raster could be used for all sample years.

For each combination of groundwater contributing area shapes and rasters, the shapes were intersected with the rasters and the total count of intersected pixels by land use class was obtained (using R package *exactextractr*). To account for contributing areas of varying size, pixel counts were normalized by converting them into proportions of total area. From this data were ran simple linear regressions to investigate the correlation between log-normalized neonicotinoid concentration and landscape composition within groundwater contributing areas. For simplicity of presentation, we focus on the 20-

year groundwater contributing areas for regression against the NLCD raster, and the 10-year contributing areas for regression against the CDL rasters (CDL rasters are not available prior to 2008). Considering neonicotinoid use in the Central Sands began at scale in the late 1990's/early 2000's, we feel these groundwater contributing areas are appropriate for this analysis.

## Objective 2

The second objective was to refine our understanding of the spatial and temporal variations in groundwater and base flow along discrete sections of Fourteenmile Creek, relating groundwater flow to surface water insecticide detection frequency and concentrations. The main channel of Leola Ditch, along the northside of County Highway D (CTH D) from State Highway 73 (STH 73) to Sixth Avenue was identified as an ideal field area to evaluate for this study (**Figure 6**). This portion of Leola Ditch is located within the larger Leola Ditch Subwatershed, also referred to as Hydrologic Unit Code (HUC) 12 watershed 070700030603. The Leola Ditch HUC 12 watershed is one of three HUC 12 watersheds contained within the larger Fourteenmile Creek HUC 10 watershed 0707000306. The main channel of Leola Ditch, which runs parallel to CTH D from STH 73, west past Sixth Avenue contained easily accessed from CTH D as well as multiple north-south road crossings.

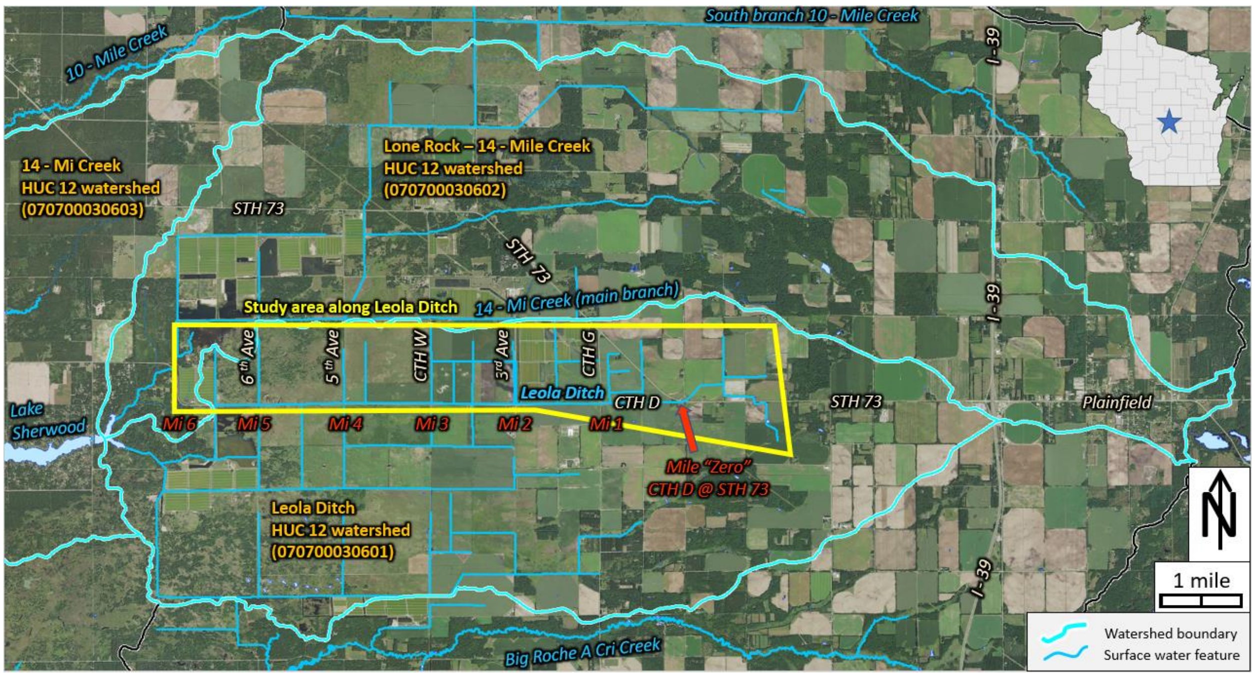



***Figure 6.*** Location map for main study area along Leola Ditch. For the purposes of this study, mile marker zero (0) was assigned to the intersection of CTH D with STH 73 and is referred to in the discussion for Objective 2 when describing field work along Leola Ditch. Leola Ditch was accessed directly from CTH D as well as several intersecting roads, such as CTH G, 3rd Ave, CTH W, 5th Ave, and 6th Ave. Leola Ditch is located within the Leola Ditch Watershed (HUC 12 = 070700030601), which is contained within the larger Fourteenmile Creek Watershed (HUC 10 = 0707000306).

As part of **Objective 2**, multiple field methods were employed to better characterize groundwater surface water dynamics along Leola Ditch. Several baseline water quality parameters (e.g., pH, conductivity, temperature, and dissolved oxygen) were performed in the field while water quality samples were analyzed at the Water and Environmental Analysis Lab (WEAL) at UW-Stevens Point and additional

ELISA assays for neonicotinoids were performed by the Entomology Department at UW-Madison. The range of methods included the use of:

- **Canoe floats** to collect continuous stream water quality data and streambed sediment conductivity;
- **Streamgaging** to estimate streamflow;
- **Water Chemistry** including collecting samples for testing at WEAL/UW-Madison and the measurement of in-situ baseline water-quality parameters,
- **Mini-piezometers** to a) collect shallow streambed groundwater samples for testing at WEAL/UW-Madison, b) measure baseline water-quality parameters of shallow streambed groundwater, and c) estimate vertical-head differences between the surface water and shallow groundwater;
- **Streambed temperature flux** measurements to estimate groundwater flux into and out of the streambed as well as hydraulic conductivities of the streambed;
- **Continuous stream conductivity** measurements to identify the potential change in water quality over extended periods of time;

### *Canoe floats*

The Wisconsin Geological and Natural History Survey (WGNHS) conducted instrumented canoe surveys from First Avenue and CTH D near STH 73 to the west to Sixth Avenue and CTH D on July 16, 2020, and August 26, 2020. The extent of the survey and stream stage of Leola Ditch is shown in **Figure 7**. Note the land cover and use around the ditch. Stream channels run to the north and south, there are wetlands to the west and agricultural fields, to the east in the head waters and in the center of the map.

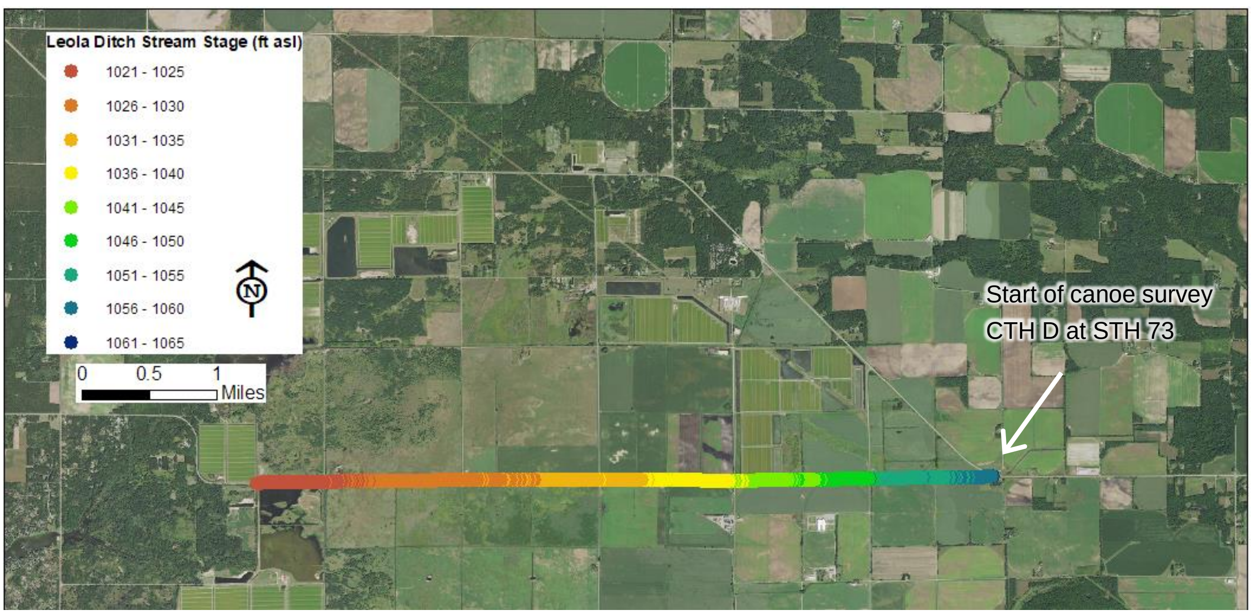



***Figure 7****. Shows the profile of Leola Ditch stream stage over the survey length. The stream stage at State Highway 73 starts at 1057 ft and decreases to the west to 1023 feet above sea level over the 5 ½ miles of the survey for an average slope of 0.0012 ft/ft. This slope is not constant. It is steepest for the first several miles, flattens for a mile or so and then steepens again.*

The area of Leola Ditch with a lower slope (orange and dark orange in **Figure 7**) corresponds to the Adrian and Houghton Mucks in the NRCS soils map while the areas at the start and end of the surveys are in areas of higher stream gradients and loamy or sandy soils such as the Newsome Loamy Sand or the Manawa Silt Loam. These soils and the stream stage are shown together in **Figure 8**. The change in stream gradient is apparent as the lengths of the individual colors. The orange and dark orange are portions of Leola Ditch with lower slopes. It seems likely that the organic soils formed where water was at or near the surface. A change from higher to lower hydraulic gradient may have contributed to the formation of these conditions.

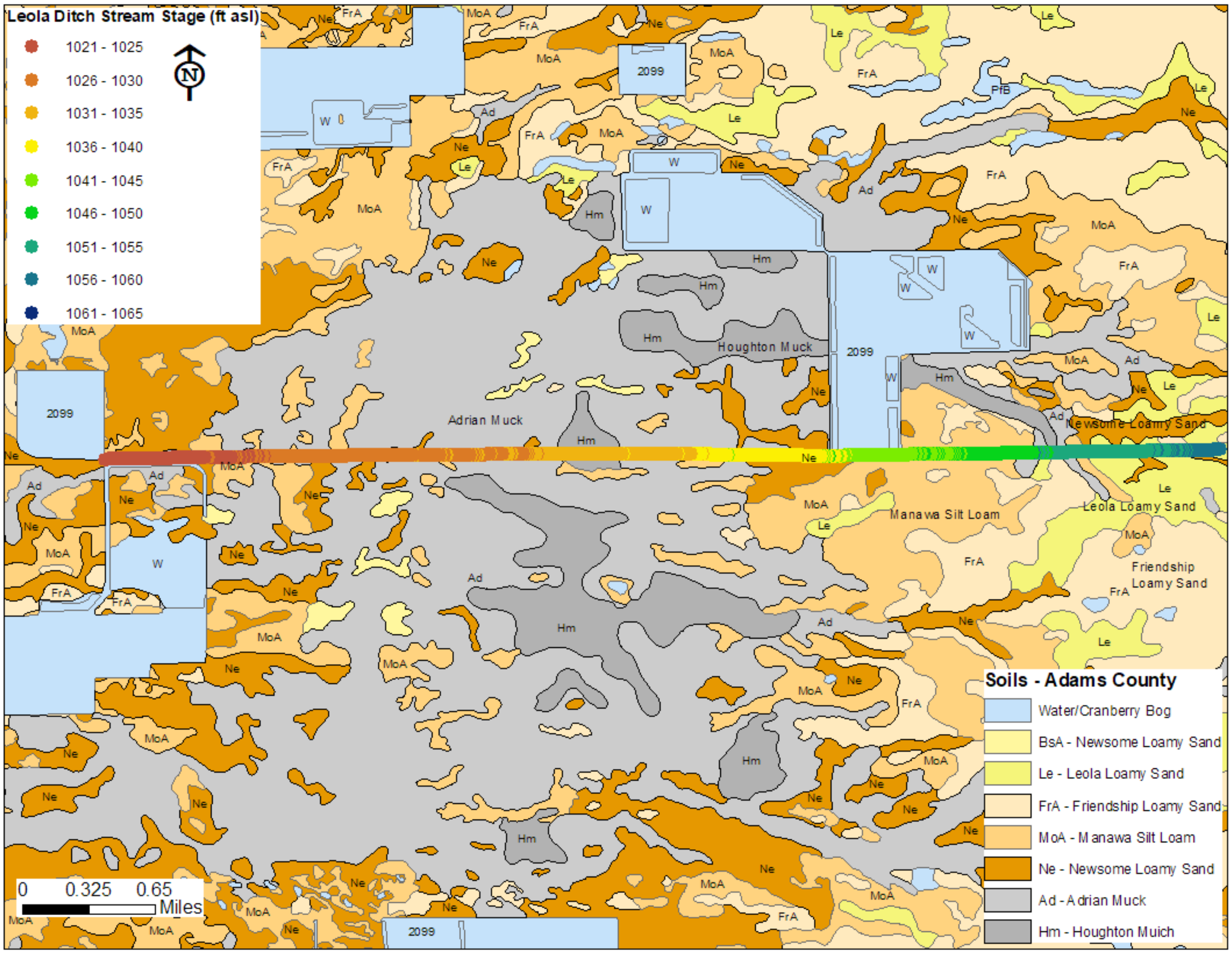



***Figure 8.*** *Soil composition map showing stream gage.*

The canoe was instrumented with a Geonics EM-31 electromagnetic conductivity meter to measure water and sediment conductivity, a Lowrance Hook 5 depth finder with temperature, and a GoPro video camera. The EM31 conductivities were recorded and located using an Arduino based datalogger and GPS. The EM31 was oriented in the horizontal dipole direction to provide greater sensitivity to shallower materials (McNeill, 1980) located by the depth finder and its internal GPS. We attempted to measure water quality using instrumentation that recorded water temperature, conductivity, pH, dissolved oxygen, chloride, and nitrate concentrations, however the instrumentation malfunctioned, and the data was not useful. A full description of the instruments and the canoe survey method is available in Christenson (2019) in **Figure 9** below.

***Figure 9.*** *Canoe instrumented with EM-31 conductivity meter, depth finder, water temperature and quality instruments, and Go Pro video cameras. Photo by David Hart.*

### *Streamgaging*

WGNHS performed periodic streamgaging measurements along portions of Leola Ditch, west of Plainfield, WI, from May 2020 until April 2022. Measurements were conducted at varying locations along Leola Ditch from CTH D at STH 73, west to Sixth Avenue. Additional field measurements and laboratory analysis of water samples collected in the field were often performed in association with streamgaging events.

Streamflow measurements were performed using a Marsh McBurney Flo-Mate 2000 electromagnetic flow meter. At each location, point velocity measurements and depths were recorded at approximately 20 stations along a transect, perpendicular to flow, with each measurement representing approximately 5% of total streamflow. Streamgaging sites were often located near culverts and bridges at road crossings or along sections of the stream where the channel was straight, and the depth and width of the streambed was consistent. Back in the office, field-recorded velocities were multiplied by the corresponding depth and width for each station along the transect and summed to provide an estimate of streamflow at locations along Leola Ditch. Methods generally follow those outlined by Turnipseed and Sauer (2010).

Streamgaging campaigns were performed several times throughout the year and captured a variety of stream stage conditions. Campaigns were typically performed as a “synoptic” survey with streamflow measurements collected along several miles of stream over a short period of time (typically 4-8 hours) on a single day. This approach allowed us to associate changes in streamflow with progressively more or less groundwater inflow to the stream and the extent to which lateral tributary ditches were contributing surface water to the main channel of Leola Ditch. As will be discussed in the Discussion and Results section, and covered through the application of other field methods, the synoptic stream survey approach provided a reliable way to evaluate the "gaining" (groundwater discharge contributes to streamflow) and "losing" (surface water loses water to surrounding aquifer) dynamics along Leola Ditch.

### *Mini-piezometer installation and use*

A total of 20 mini-piezometers were built and installed along a 2-mi section of Leola Ditch, between Third Avenue and Fifth Avenue, where landowner permission was obtained (**Figure 10**). Our approach for using mini-piezometers in conjunction with a variety of other field methods (e.g., streamgaging, streambed temperature flux measurements, vertical-head-difference measurements) to characterize surface water groundwater dynamics was greatly aided and leveraged by prior fieldwork conducted by DeVita and McGinley (2021), Dr. William DeVita (personal communication, 2020), and Dr. David Hart (personal communication, 2020) in the Central Sands Region of Wisconsin. Winter and others (1998), Rosenberry and LaBaugh (2008), and Rosenberry and others (2016) provided a conceptual and applied framework for our field-based approach to characterize surface water-groundwater interactions.

Mini-piezometers were constructed from 0.5-in diameter clear LDPE flexible tubing. The bottom 2-in of tubing was incised with five 0.25-in wide by 0.5-in long slots for groundwater ingress with fine stainless-steel mesh wrapped around the openings and secured with plastic zip ties and electrical tape to prevent entry of sediment. Mini-piezometers were installed by first driving a section of 1.125" diameter galvanized steel pipe into the streambed. The bottom of the pipe was outfitted with a disposable plastic drive-point and the pipe was driven to the desired depth using a steel fence-post driver. The fence-post driver was then removed, and the pre-assembled mini-piezometer tube was pushed down the pipe. Holding the tube in place, the galvanized pipe was slowly pulled back, pushing the plastic drive-point out of the pipe, and agitated up and down to "construct" the mini-piezometer. The up and down motion allowed streambed sediment to collapse around the tube, ensuring the mini-piezometer was in connection with the shallow groundwater system and isolated from the surface water feature.

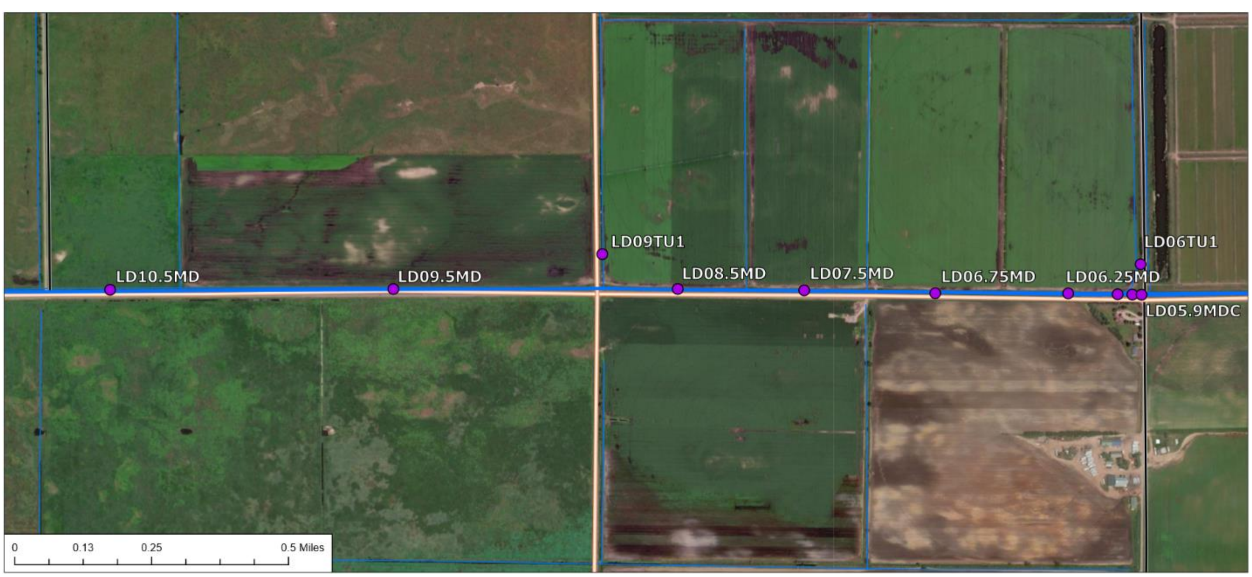



***Figure 10.*** *Location map of mini-piezometer installations along Leola Ditch marked by labelled purple dots.*

Generally, the standard installation depth was about 2.5-ft below the streambed; however, one cross section of closely spaced east-west oriented mini-piezometers were installed to 3.5-ft and 1.8-ft. Two cross sections of mini-piezometers were also installed running north to south, perpendicular to stream flow (**Figure 11**).

***Figure 11.*** *Example of mini-piezometer field installation at station LD5.9MD. Here three mini-piezometers were installed in a fence across the width of the ditch. This picture taken facing downstream to the west. Photo by Michael Parsen.*

Mini-piezometers were sampled using a Geotech geopump peristaltic pump with 0.25-in diameter flexible plastic tubing during quarterly sampling events that simultaneously collected groundwater and surface water at a number of monitoring stations. The pumping rate was set to roughly 0.7 liters per minute. As part of the sampling process water was pumped continuously out of the piezometer into a small vessel fitted with an Oakton PC450 pH/conductivity/temperature probe and an Oakton DO6+ dissolved oxygen probe. The parameters measured by these instruments were monitored continuously during pumping until readings stabilized to ensure water samples were representative of groundwater. Pumping time to parameter stabilization varied from 3 minutes 30 seconds to 6 minutes. Mini-piezometer water height, relative to surface water, provided an estimate of vertical head-difference and was measured using a ruler accurate to 0.005-ft.

***Water chemistry***

Water chemistry data was collected from a series of stations positioned along Leola Ditch specifically placed to bracket the tributaries which drain the area north of the main channel. Additional stations were concentrated along a three-kilometer section of the ditch where landowner permission was granted for installation of mini-piezometers into the streambed allowing for groundwater sampling (**Figure 12**). During field work, temperature, specific conductance, pH, and dissolved oxygen were measured in situ in surface waters and on groundwater pumped from mini-piezometers into a flow through cell. Temperature, specific conductance, and pH were measured with an Oakton PC450 calibrated for pH using pH 4, 7, and 10 standards, and conductivity using 1413 µS/cm standard before each field session. Dissolved oxygen was measured with an Oakton DO6 meter calibrated at the beginning of a field day using either air or a zero dissolved oxygen standard. Samples of surface water and groundwater were captured in 500 mL polyurethane bottles and shipped in coolers with ice to the UW-Stevens Point Water and Environmental Analysis Lab (UWSP WEAL) for analysis. Samples for neonicotinoid analysis were collected separately in 1L amber glass bottles. Groundwater samples were collected via peristaltic pump from mini-piezometers installed at selected stations along Leola Ditch and screened to depths from 1.5-3.5-ft below the streambed.

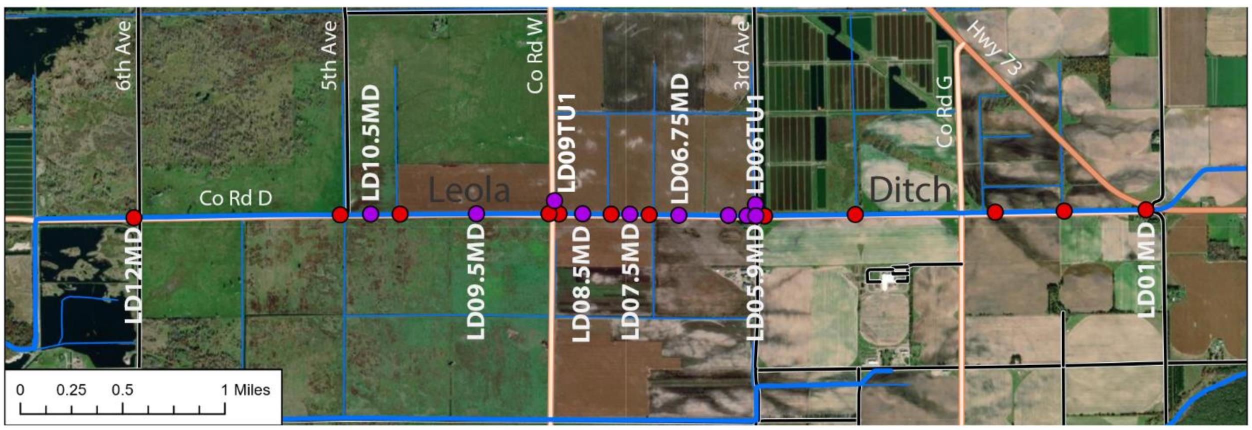



***Figure 12.*** *Overview map with satellite imagery over hillshade from 5ft LIDAR data for section of Leola Ditch monitored during this study. Thick blue lines mark larger drainages, thin blue lines represent small tributary ditches. Red circles are surface water sampling stations, purple circles are stations with mini-piezometer installations.*

Two different methods of analysis for neonicotinoid concentrations were utilized for this study: enzyme-linked immunoassay (ELISA) kits performed by collaborators at the UW-Madison Entomology department, and liquid chromatography-mass spectrometry (LC-MS) at UWSP WEAL. ELISA kits analyzed for thiamethoxam and imidacloprid, while LC-MS analyses done by UWSP WEAL quantified thiamethoxam, imidacloprid, clothianidin, acetamiprid and dinotefuran. Early efforts at identifying neonicotinoid concentrations in Leola Ditch (prior to contracting with UWSP WEAL) used commercially-available ELISA kits. The specifics and use of these kits are outlined earlier in the methods, under the Landscape Analysis section. The most significant limitation of the use of these kits is their relatively high limit of quantification, relative to LC-MS technology used by UWSP WEAL, which can detect down to the ng/L concentration (up to 100 times lower concentrations relative to ELISA methods).

To complement water chemistry data and to characterize the aquifer materials in the shallow sub surface around Leola Ditch, five hand auger boreholes were dug on the stream banks. Borings were driven down three feet with sediment samples collected every foot. Sediments were then dried and examined visually with a microscope.

***Streambed temperature flux***

We used temperature profile measurements combined with mini-piezometer heads to estimate groundwater flux into and out of the streambed and hydraulic conductivities of the streambed. In this method, multiple temperatures are measured from the streambed to depths of around 1 meter over several days. The measured temperature profile is a function of the advective transport of heat by groundwater flowing into or out of the stream and conductive transport of heat through the saturated streambed sediment. These two mechanisms of transport depend on the porosity and hydraulic gradient and conductivity beneath the streambed and the thermal conductivity and specific heat of streambed sediments.

Measuring the profiles over several days provides a variable temperature boundary at the streambed that helps to constrain the parameters. The hydraulic gradient is necessary to constrain the results as well. The method is described in more detail in Koch and others (2015). Our instrumentation uses waterproof DS18B20 sensors threaded through a PVC casing and taped to the outside. The stick or string of temperature sensors is shown in **Figure 13A**. Arduino Uno microprocessors and an Adafruit real-time clock and micro-SD card shield connected to the Arduino and housed in a waterproof Pelican case with a battery pack of eight D-cells. The D cells were arranged so that four cells each were in series to produce 6 volts each. These two sets were then set in parallel to provide additional battery life. The electronics and battery pack are shown in **Figure 13B**. The installed temperature profiler and mini-piezometer are shown installed in Leola Ditch in **Figure 13C**.

***Figure 13.*** *Upper photo, temperature probe stick used of obtaining profile measurements when driven into a streambed (Photo by David Hart). Lower left, Arduino control box used for recording data stick data (Photo by David Hart). Lower right, stick driven into streambed, with attached control box, to left of previously-installed stream-bed piezometer (long flexible tube on right) (Photo by Michael Parsen).*

### *Continuous stream conductivity*

Conductivity probes, capable of measuring continuous conductivity and temperature were deployed along a portion of Leola Ditch to complement the other in-stream monitoring methods. By measuring continuously, the goal was to observe fluctuations in stream-water chemistry that may be associated with large rainfall events, snow melt, or extended dry periods when the stream may become increasingly dominated by groundwater flow. As baseline water-quality indicators, conductivity and temperature often serve as reliable proxies for identifying pulses of new water that are introduced into streams from adjacent land or roadways. One such example would be overland flow of water during heavy rainfall events where surface runoff incorporates dissolved solids. Such events would be short lived and likely to be completely missed by quarterly sampling events.

For this project, six Levelogger 5 LTC transducers were installed along a 2-mi section of Leola Ditch, between Third Avenue and Fifth Avenue, where the project team obtained landowner permission (**Figure 14**).

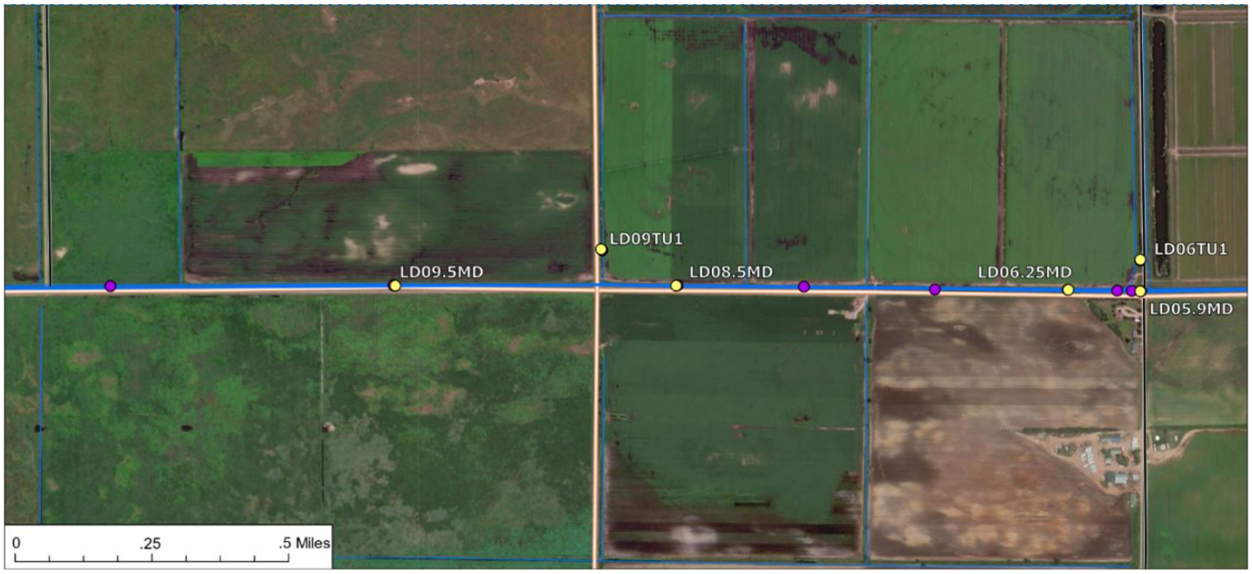



***Figure 14.*** *Location map of Leola Ditch with installations of Solinst conductivity probes marked by labeled yellow dots. Mini-piezometer installations marked by purple dots. The goal was to measure changes in water chemistry associated with lateral tributary ditches to Leola Ditch at Third Avenue and Fifth Avenue.*

The transducers were programmed to record temperature and conductivity every 15 minutes. Calibration was performed prior to the initial deployment using a two-point method with 1413 and 5000 µS/cm standards. Real-time tests were performed at each data-collection period to evaluate instrument drift relative to 1413 µS/cm standard and the transducers were re-calibrated as necessary. Several different installation methods were tested, with varying levels of success, due to the variability of in-stream conditions. In the first field design, transducers were attached vertically to a 4-ft metal fence stake, which was securely driven into the streambed. About 1-ft of each stake was exposed and the transducer orifice was close to the streambed. In this design, the stake quickly accumulated floating debris (e.g., grass, sticks, leaves) and the project team was concerned that the transducer may not record readings representative of stream water and that the stake (and transducer) may be pulled out by the fast-moving water and lost (**Figure 15**).

***Figure 15.*** *Left photo, original field design with Solinst conductivity probes oriented vertically (Photo by Michael Parsen). Right photo, Solinst conductivity probe deployment in Leola Ditch with accumulation of floating debris over the stake and probe (Photo by William Fitzpatrick).*

In the next installation design, the transducer was attached to a 90-degree elbow bracket mounted by bolts to the top of a 3-ft fence stake (**Figure 16**). This assembly was driven into the streambed so that the transducer was only suspended a few inches from the streambed. To intercept floating debris and reduce the accumulation over the transducer, additional fence stakes were installed a few feet upstream. While this lower-profile design resulted in less debris accumulation, current-transported sediment entered the transducer orifice leading to persistent conductivity measurement error. The final installation design maintained the low-profile with debris-protecting stakes but added a section of slotted 1.25-in diameter PVC pipe. The pipe was closed on the upstream end and left open on the downstream end. This design significantly reduced debris accumulation while eliminating the flow of particulates into the transducer orifice that could lead to erroneous conductivity measurements.

***Figure 16.*** *On left, second installation design with the conductivity probe oriented parallel and configured so the stake could be driven close to the streambed to reduce debris accumulation. Photo by Michael Parsen. On right, final installation method for Solinst conductivity probes utilizing protective stilling well in same geometry left image. Photo by William Fitzpatrick.*

# RESULTS AND DISCUSSION

## Objective 1: Neonicotinoid detections, relationships to cropping systems

### *Landscape analysis*

Prior to conducting this analysis, our hypothesis was that higher neonicotinoid detections in stream water grab samples would be associated with higher fractions of cultivated crops generally, and cultivated crops known to receive neonicotinoid application specifically, in the groundwater contributing areas upstream of a given sampling location. But it is not simply the sign of the relationship that is valuable, but the magnitude of it, and the specific crop or crops most associated with neonicotinoid detections, that we hoped to glean from this analysis.

In **Figure 17** below, we illustrate the diversity of landcovers present in the 20-year groundwater contributing areas for our sampling locations. A good regression analysis requires a wide and representative range of values for both the explanatory variable (landcover percentage, in our case), and the observed variable (neonicotinoid concentration). For thiamethoxam, our dataset included detections ranging up to 1.05 µg/L (average 0.06 µg/L), and for imidacloprid, we had detections ranging up to 0.86 µg/L (average 0.07 µg/L). These detections span roughly 3 orders of magnitude, and for many landcover classes, the proportion of the groundwater contributing areas across sites spanned a broad range, making this dataset a good candidate for our analyses.

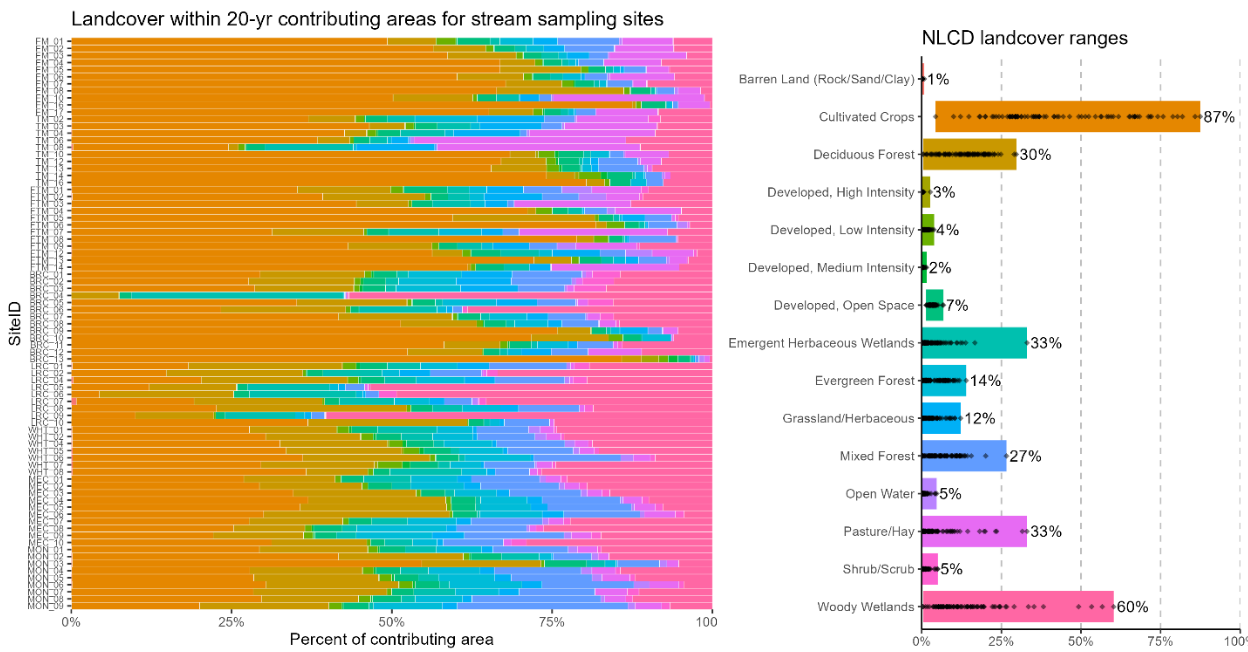



***Figure 17.*** *Range of landcover compositions across grab sampling locations from the National Land Cover Dataset. Diversity of landcover across sites is illustrated (left), and range of values at individual sites by land cover class is shown (right).*

As described in the Methods section, we generated regression models for each land cover class within the NLCD raster dataset and the CDL raster dataset. Many landcover classes do not represent a significant fraction of the landscape in our study area, so for clarity we will discuss a select set of major landcover types. **Figure 18** below illustrates the relationships we found between thiamethoxam concentration in stream water grab samples across the Wisconsin and Fox River watersheds, and the relationship between increasing concentrations and increasing landscape fraction of each landcover class. We found a strong positive relationship between high fractions of cultivated crops in the landscape and higher average thiamethoxam detection (slope 1.48, R2 = 0.22). In these regression equations, the slope of the best-fit line represents the log10-fold increase or decrease in expected mean detection when moving from 0% landcover to 100% landcover. In the case of cultivated crops, the equation suggests a 31-times (101.5) higher thiamethoxam concentration in a 100% cultivated crop landscape versus a 0% cultivated crop landscape (with the caveat that these best-fit line models should not be extended outside of the data range used to generate them).

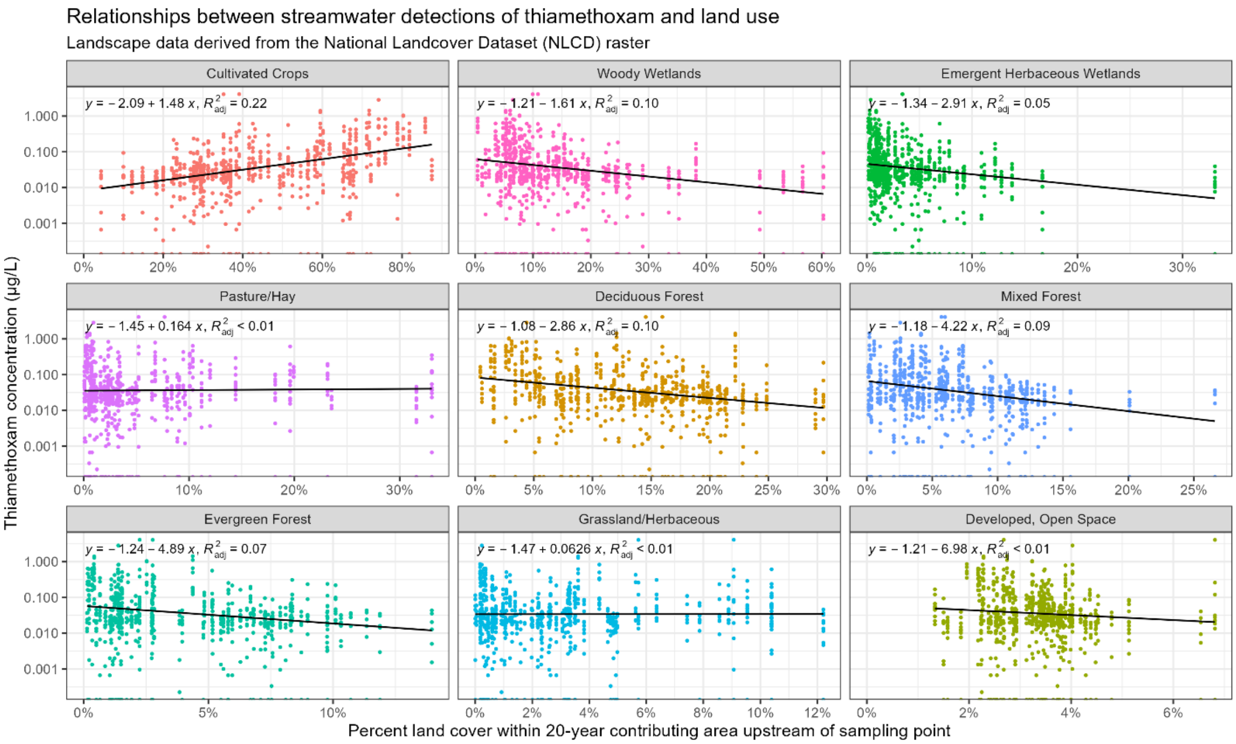



***Figure 18.*** *Relationships between thiamethoxam concentration in surface water grab samples and landscape composition of 20-year groundwater contributing areas (National Land Cover Dataset). Thiamethoxam concentration determined using ELISA kit method, limit of quantification 0.05 µg/L. Concentrations below this limit are included in these plots, but should be recognized as less reliable than higher concentrations*

The analysis also reveals negative relationships between increasing landscape fractions of wetlands (woody or herbaceous) and forests (deciduous, mixed, evergreen). No relationship was found for pasture/hay, grassland/herbaceous, or developed land. One interpretation of these results is that higher fractions of wetlands and forests in the landscape can help to cleanse or detoxify surface and groundwater, resulting in lower concentrations in such landscapes, though perhaps a simpler explanation is that watersheds with more cultivated crops have less wetlands and forests, and vice versa, because of the finite nature of the landscape. Pastures and grasslands may not be subject to this same tradeoff, explaining the flat relationship.

A similar set of regressions as those discussed above for thiamethoxam were also performed for imidacloprid, but no significant relationships (either positive or negative) were found between imidacloprid and any of the landcover types (**Figure 19**). In our sampling efforts, imidacloprid was detected less frequently than thiamethoxam and at lower concentrations. The different use patterns and lower water solubility of imidacloprid may help explain why these results differ from those for thiamethoxam. This non-relationship between land use and imidacloprid concentrations was also observed within the CDL analysis.

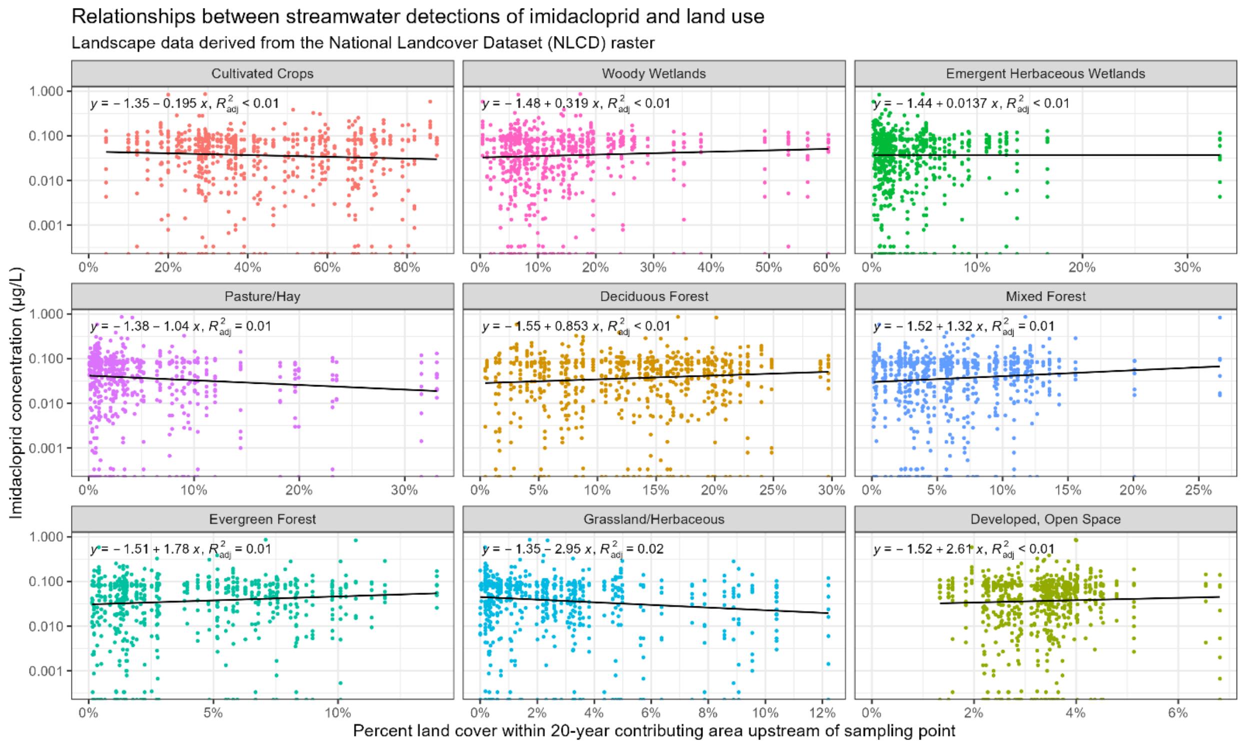



***Figure 19.*** *Relationships between imidacloprid concentration in surface water grab samples and landscape composition of 20-year groundwater contributing areas (National Land Cover Dataset). Imidacloprid concentration determined using ELISA kit method, limit of quantification 0.2 µg/L. Concentrations below this limit are included in these plots, but should be recognized as less reliable than higher concentrations.*

Moving on to our analyses using the Cropland Data Layer (CDL), we see a different distribution of landcover classes across our groundwater contributing areas as compared to the NLCD analysis, because we used the smaller 10-year contributing areas (**Figure 20**) instead of the larger 20-year contributing areas. Deciduous forest, grassland/pasture, and herbaceous/woody wetlands make up the largest non-crop landcover classes, with potatoes, sweet corn, dry beans, corn, soybeans, and alfalfa making up the most substantial cultivated crop classifications present in these groundwater contributing areas. Because the non-crop landcover classifications are the same in the CDL as in the NLCD raster dataset, we will focus only on the crop classifications for our CDL regression analysis.

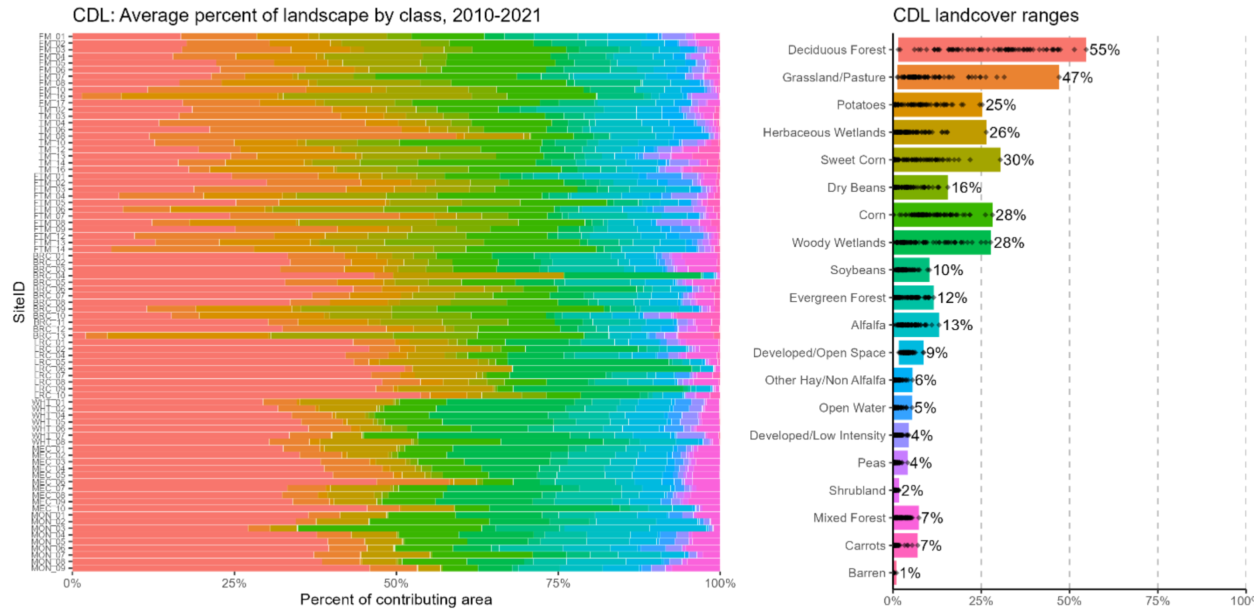



***Figure 20.*** *Range of landcover compositions across grab sampling locations from the Cropland Data Layer. Diversity of landcover across sites is illustrated (left), and range of values at individual sites by land cover class is shown (right).*

Recall from the previously discussed NLCD regression analysis that the relationship between thiamethoxam concentration and cultivated crop land cover fraction had a slope of 1.48 and an R2 of 0.22. Specific crop type relationships will be compared against these values and will be either more strongly associated with thiamethoxam concentrations (slope>1.48, R2>0.22), or less strongly associated than in aggregate. Figure 21 illustrates several of these specific crop relationships. Potatoes, sweet corn, beans, and peas were all strongly associated with increased thiamethoxam concentrations, while field corn was moderately associated with thiamethoxam concentrations, and alfalfa had little to no relationship. Neonicotinoids are not typically used in alfalfa production, so the flat relationship is justified here.

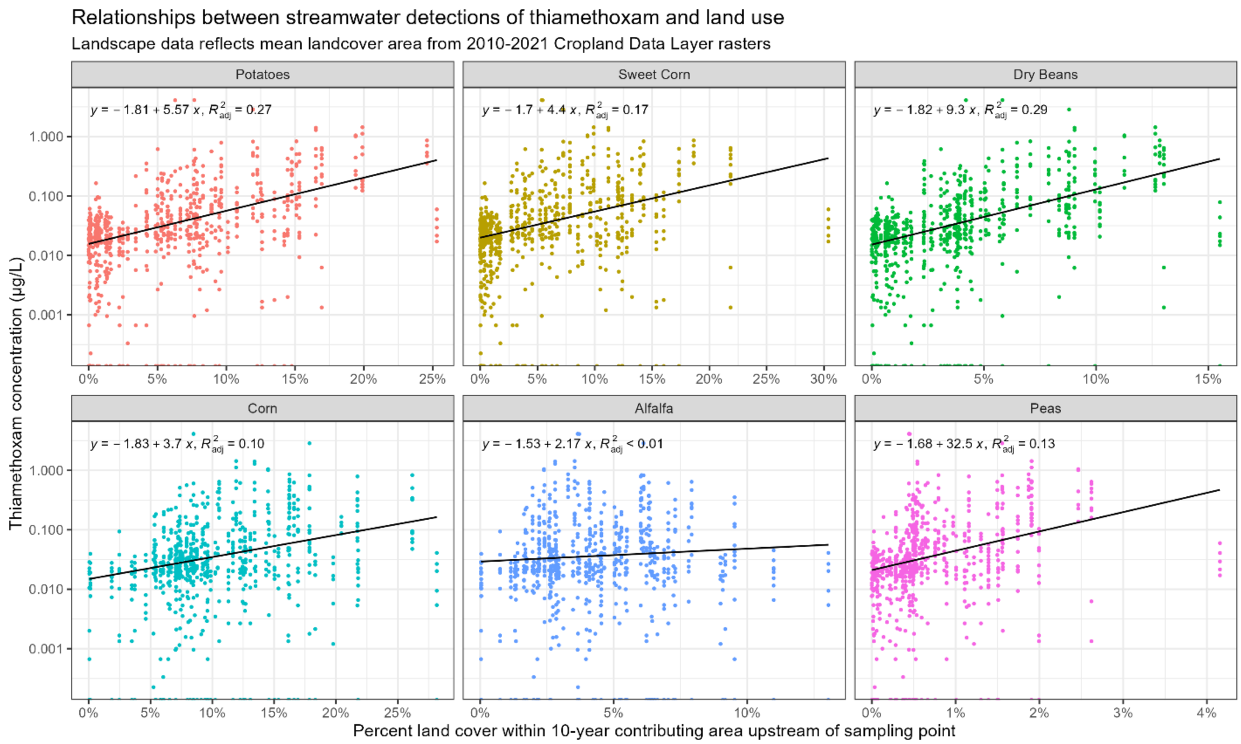



**Figure 21.** *Relationships between thiamethoxam concentration in surface water grab samples and landscape composition of 10-year groundwater contributing areas (Cropland Data Layer).*

There is another observation worthy of note here: for potatoes, sweet corn, beans, and peas, the distribution of individual points on the scatterplots, and the best-fit line, are all strikingly similar to one another. In our analysis, the landcover fraction for each land use class is represented as the average across 10 CDL years, within each groundwater contributing area. This will result in crops that rotate sequentially from year to year in the same field acquiring the same spatial patterning, and when regressed against our pooled neonicotinoid dataset, their relationships will be strongly correlated. As long as one crop type rotated onto a particular field parcel is treated with a neonicotinoid, that crop and all associated rotational crops will show up as correlated with neonicotinoid detections. It is important then that we use this data as a starting point in identifying crops that contribute to neonicotinoid detections in groundwater contributing areas but recognize that there may be "innocent bystander" crops that rotate in sequence with those neonicotinoid-treated crops and appear to be themselves related to neonicotinoid detections.

There is another confounding variable at play in this analysis, though one we intended to include: whether the streams are located in the Central Sands, or in the Fox River valley. As discussed in the methods, these two regions were included in our sampling efforts because they provide a strong contrast to one another in terms of the crops grown, the patterns of neonicotinoid use, the fraction of the landscape dedicated to cultivated agriculture, among other differences. If we take the analysis from Figure 21 and split each regression by region, we can clearly see that both the range of thiamethoxam detections and the range of land use fractions by land cover type differ greatly between the regions (**Figure 22**). For potatoes, sweet corn, dry beans, and peas, in particular, the figure illustrates that these crops make up a very small fraction of the landscape in the Fox valley rivers. Corn and alfalfa make up similar fractions of the landscape in each region, but thiamethoxam concentrations were positively correlated with corn in the Central Sands but had no relationship with corn in the Fox valley.

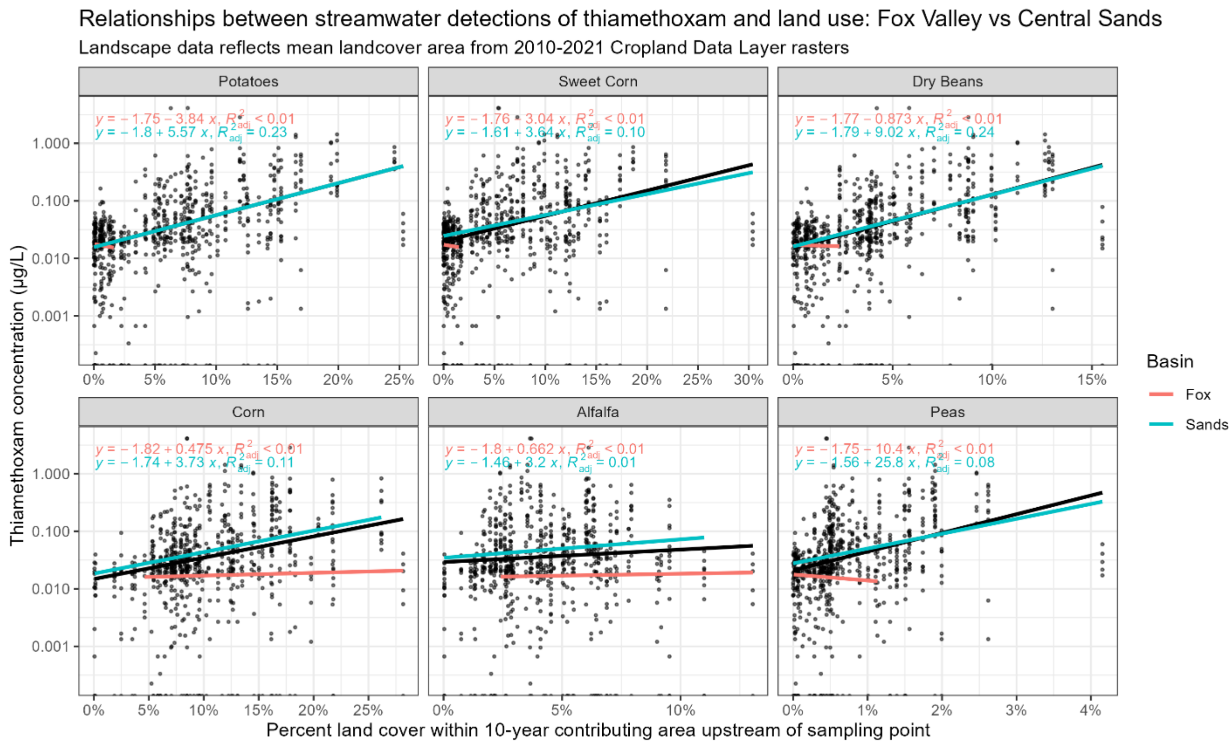



***Figure 22.*** *Relationship between thiamethoxam concentration in surface water grab samples and landscape composition of 10-year groundwater contributing areas (Cropland Data Layer). Regression best-fit lines are separated by region, with the Central Sands rivers shown in teal and the Fox valley rivers shown in red. The pooled regression line is shown in black.*

## Objective 2: Leola Ditch stream characterization

### *Canoe floats*

The resulting data are shown in **Figure 23**. The data are shown as colored squares collected as the canoe moved downstream from east to west. The temperature of Leola Ditch is shown in **Figure 23A**. The stream temperature is coolest (14.5 C) upstream at the start of the survey and increases to more than 18 C downstream to the west at the survey end. Part of this increase is likely due to the stream warming over the several hours required to canoe the six miles downstream. However, the coolest temperatures are found in the second mile of the survey. We attribute these lower temperatures to groundwater inflows.

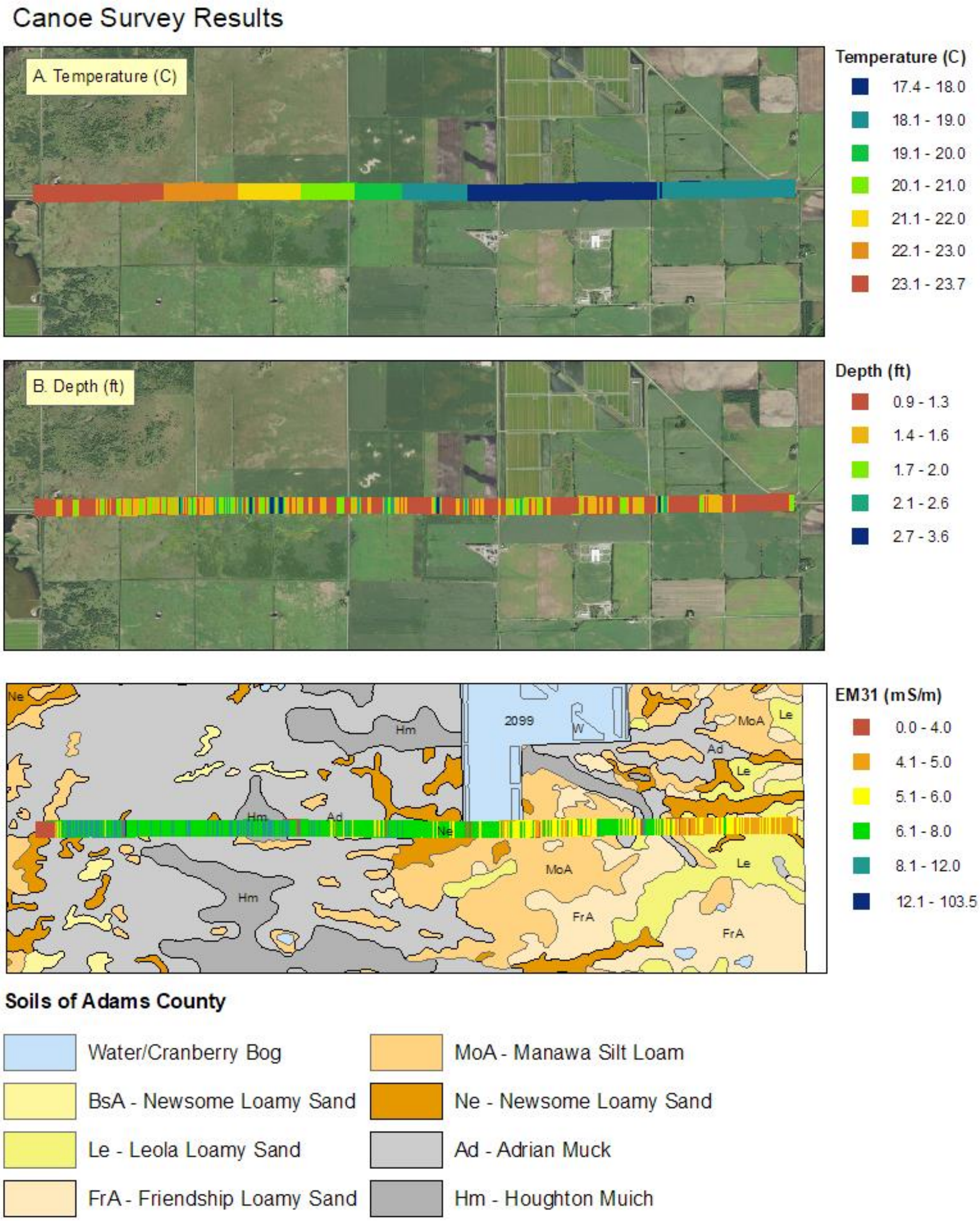



***Figure 23A-C.*** *Canoe float results showing variation in stream water temperature (A), stream water depth (B), and streambed conductivity shown in relation to native soil types (C).*

The stream depths vary from less than 1 ft to more than 3 feet along the survey (**Figure 23B**) without any strong patterns along the survey. The variation often occurs over shorter length scales. This is likely due to the way the data was acquired. The depth finder transducer was located at the rear of the canoe. Leola

Ditch is narrow and sinuous and so the transducer was not always located in the thalweg but would have sometimes been close to the shore in shallow water and at other times over the thalweg. The fish finder could not measure depths of less than 0.9 feet.

The EM31 results are overlain on the soils map (**Figure 23C**). Lower conductivity EM31 readings are shown in reds, oranges, and yellow while higher conductivity readings are shown in greens and blue. There is a good correlation between the conductivity and the soils. We would expect soils without organics and clays (yellows and oranges in the soils key) to have lower conductivities while mucks with more organics (greys in the soils key) would have higher conductivities. We attempted to remove the contribution of the stream water from the reading. We found our analysis gave negative streambed conductivities. We suspect this is because of the assumption that there was no contribution to the conductivity reading from the stream bank while in fact, the EM31 coils were often right at the stream banks, again due to the narrow width and sinuosity of Leola Ditch. The uncorrected readings are shown in **Figure 23C**. Because the stream water fluid conductivity and stream depth are relatively constant, the EM31 gave a reasonable measure of the sediment in and around the stream.

***Streamgaging***

Streamflow results over the course of the study were plotted showing streamflow in cubic feet per second (cfs) at discrete monitoring stations along Leola Ditch. Due to the large number of lateral tributary ditches contributing water to Leola Ditch from the north, many measurements were performed immediately upstream or downstream of these tributary features. **Figure 24** below presents streamflow measurements during three synoptic gaging surveys along the entire study area. This was performed over a 5-mile stretch of Leola Ditch, running parallel to CTH D, from STH 73 in the east to Sixth Avenue in the west. Dotted lines show the location of lateral tributaries to Leola Ditch and lines have been added between points to help illustrate the overall streamflow trend along this 5-mi section. In each case, streamflow rapidly increases (nearly doubling or tripling) over the first few miles (0-3 mi) before flattening off or even slightly decreasing, as is the case in September 2020. Many of the observed jumps in streamflow across lateral tributary ditches occur over relatively small distances along Leola ditch. These jumps, on the order of 1-2 CFS, may partially be attributable to surface water input/output from tributary ditches; however, measurement error may also be a factor. Measurement error for the streamgaging method we employed is estimated to be five percent (Turnipseed 2010), while our own analysis of repeat measurements suggested a percent error of four percent. Measurement error of this magnitude could potentially result in streamflow variations of 0.1-1.0 CFS for an associated streamflow range of 2.5-20 CFS along portions of Leola Ditch.

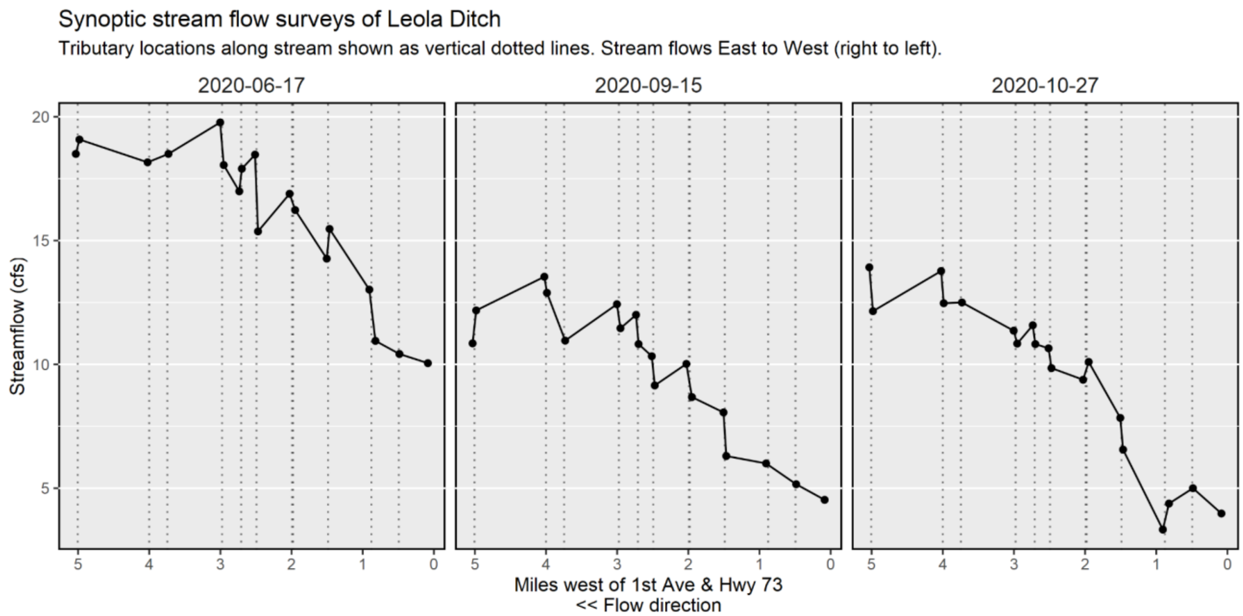



***Figure 24.*** *Streamflow measurements in cubic feet per second (CFS) along a 5-mi section of Leola Ditch from State Highway 73, west to Sixth Avenue. Locations of lateral tributary ditches, entering Leola Ditch from the north, are represented with vertical dotted lines.*

Following our initial evaluation in summer and fall 2020, streamflow measurements resumed in March 2021. From this period onwards, the detailed streamgaging surveys, including upwards of 20 monitoring stations, were reduced to an abbreviated survey consisting of stations (**Figure 25)**. The change to an abbreviated survey significantly reduced field time for streamgaging during field campaigns, allowing for the collection of more surface-water and groundwater field measurements and water-quality samples. Similar to what was observed during the synoptic surveys the prior year, in most cases, streamflow appears to double or even triple (September 2021) over the first three miles of Leola Ditch before levelling off or slightly decreasing over the remaining portion. While the overall flow regime shifts up (higher stream stage and increased flow rates) and down (lower stage, reduced flow rates), the general spatial pattern or distribution in flow along the Leola Ditch remained rather consistent. Taken as a whole, the abbreviated surveys provided sufficient data to provide “snapshots” of the overall flow regime, which were largely comparable to the synoptic surveys.

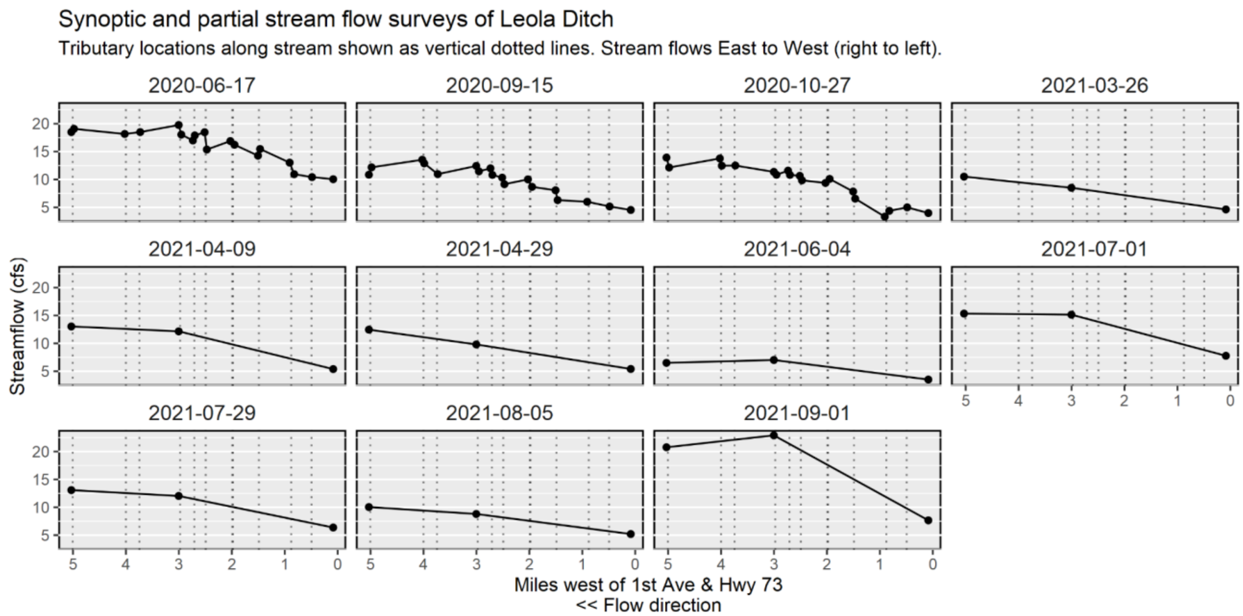



***Figure 25.*** *Streamflow measurements in cubic feet per second (CFS) along a 5-mi section of Leola Ditch from State Highway 73, west to Sixth Avenue. Locations of lateral tributary ditches, entering Leola Ditch from the north, are represented with vertical dotted lines. The first three gaging dates included multiple closely-spaced gaging locations, while subsequent stream gaging campaigns were scaled back to three diagnostic locations which still illustrate the overall streamflow conditions along this portion of Leola Ditch.*

Comparing stream discharge to hydraulic head ("head") measurements in mini-piezometers from late October 2020 may provide an explanation for why streamflow stabilizes or decreases further downstream. As shown in **Figure 26**, heads in mini-piezometers change from upward vertical head differences (positive head) to downward vertical head differences (negative head), providing evidence that Leola Ditch transitions from a gaining (groundwater inflow) to losing (stream-water outflow) system over this section of stream.

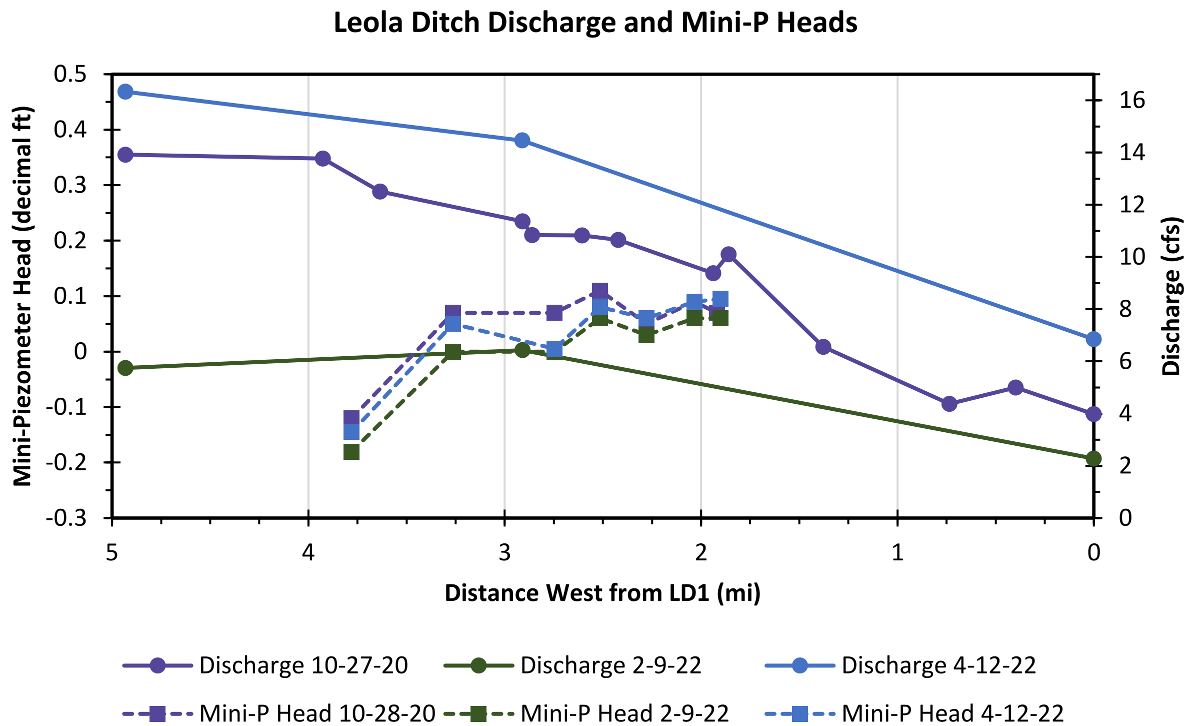



***Figure 26.*** *Streamflow (cfs) and mini-piezometer hydraulic head (decimal ft) as measured at different dates along a 5-mi (8,000m) section of Leola Ditch from State Highway 73, west to Sixth Avenue. This figure is presented again below as **Figure 37**, in the section on vertical-head measurements.*

### *Water chemistry: Summary of detections*

Between September 15, 2020, and April 12, 2022, water samples were collected at several locations along the 2- to 4-mile sections of Leola Ditch (from Third Avenue to Fifth Avenue), where we had previously established mini-piezometer installations, and delivered to UWSP WEAL for analysis. These piezometer installations allowed us to pair water-quality analyses of shallow groundwater (1- to 3-ft below the streambed) to the water quality of Leola Ditch at each monitoring station. While each piezometer provided a spatially-discrete indicator of groundwater quality, the corresponding surface water sample represented an integrated average of all upstream surface water and groundwater contributions. In total we collected samples at 5-12 sites during each of 6 sampling campaigns, for a total of 90 samples. These results, summarized by analyte and pooled across all samples, are presented in **Table 1**.

**Table 1.** Summarized results from surface and groundwater samples collected in Leola Ditch and analyzed by LC-MS at UWSP WEAL.

| Category | Analyte | LOD | Units | Count | Detects | Det % | Min | Mean | Max |
|---|---|---|---|---|---|---|---|---|---|
| Metals package | Alkalinity | 4 | mg/L | 32 | n/a | n/a | 141.0 | 181.7 | 348.0 |
| Metals package | Arsenic | 0.005 | mg/L | 32 | 10 | 31% | 0 | 0.002 | 0.012 |
| Metals package | Calcium | 0.026 | mg/L | 32 | 32 | 100% | 52.70 | 68.52 | 123.31 |
| Metals package | Copper | 0.001 | mg/L | 32 | 32 | 100% | 0.001 | 0.009 | 0.043 |
| Metals package | Hardness | 0 | mg/L | 32 | n/a | n/a | 237.2 | 291.7 | 493.4 |
| Metals package | Iron | 0.008 | mg/L | 32 | 29 | 91% | 0 | 0.09 | 0.67 |
| Metals package | Lead | 0.004 | mg/L | 32 | 0 | 0% | 0 | 0 | 0 |

| Category | Analyte | LOD | Units | Count | Detects | Det % | Min | Mean | Max |
|---|---|---|---|---|---|---|---|---|---|
| Metals package | Magnesium | 0.017 | mg/L | 32 | 32 | 100% | 22.05 | 29.30 | 53.66 |
| Metals package | Manganese | 0.001 | mg/L | 32 | 32 | 100% | 0.028 | 0.195 | 1.412 |
| Metals package | pH | 0.1 | SU | 32 | n/a | n/a | 7.51 | 7.96 | 8.11 |
| Metals package | Phosphorus | 0.004 | mg/L | 32 | 32 | 100% | 0.006 | 0.018 | 0.055 |
| Metals package | Potassium | 0.05 | mg/L | 32 | 32 | 100% | 3.22 | 5.06 | 12.50 |
| Metals package | Sodium | 0.234 | mg/L | 32 | 32 | 100% | 1.25 | 4.78 | 6.00 |
| Metals package | Sulfate | 0.06 | mg/L | 32 | 32 | 100% | 33.27 | 63.54 | 108.60 |
| Metals package | Zinc | 0.003 | mg/L | 32 | 8 | 25% | 0 | 0.004 | 0.030 |
| Neonicotinoids | Acetamiprid | 1.7 | ng/L | 45 | 0 | 0% | 0 | 0 | 0 |
| Neonicotinoids | Clothianidin | 1.5 | ng/L | 45 | 27 | 60% | 0 | 27.43 | 446.00 |
| Neonicotinoids | Dinotefuran | 0.7 | ng/L | 45 | 0 | 0% | 0 | 0 | 0 |
| Neonicotinoids | Imidacloprid | 2.4 | ng/L | 45 | 23 | 51% | 0 | 5.16 | 16.80 |
| Neonicotinoids | Thiamethoxam | 1.5 | ng/L | 45 | 40 | 89% | 0 | 138.75 | 390.00 |
| Nitrate | NO3+NO2(N) | 0.1 | mg/L | 90 | 70 | 78% | 0 | 11.41 | 71.80 |
| Water chemistry | Chloride | 0.5 | mg/L | 90 | n/a | n/a | 13.60 | 23.76 | 36.10 |
| Water chemistry | Conductivity | 1 | µS | 90 | n/a | n/a | 513.0 | 655.1 | 1150.0 |

*Samples were collected on Sep 15 and Oct 28, 2020, on Feb 24, Jun 4, and Dec 23, 2021, and Apr 12, 2022*.

***Water chemistry: Major ions***

A full suite of inorganic analyses was performed on surface and groundwater samples during two sampling campaigns in the fall of 2020. The most salient difference in major ions between groundwater and surface water, as illustrated with Piper (1944) diagrams, was the larger proportion of bicarbonate in groundwater relative to Cl and $SO_4$ (**Figure 27**), with groundwater also being overall higher in total dissolved solids (TDS) than surface water (333-411 mg/L vs 377-732 mg/L). Exceptions to this trend were observed at stations LD9.5MD and LD10.5MD, towards the western end of our study area Leola Ditch. At these two sites, the major ion signature measured in groundwater closely resembled the surface water signature. This east to west trend is presented in **Figure 28** as a series of Stiff (1951) diagrams for stations LD6.75MD, LD9.5MD, and LD10.5MD. When compared to the streamgaging results, which suggest that Leola Ditch transitions from a gaining to losing surface water system along our study area, the major ion signature for surface water and groundwater provide another line of evidence for this phenomenon. In other words, while the distinct major-ion signature of groundwater is observable in the shallow streambed mini-piezometer at station LD6.75MD, water sampled from the mini-piezometer at LD9.5MD and LD10.5MD suggest that Leola Ditch is losing water to the surrounding aquifer and the mini-piezometers are dominated by surface water.

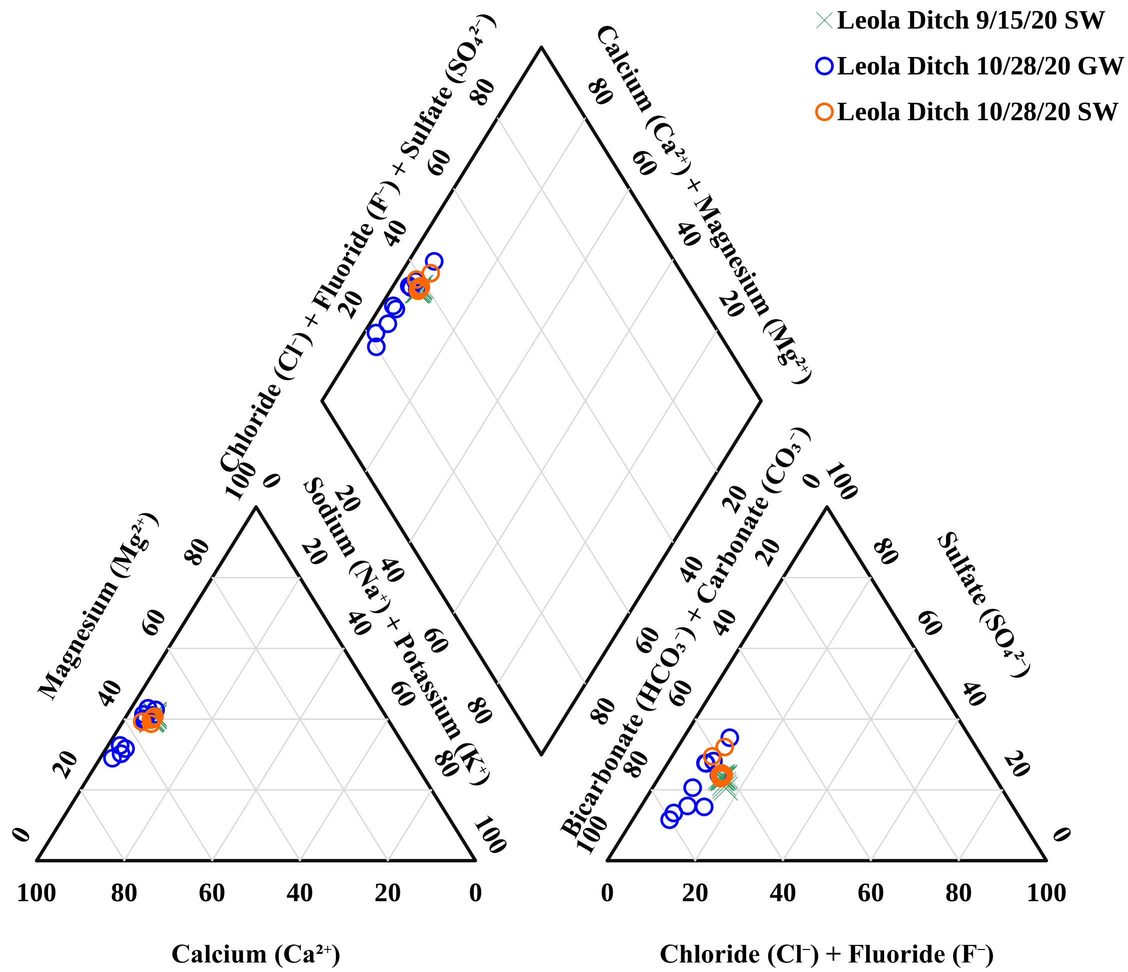



***Figure 27***. *Diagrams after Piper (1944) showing proportions of major ions in groundwater (GW, blue circles) and surface water (SW, orange circles and green Xs) samples collected from Leola Ditch.*

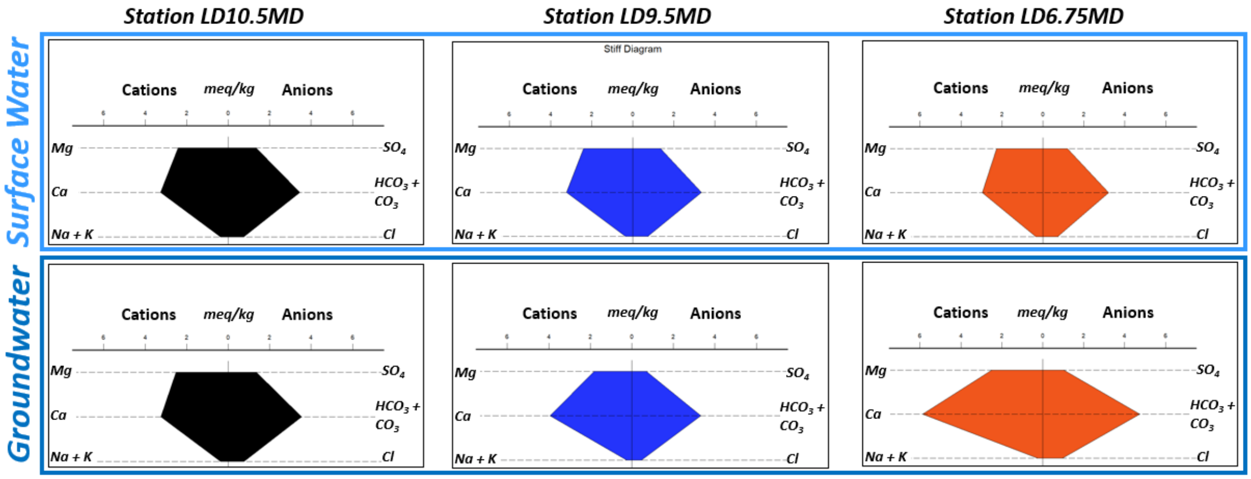



***Figure 28.*** *Diagrams after Stiff (1951) for surface water and groundwater collected at monitoring stations LD6.75MD, LD9.5MD, and LD10.5MD on 10/28/2020. At station LD6.75MD, the major ion signature for groundwater is distinctly different from that of surface water, most notably with enrichment of Ca and* $HCO_3$ + $CO_3$*. Moving west to stations LD9.5MD and LD10.5MD, the signature for groundwater increasingly resembles that of surface water, providing another line of evidence for losing sections of Leola Ditch, where surface water is moving into the shallow aquifer.*

A number of different chemical variables were compared to variations in specific conductance to determine what ions are responsible for trends observed in surface and groundwater. In surface water, specific conductance increases going downstream to the west by roughly 50 µS/cm over the 8 km of Leola Ditch monitored (**Figure 29**). By contrast, groundwater along the course of the ditch varied markedly in conductivity both with distance downstream and between different sampling dates. Starting at LD6.25MD and continuing to LD7.5MD and LD8.5MD, groundwater has elevated specific conductance before returning to conductivity values comparable to surface water by LD10.5MD. Groundwater at station LD9.5MD varies, exhibiting elevated conductivity on a few sampling dates and conductivity very close to surface water on most sampling dates.

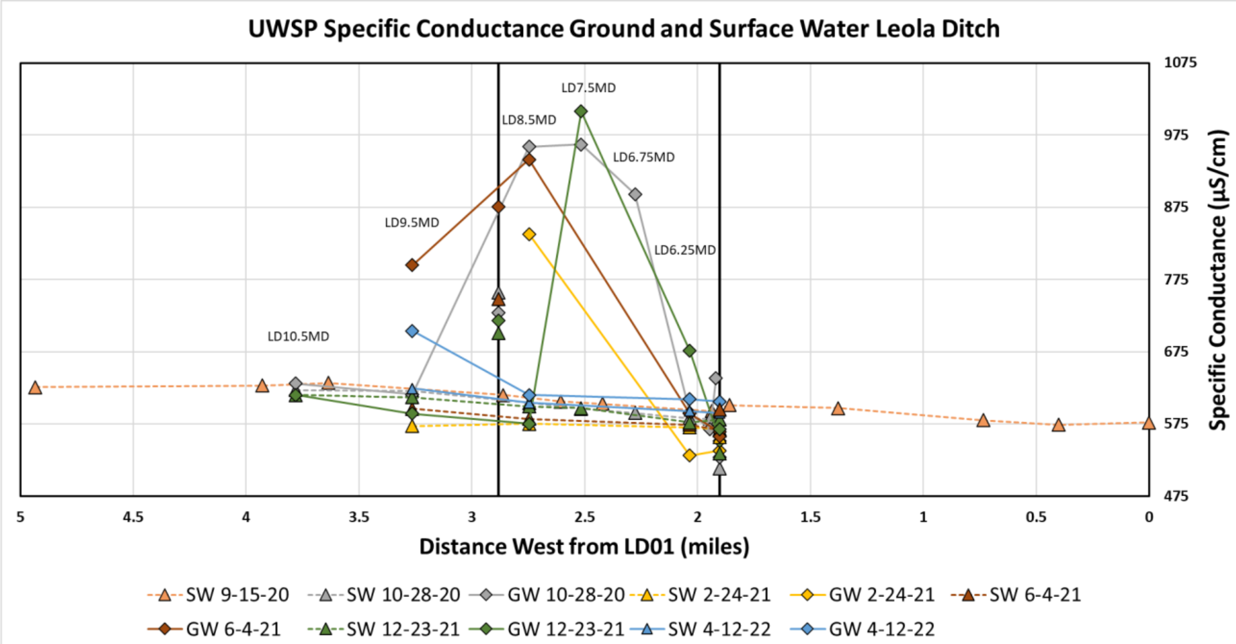



***Figure 29.*** *Plot of specific conductance as measured by UWSP WEAL versus distance downstream from station LD1 (see map,* ***Figure 12****). Surface water is marked by triangular symbols with dashed lines, groundwater by diamond symbols with solid lines. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with surface and groundwater data from these tributaries plotted as single points on the black lines.*

Differing concentrations of chloride, TDS, Ca + Mg, and Nitrate were investigated as possible factors contributing to changes in conductivity between samples. All exhibited positive linear relationships with specific conductance but Ca + Mg had the tightest correlation with the least scatter around the trend (**Figure 30**) indicating that these ions likely accompanied by bicarbonate are causing the majority of the variation seen in specific conductance.

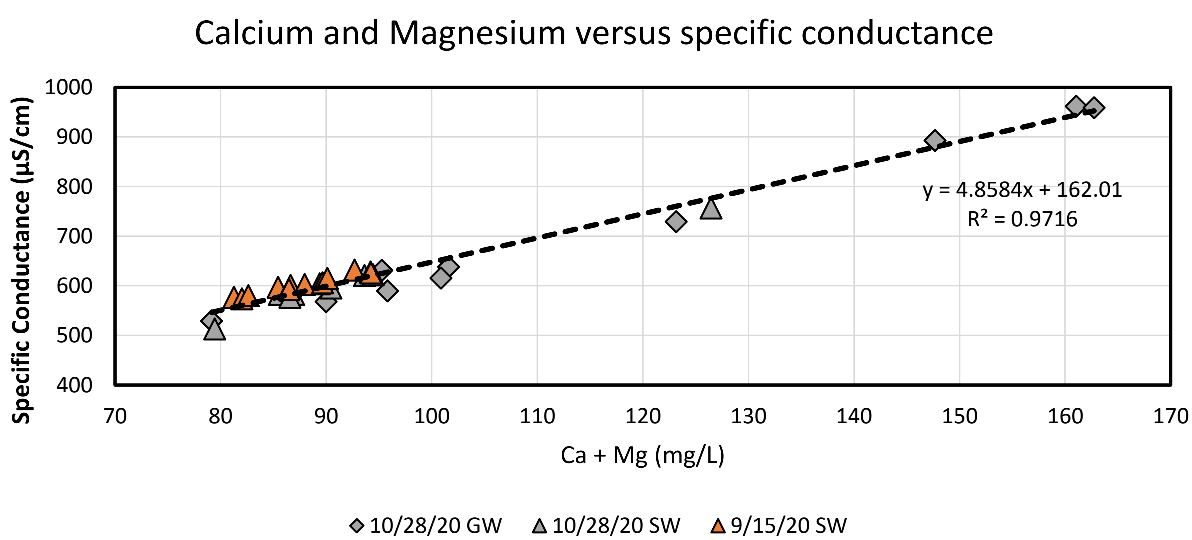



***Figure 30.*** *Plot of calcium and magnesium versus specific conductance for surface water and groundwater samples collected from Leola Ditch in the fall of 2020.*

### *Water chemistry: Nitrate*

Nitrate in surface water along Leola Ditch exhibits a pattern where relatively high concentrations (>10 mg/l) are present to the east (near STH 73) and gradually decline downstream to the west (**Figure 31**). By contrast, nitrate concentrations in groundwater displayed much more variability. Groundwater nitrate concentrations at stations LD5.9MD and LD6.25MD were consistently below surface water concentrations. At stations LD8.5MD and LD10.5MD groundwater nitrate concentrations varied from equal to or well below surface water concentrations. At stations LD6.75MD and LD7.5MD groundwater nitrate concentrations consistently rose as high as 34-72 mg/l, while concentrations at station LD9.5MD had more variability but spanned roughly 7-44 mg/l over the course of this study.

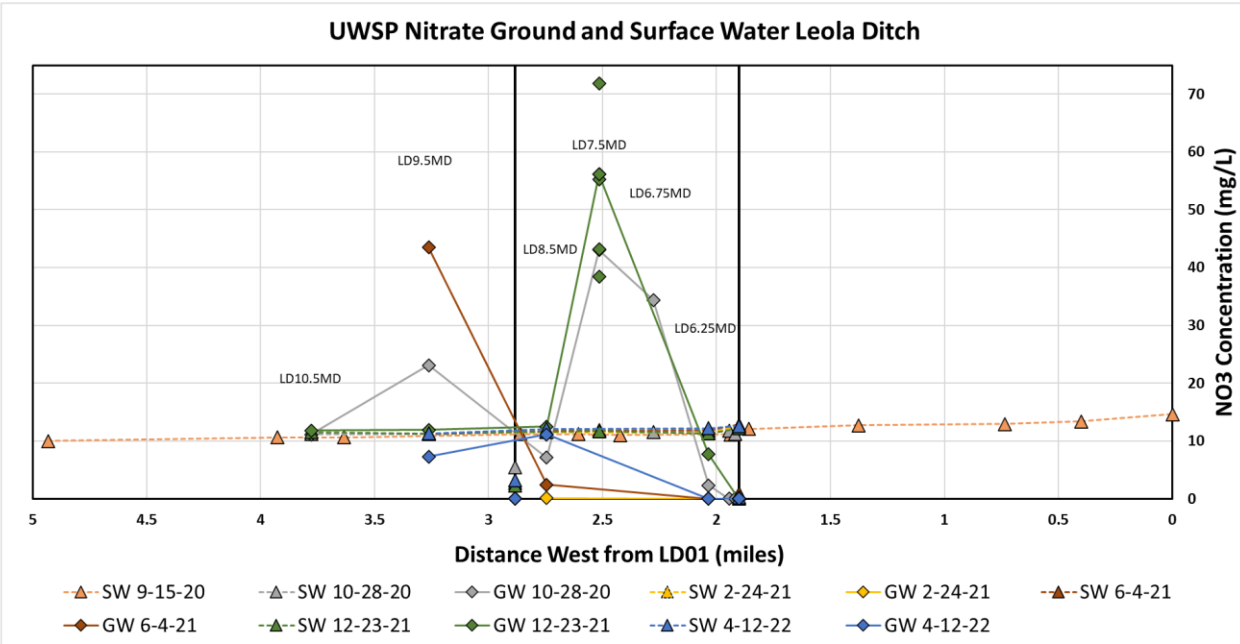



***Figure 31.*** *Plot of nitrate concentration versus distance downstream from station LD1. Triangles with dashed lines represent surface water, diamonds with solid lines represent groundwater. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with surface and groundwater data from these tributaries plotted as single points on the black lines. Five points at LD7.5MD for series GW 12-23-21 represent data from five mini-piezometers installed at the station.*

### *Water chemistry: Neonicotinoids*

LC-MS analyses of water samples never detected acetamiprid and dinotefuran in groundwater or surface water from Leola Ditch. Thiamethoxam, clothianidin and imidacloprid were commonly detected, with all three species prevalent in surface waters and groundwater from certain stations. Commonly, neonicotinoid concentrations at Leola Ditch follow a rule where thiamethoxam is present at roughly 10 times the abundance of clothianidin, with imidacloprid in turn about three times less abundant than clothianidin.

Spatially, neonicotinoid concentrations in surface water were observed to decrease slightly downstream but not at a linear rate (**Figure 32**). Three out of four sampling periods show a sharp decrease from LD5.9MD to LD6.25MD bracketing the intersection of tributary stream, which is consistently low in neonicotinoids in both groundwater and surface water samples. From LD6.25MD to LD8.5MD concentrations are generally stable in surface water before decreasing again at LD9.5MD. In groundwater, neonicotinoids are universally low at LD5.9MD and LD6.25MD, becoming elevated and similar in concentration to surface water at stations LD8.5MD and LD9.5MD (**Figure 33**). Interestingly, time of year and stream stage was not observed to influence neonic concentrations with values remarkably consistent between sampling dates for surface water. Neonicotinoids in groundwater by contrast were inconsistent between sampling dates at the same time of year or same level of stream discharge. Neonicotinoid concentrations derived from the ELISA method (**Figure 34**, **Figure 35**) are much noisier and more variable than concentrations obtained by LC-MS but are generally in agreement. Groundwater concentrations of thiamethoxam are frequently elevated and very similar to counterpart surface water at stations LD9.5MD and LD10.5MD, being at generally lower concentrations upstream.

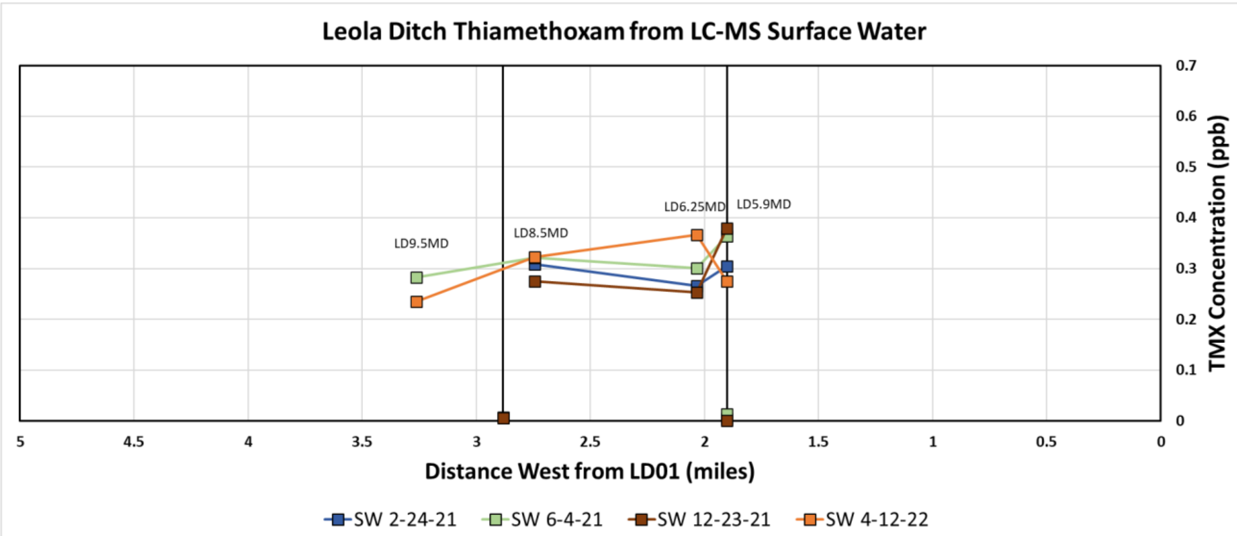



***Figure 32.*** *Plot of thiamethoxam concentrations determined by LC-MS in surface water versus distance downstream from station LD1. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with data from these tributaries plotted as single points on the black lines.*

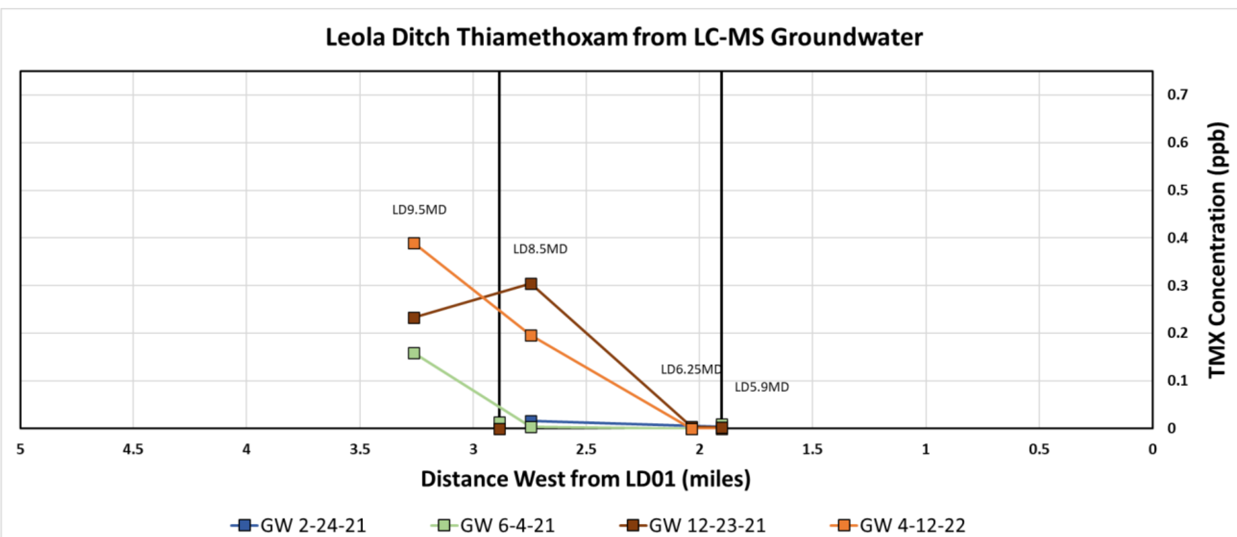



***Figure 33.*** *Plot of thiamethoxam concentrations determined by LC-MS in groundwater versus distance downstream from station LD1. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with data from these tributaries plotted as single points on the black lines.*

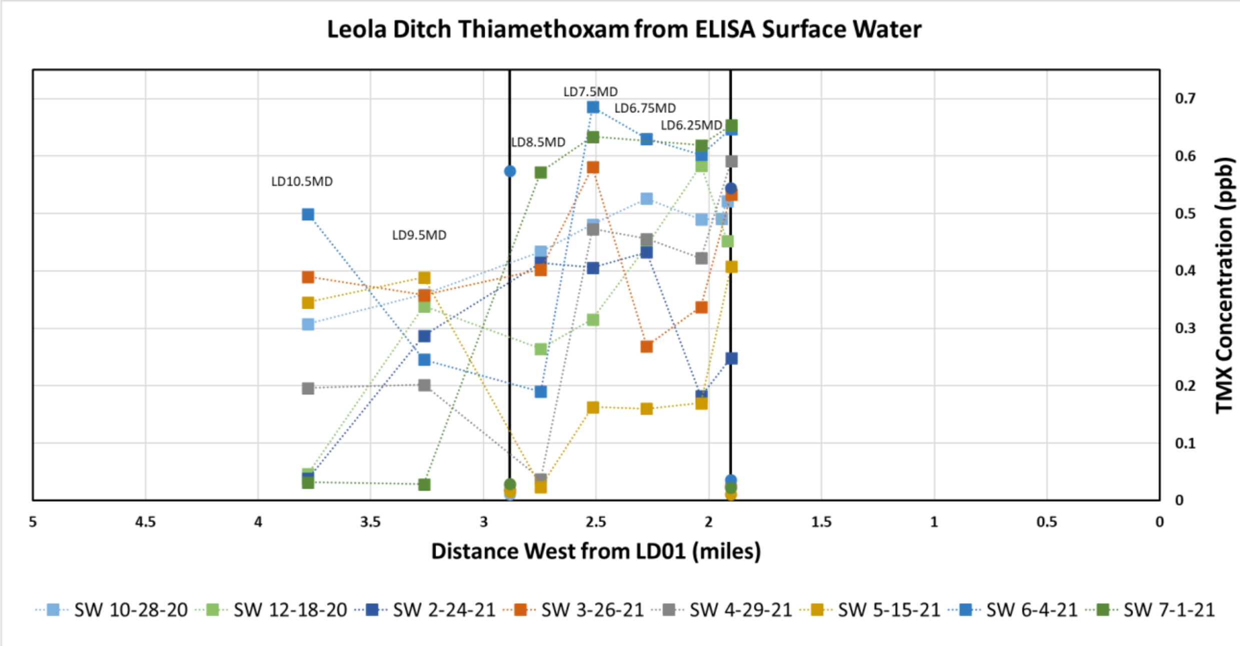



***Figure 34.*** *Plot of thiamethoxam concentrations determined by the ELISA method in surface water versus distance downstream in Leola Ditch. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with data from these tributaries plotted as single points on the black lines.*

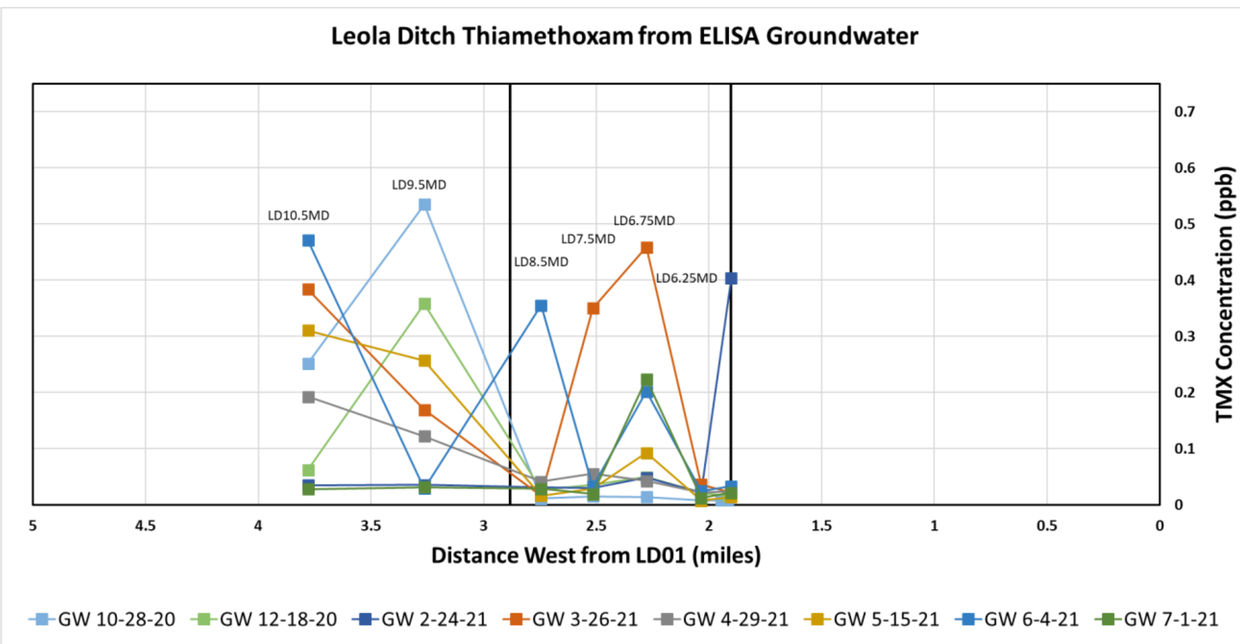



***Figure 35****. Plot of thiamethoxam concentrations determined by the ELISA method in groundwater versus distance downstream in Leola Ditch. Black vertical lines represent the intersection of tributaries LD6TU1 and LD9TU1 with data from these tributaries plotted as single points on the black lines.*

All three of these neonicotinoids show a distinct positive correlation with nitrate, with either surface water or groundwater impacted by nitrate concentrations ≥10 mg/L almost universally showing elevated concentrations of all three chemicals, while groundwater or surface water ≤10 mg/L nitrate have very low or no detectable neonicotinoids with only a few exceptions (**Figure 36**).

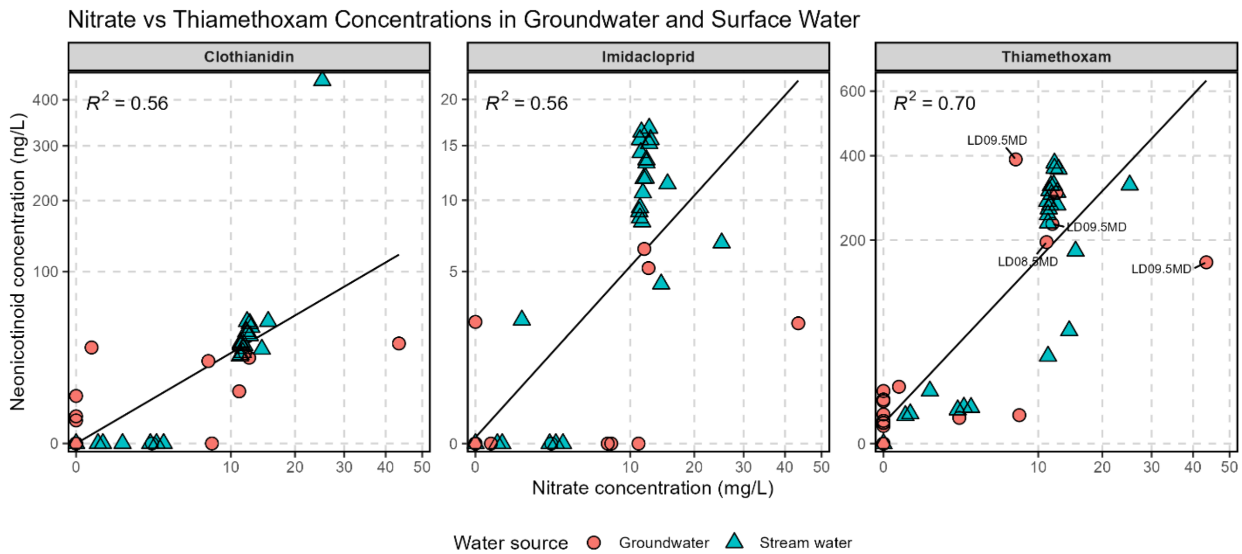



***Figure 36.*** *Correlation plot of nitrate and neonicotinoid concentrations in groundwater and surface water samples collected from Leola Ditch. Surface water samples represented by triangles, groundwater samples by circles. Best fit line indicated on plot, with accompanying $R^2$ correlation statistic. Stations with high groundwater concentrations of thiamethoxam are labelled with the station name.*

### *Water chemistry: Interpretations*

From the preceding data, several important findings are evident. The first is that groundwater captured in mini-piezometers from LD6.75MD to LD8.5MD has a distinctive high TDS signature driven primarily by the soluble products of carbonate minerals. Several different hypotheses can provide an explanation for this. In acidic soils with low buffering capacity like those making up the quartzose sediments of the Wisconsin Central Sands Region, lime is often added to agricultural fields to increase pH and provide extra nutrients to crops. Groundwater modeling runs performed for this project, using the regional central sands model (Kraft and Mechenich, 2010), suggest that groundwater discharge to Leola Ditch is dominated by groundwater flow from the east and north. Comparing land use from aerial photography to groundwater flow paths, mini-piezometers with high TDS likely intersect groundwater passing underneath large sections of agricultural fields. By contrast, mini-piezometers to the east with low TDS receive flow from groundwater that has passed through a complex of cranberry bogs which may not apply lime at the same intensity. There also seems to be a correlation between high TDS groundwater and elevated nitrate concentration at station LD6.75MD and LD7.5MD, suggesting that agricultural activities may be causing the distinctive groundwater signature. However, at station LD8.5MD, sampling intervals with high TDS have low nitrate and importantly, low neonicotinoids, indicating that agricultural impacts may not entirely be the cause of this distinctive groundwater chemistry.

Another possibility is that groundwater through the stretch with high TDS values is interacting with distinctive aquifer materials containing a greater fraction of carbonate minerals. From microscopic examination of stream sediments and sediments drilled in shallow borings on the stream banks, sand and gravel sized dolomite grains were recognized at fluctuating abundance (~5-10%). Greater proportions of these grains would reasonably explain the difference in Ca + Mg concentration primarily driving the increase in specific conductance. A related possibility is that high TDS groundwater is discharging from flow paths that have interacted with areas of the aquifer that have not been leached of their carbonate fraction. In the Clayton (1987) report on the Pleistocene geology of Adams County, leaching of carbonate from the uppermost few meters to few tens of meters is reported ubiquitously with a thicker leached horizon associated with areas of sandy sediment. The area from LD6.25MD westward through the area of high TDS groundwater has a layer of muck at the surface encountered both in shallow borings and in an EM31 conductivity survey. This layer of muck is fine grained and may have provided an impermeable cap shielding underlying sediment from carbonate leaching and resulting in the distinctive groundwater signature.

Another implication of the water chemistry data is the trend from LD8.5MD westwards where identical chemistry between groundwater and surface water, observed for a variety of measured parameters, provides another line of evidence for a "losing" stream system where water from Leola Ditch discharges to groundwater and reenters the aquifer. Groundwater collected from mini-piezometers at LD10.5MD, LD9.5MD, and LD8.5MD, at some sampling periods, is very similar to the corresponding stream water result for specific conductance, nitrate and neonicotinoids. As such, the groundwater results for these western mini-piezometer sites cluster together with surface water on Piper and Stiff diagrams; contrasting with groundwater pumped from more eastern sections of Leola Ditch. Having a losing stretch of a ditch system impacted by agricultural activity is an important factor to consider as soluble contaminants such as nitrate and neonicotinoids are effectively being transported from intensively farmed areas upstream into the relatively natural and less-impacted landscape surrounding Leola Ditch west of LD9TU (Fifth Avenue).

A final important take away from the analytical results presented above is in the use of nitrate as an effective proxy for neonicotinoid contamination in the groundwater and surface waters of Leola Ditch. A nitrate concentration of >10 mg/L is with only one exception correlated with significant concentrations of neonicotinoids, highlighting the connection between neonicotinoids and nitrate in waters impacted by agricultural activity in this system.

***Streambed vertical-head difference***

The static height of water inside the mini-piezometer relative to the height of the stream surface in Leola Ditch was measured to provide an indication of head differences between the shallow groundwater system and surface water at each mini-piezometer location. Three sets of head measurements relative to stream level are presented in **Figure 37**, paired with concurrent discharge measurements from streamgaging. Overall, relative heads are positive from LD5.9MD to LD7.5MD, slightly positive or zero from LD8.5MD to LD9.5MD and negative at LD10.5MD. Comparing stream discharge to the trend in mini-piezometer heads relative to stream level, a positive correlation is apparent. Dates with positive relative heads at station LD9.5MD correspond to dates where discharge increased between LD9MD and LD12MD while a negative relative head at LD9.5MD is associated with a decrease in discharge over the westernmost 2.5 mi (~4 km) of Leola Ditch.

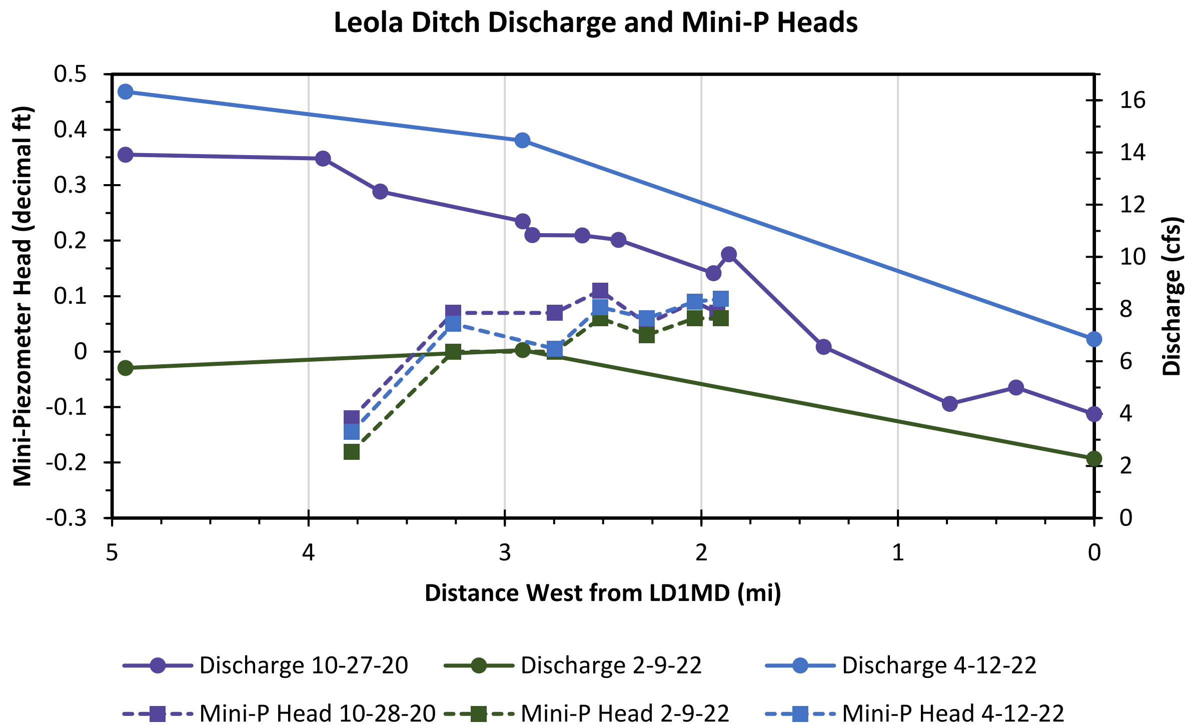



***Figure 37.*** *Combination chart of measured mini-piezometer heads (left axis) and stream discharge (right axis) with distance downstream in Leola Ditch. Stream discharge trends marked by solid lines with circular markers, head measurements marked by dashed lines with square markers. Black vertical lines represent intersection of tributary ditches with Leola Ditch.*

In addition to trends in relative head going from east to west over 2 miles of Leola Ditch from Third Avenue to Fifth Avenue, consistent patterns in head were also observed along several mini-piezometers fences, consisting of multiple mini-piezometers installed along a north-south transect, perpendicular to streamflow. **Figure 38** shows relative head measurements across the mini-piezometer fences at LD5.9MD, LD7.5MD and LD10.5MD on two dates. Comparing the two measurement dates, mini-piezometer heads are consistently higher on the date where greater discharge was measured in the stream. Among the mini-piezometers arranged in north-south fences, there are noticeable differences between head measurements collected on the same date. At LD5.9MD heads increase slightly going from north to south. By contrast at LD7.5MD heads decrease slightly between the northern and southern piezometers and are consistently zero in the middle. At LD10.5 roughly equivalent negative heads are present at the northern and southern wells while the well in the middle is less negative. At LD7.5MD, where shallow and deeper mini-piezometers were driven on the north side of the stream, the pattern of relative heads are less clear, with the water level in the deeper mini-piezometer always slightly lower than the one at 2.5 ft and the water level in the shallow mini-piezometer either lower or at the same level as the one at 2.5 ft.

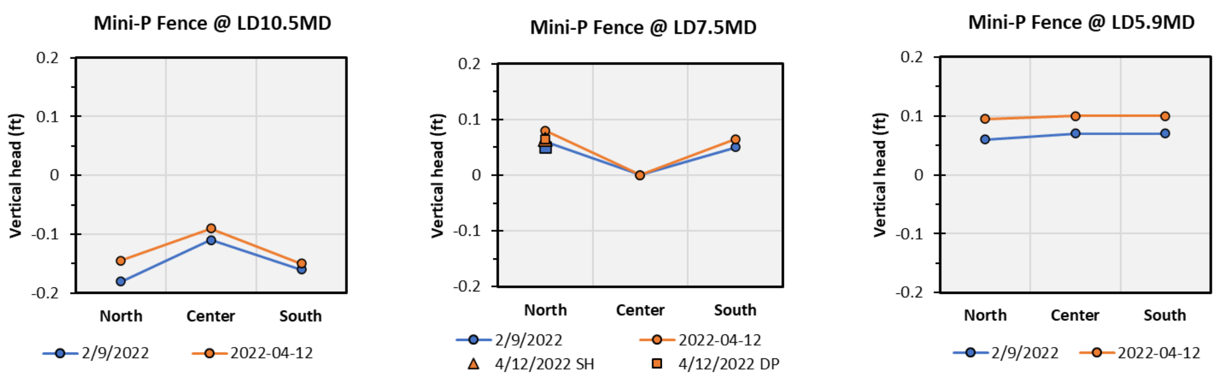



***Figure 38.*** *Measured height of water level in mini-piezometers relative to stream surface for a transect (or fence) perpendicular to streamflow direction at stations LD5.9MD (upstream), LD7.5MD, and LD10.5MD (downstream). At each station, a fence of piezometers was installed with one each near the north bank, center, and south bank of the stream. At LD7.5MD, two additional piezometers were installed near the north bank shallower (SH) and deeper (DP) than the typical piezometer.*

**Interpretations.** This section will focus on information collected about physical groundwater conditions and dynamics between groundwater and surface water along the monitored section of Leola Ditch. See previous section for in-depth presentation of groundwater chemistry results. The trends in heads both parallel and perpendicular to the streamflow direction have important implications for the dynamics of groundwater within the Leola Ditch system and the potential for contamination by nearby land use. Based on the pattern of observed mini-piezometer heads from east to west along the section of ditch monitored in this study, a clear distinction exists between the area from LD1MD to LD9MD and the area from LD9MD to LD12MD. Corroborated by discharge data (**Figure 24**, **Figure 25**, **Figure 26**, **Figure 37**) and groundwater chemistry (**Figure 28**), LD1MD-LD9MD is considered to be a “gaining” section with groundwater actively discharging to surface water. By contrast, the section of Leola Ditch from LD9MD to LD12MD, further to the west, displays comparatively minimal groundwater discharge transitioning into loss of stream water to the surrounding aquifer. This transition from “gaining” to “losing” conditions was consistent with observations and interpretations from several other field investigation methods (e.g., streamgaging, streambed temperature-flux, water chemistry) and generally consistent with particle-tracking results from the Central Sands groundwater model (Kraft and Mechenich, 2010). Previous investigations by Meigs and Bahr (1995) identified similar dynamics where complex three-dimensional groundwater flow fields were observed and modeled near surface features of the Central Sands Region.

A further demonstration of the gaining versus losing character of the different parts of Leola Ditch is illustrated by the distribution of ice cover along the stream during winter months (**Figure 39**). Looking east from LD6.25MD, the stream is completely free of ice and has minimal snow at the base of the ditch on the south bank of the channel. Going 1 mi west to LD7.5MD, some ice was observed lining the stream channel with snow accumulation visible along the south bank. Going further west, to the losing section of the stream at LD9.5MD, the difference in ice cover is immediately apparent with ice mostly covering the channel. Further west, at station LD10.5MD, the channel is completely covered with over an inch of ice accumulation.

LD6.25 facing east (upstream)

LD8.5MD facing west (downstream)

LD9.5MD facing west (downstream)

LD10.5MD facing west (downstream)

***Figure 39.*** *Photos taken at four stations during fieldwork on Feb 9, 2022. See site map (**Figure 12**) in the methods section for locations. Note the differences in ice cover at each station. Lack of ice is suggestive of groundwater discharge to the stream, while the presence of ice suggests lack of groundwater discharge and the transition from a gaining to losing stream condition.*

### *Stream-groundwater comparisons using mini-piezometers*

**Neonicotinoids.** Our initial efforts at characterizing neonicotinoid concentrations in Leola Ditch used methods previously employed by the Groves lab, namely commercially-available ELISA kits developed to quantify thiamethoxam or imidacloprid in water samples. We focused on establishing baseline neonicotinoid concentrations in the stream, as well as profiling changes in concentration along our study section of Leola Ditch and, using the mini-piezometers, investigating differences in neonicotinoid concentration between surface water and groundwater below the streambed. For these investigations we typically collected paired surface water and groundwater samples at 11-13 locations along Leola Ditch between the 2-mile and 4-mile markers west of the intersection of First Avenue and STH 73 (the easternmost/upstream section of the study area). Collections were performed on Oct 28 and Dec 18 in 2020, and Feb 24, Mar 26, Apr 29, May 15, Jun 4, and Jul 1 of 2021. We only tested for imidacloprid on the first survey date, due to the low concentrations detected on that date, the high cost of the imidacloprid kits, and the higher limit of quantification on the imidacloprid kit compared to the thiamethoxam kit.

From these investigations we can highlight a few trends (**Figure 40**). Imidacloprid was detected at low concentrations (n=22, max 24 ng/L, average 4 ng/L), at similar concentrations in stream and groundwater, and at similar concentrations along a two-mile stretch of Leola Ditch. Thiamethoxam, in contrast, was detected at an average concentration of 183 ng/L (n=190, max 686 ng/L). Additionally, the average concentration of thiamethoxam in stream water and groundwater changes from the first mile to the second mile. In the upstream section, substantially higher concentrations were found in the stream relative to the groundwater, but moving downstream, we notice the stream water and groundwater having similar concentrations of thiamethoxam at each location. As discussed in our streamgaging section, we identified these two sections of stream as having different stream water/groundwater dynamics, where the upstream section is gaining water from the ground, and the lower section is losing water to the groundwater. Thus, groundwater drawn up through the mini-piezometers is water that exited the stream, explaining the similar thiamethoxam concentrations.

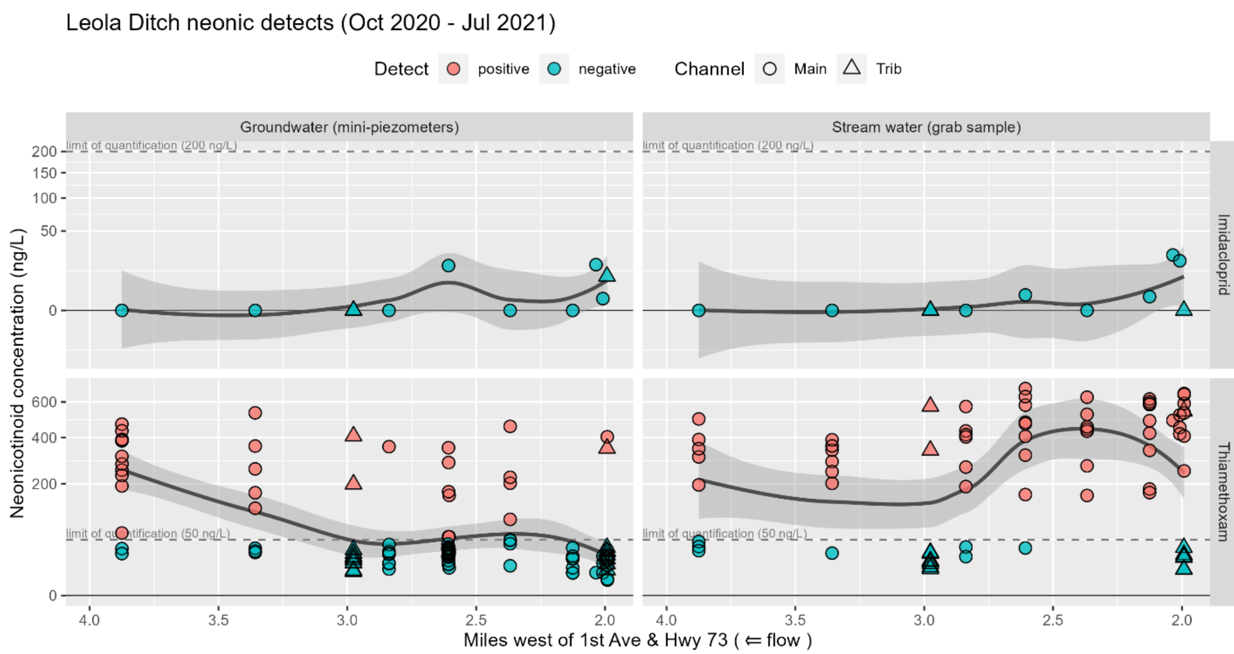



***Figure 40.*** *Thiamethoxam and imidacloprid detections in surface and groundwater along a discrete section of Leola Ditch, 2020-2021. The figure highlights detections in the main stream channel (circles) and in tributaries feeding into the main channel (triangles). Concentrations above the limit of quantification of each kit are considered positive detections and highlighted in red, those below in teal. Mean detection at each mile marker is highlighted with a smooth-fit line. Note stream flow direction is East to West (right to left).*

While we were able to test for thiamethoxam (and imidacloprid to a lesser extent) in stream and groundwater using ELISA kits, the UW Stevens Point Water and Environmental Analysis (WEAL) lab offers a neonicotinoid package that tests for all 5 neonicotinoid compounds (acetamiprid, clothianidin, dinotefuran, imidacloprid, and thiamethoxam) at very high levels of accuracy (0.7-2.4 ng/L limits of detection). As described in the ELISA results above, paired stream/groundwater samples were collected in 1L bottles at several locations along Leola Ditch and assayed for neonicotinoids on Feb 24, Jun 4, Dec 23, 2021, and Apr 12, 2022. Pooled results for all UWSP WEAL analyses are available in **Table 1**. Acetamiprid and dinotefuran were never detected in any of the samples. Clothianidin was detected in 60% of samples at an average concentration of 45.7 ng/L and a maximum of 446 ng/L. Imidacloprid was detected in 51% of samples, at an average of 10.1 ng/L and a maximum of 16.8 ng/L. Thiamethoxam was detected in 89% of samples, at an average concentration of 156.1 ng/L and a maximum of 390 ng/L. The imidacloprid and thiamethoxam results match relatively closely with our results from the ELISA kits, though more weight should be given to the numerical values produced by UWSP WEAL considering the precision of their equipment.

In **Figure 41** we illustrate the differences between neonicotinoid detections in stream water and groundwater at each sampling location from UWSP WEAL analytical results. Stream water had consistently higher neonicotinoid concentrations across all three detected compounds, though as discussed earlier, the differences were more pronounced in the upstream half of the stream section (east of mile 3) compared to the downstream half (west of mile 3).

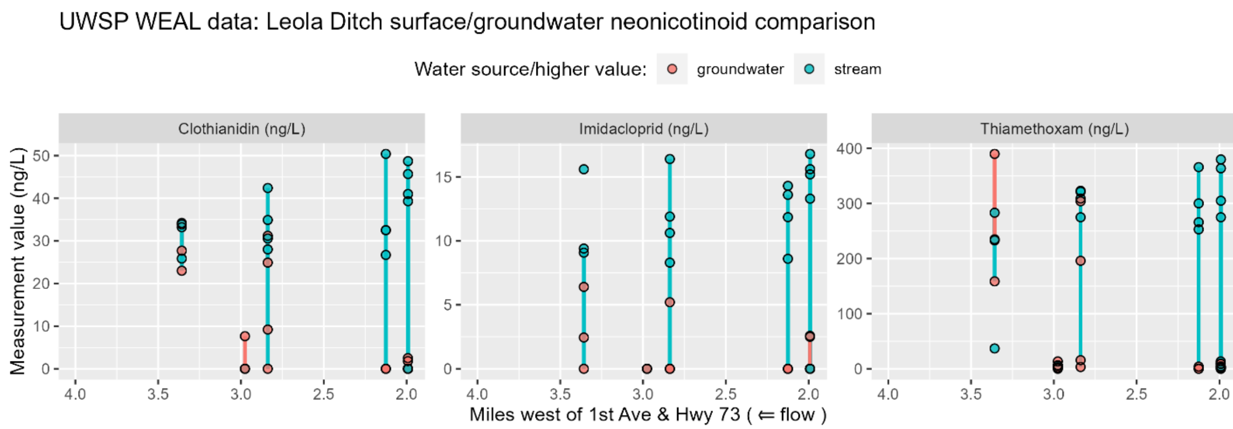



***Figure 41.*** *Surface water/groundwater paired comparisons of neonicotinoid concentrations at sampling locations along Leola Ditch. Acetamiprid and dinotefuran were never detected so are omitted from this figure. Data are from water samples collected on Feb 24, Jun 4, Dec 23, 2021, and Apr 12, 2022. In the figure, groundwater detections are shown as red circles, while stream detections are shown in teal. A vertical line connects each paired sample, colored to indicate which water source had a higher concentration. All sampling dates are included, shown as multiple points per location.*

**Metals.** On one of our sampling dates (Oct 28, 2020), we purchased the metals analysis package for water samples collected and sent to UWSP WEAL. While this data only reflects water quality at one time point, the differences between stream water and groundwater and the changes in those differences along the stream highlight the changing interactions between stream water and groundwater along Leola Ditch. For many of the analytes, we observe significantly higher concentrations of dissolved metals in the groundwater relative to the stream water in the eastern half of the study area. These departures are generally reduced or eliminated toward the downstream section of Leola Ditch, where stream water discharges into the streambed and into our mini-piezometers. These results are illustrated in **Figure 42** and detailed values are available in **Table 1**.

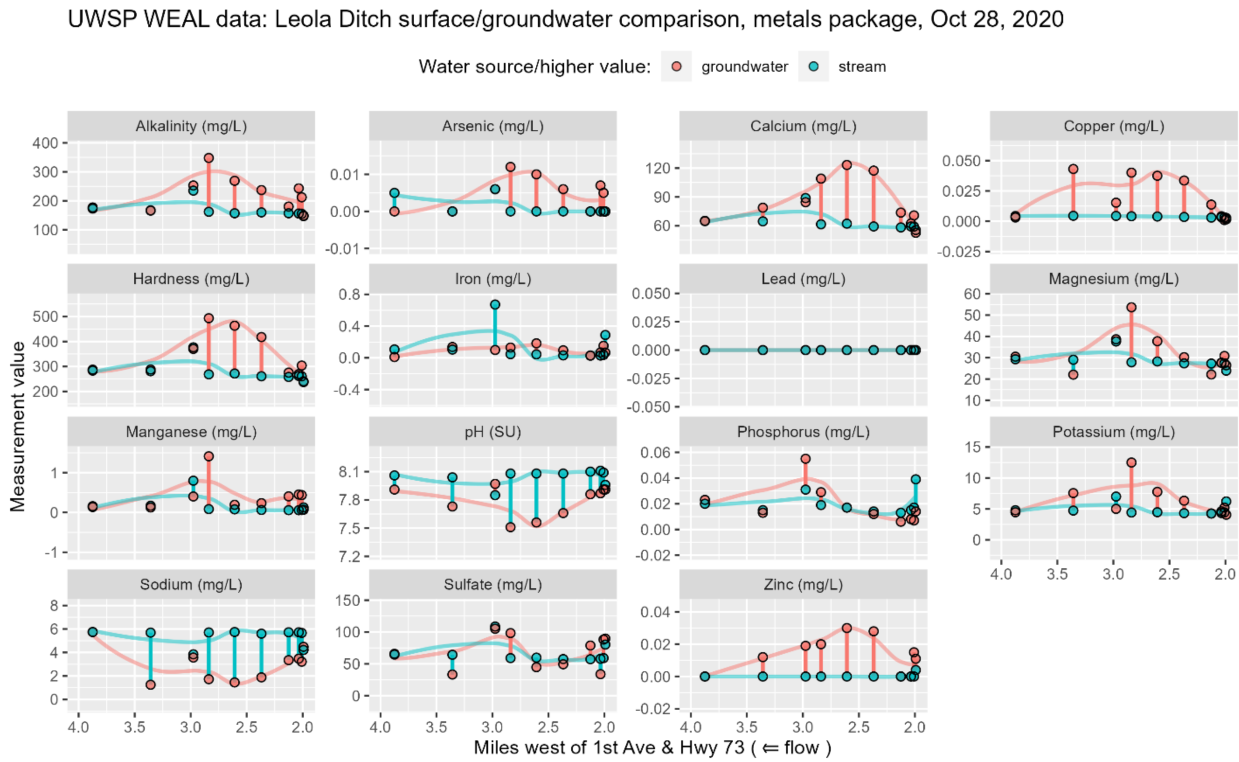



***Figure 42.*** *Surface water/groundwater paired comparisons of select metal concentrations at sampling locations along Leola Ditch on Oct 28, 2020. Groundwater detections are shown in red, surface water detections are shown in teal. A vertical line connects each paired sample, with the color of the line reflecting the source of the higher value.*

### *Streambed temperature flux*

We deployed four temperature profilers at the existing mini-piezometer locations from Third Avenue west to Fifth Avenue from October 27th to November 4th, 2020. The locations are shown in **Figure 43**. We used 1DTempPro to analyze the temperature data. The analysis yielded estimates of the groundwater flux and hydraulic and thermal conductivities of the streambed sediment. We estimated each parameter individually and cycled through the parameters until there was no change from the ones that best-fit the data. **Table 2** shows these values and the hydraulic gradients measured with the mini-piezometers.

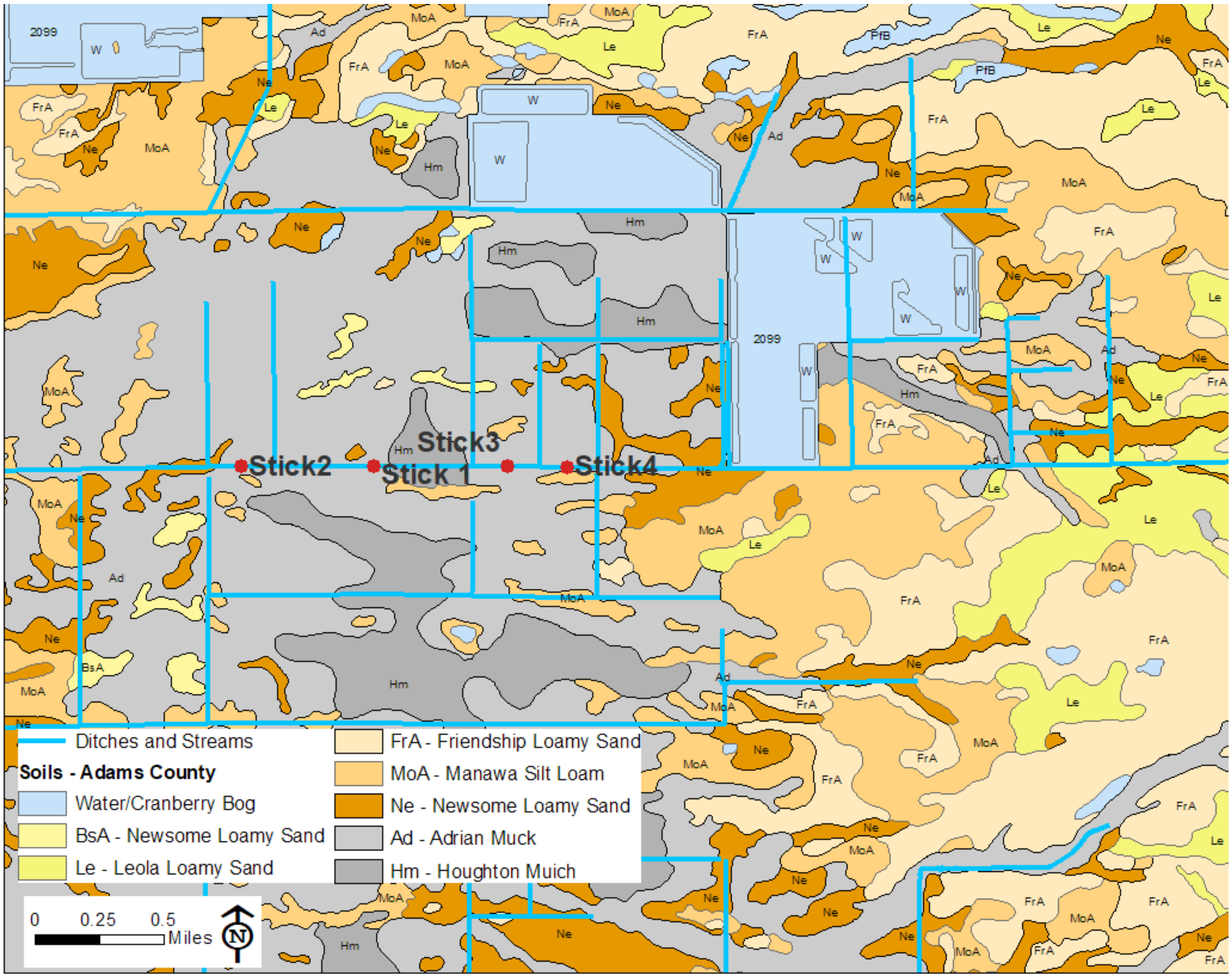



***Figure 43.*** *Soil composition map showing stick probe locations.*

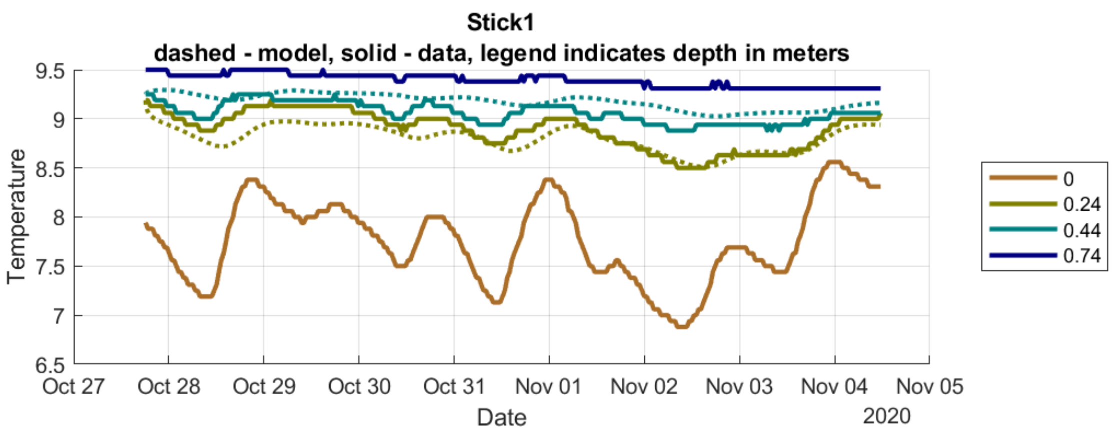
**Table 2.** Results of Temperature Profiler Groundwater Flux Estimates.

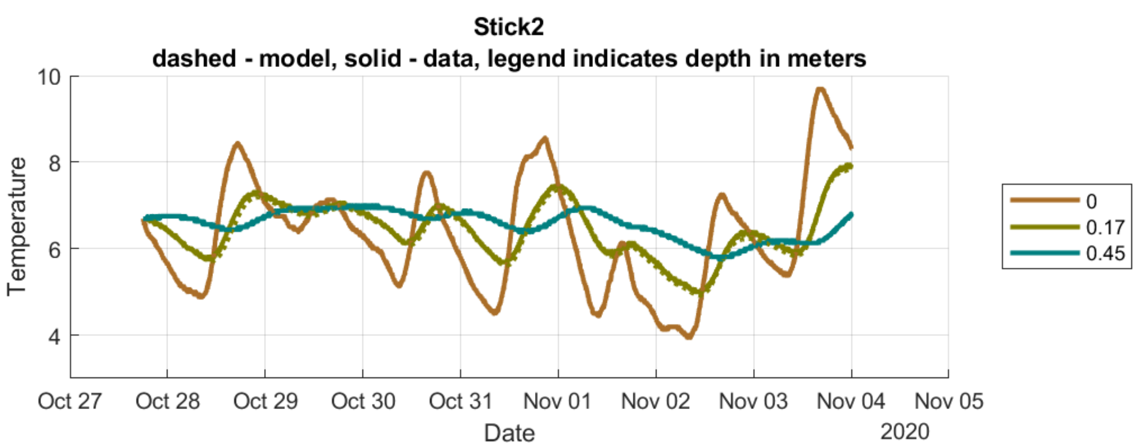
| Profile Name | Hydraulic Gradient[1] (m/m) | Hydraulic Conductivity (m/day) | Groundwater Flux[1] (m/day) | Thermal Conductivity (W/m·°C) |
|---|---|---|---|---|
| Stick 1 | -0.011 | 24.8 | -0.27 | 2.1 |
| Stick 2 | 0.029 | 1.2 | 0.08 | 2.3 |
| Stick 3 | -0.011 | 31.9 | -0.77 | 1.3 |
| Stick 4 | -0.008 | 30.5 | -0.68 | 1.3 |

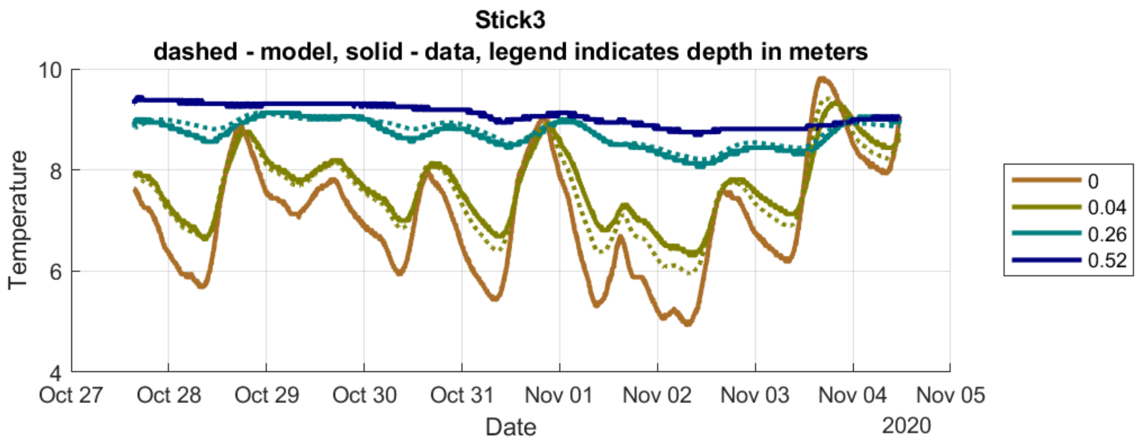
[1.] Upward gradients and fluxes from groundwater to the stream are negative since depth is positive downward.

The temperature data for each of the loggers over the time of record are shown below. The warmest temperatures are generally the deepest since the air temperature was at or below freezing during the test period. Note the different pattern shown by Stick2 versus the other profilers. In the Stick2 temperature record, all the temperature values are similar. This is because advection, the downward flux from the stream to the groundwater, carried the stream temperature signal downward. In the other profilers, only conduction carried the stream temperature signal downward while advection by groundwater acted against conduction. That is groundwater carried the cooler, less variable temperatures upward. The result is less variation in the shallow temperatures and almost no variation in the deepest and warmest temperatures in Sticks 1, 3, and 4.

The locations of the temperature profilers, soil sediments, and surface profile are shown in **Figure 43**. We were unable to gain permission to install temperature profiles to the east of Third Avenue, the portion of Leola Ditch where stream flows were observed to double from groundwater inflow. These locations would have been very useful for comparison but were not available. At the locations where we could collect temperature profiles, we see the highest fluxes and hydraulic conductivities at Sticks 3 and 4 (**Figure 44**). The hydraulic conductivity and flux at Stick 1 to the west decreases compared Sticks 3 and 4. At Stick 2, farthest to the west, the hydraulic conductivity is an order of magnitude less and flux direction has reversed. Based on the current locations and data, there does not seem to be a strong connection between the soil map showing mucks and the hydraulic conductivities measured by the temperature profilers. If data can be acquired in the upstream area where measured inflows are greater, we might be able to better test whether a connection exists or not.





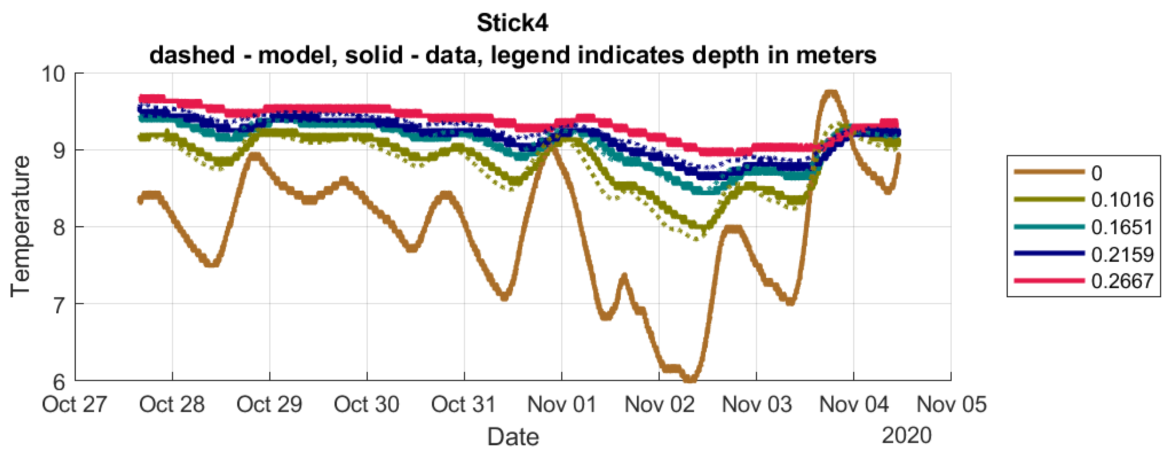




***Figure 44.*** *Temperature profiles over time at four locations (see* ***Figure 43****) in Leola Ditch. Each colored line represents a continuous temperature reading at a discrete initial depth, over a period of eight days from October 27 to November 4, 2020. Sensor depths (meters) are shown in the legend box and range from a depth of 0 m (at streambed interface) to a total depth of 0.74 m.*

### *Continuous stream conductance*

Continuous conductivity data obtained from Solinst transducers during deployments using the different installation designs yielded data of distinctly different quality. **Figure 45** compiles all datasets highlighting the obvious differences among the deployments. The first deployment from 2/6/2021-4/26/2021 was with leveloggers mounted bare horizontally near the stream bottom. The trends in specific conductance have a diurnal pattern to them, increasing steadily when the logger is coldest overnight and in the early morning before decreasing as the stream heats up during the day. Generally, the variation is only within 15 µS/cm but is seen to be ≥60 µS/cm on select days. Such a regular daily fluctuation is likely due to interference in the levelogger's ability to accurately measure conductivity when subjected to the typical change in water temperature (~3-5 °C) that takes place over the course of a day. Overall, during this first deployment period when specific conductance is averaged over a rolling 24-hour period, data displays a much smoother trend and is within ~60 µS/cm of the trendline defined by the most reliable field and lab measurements of conductivity.

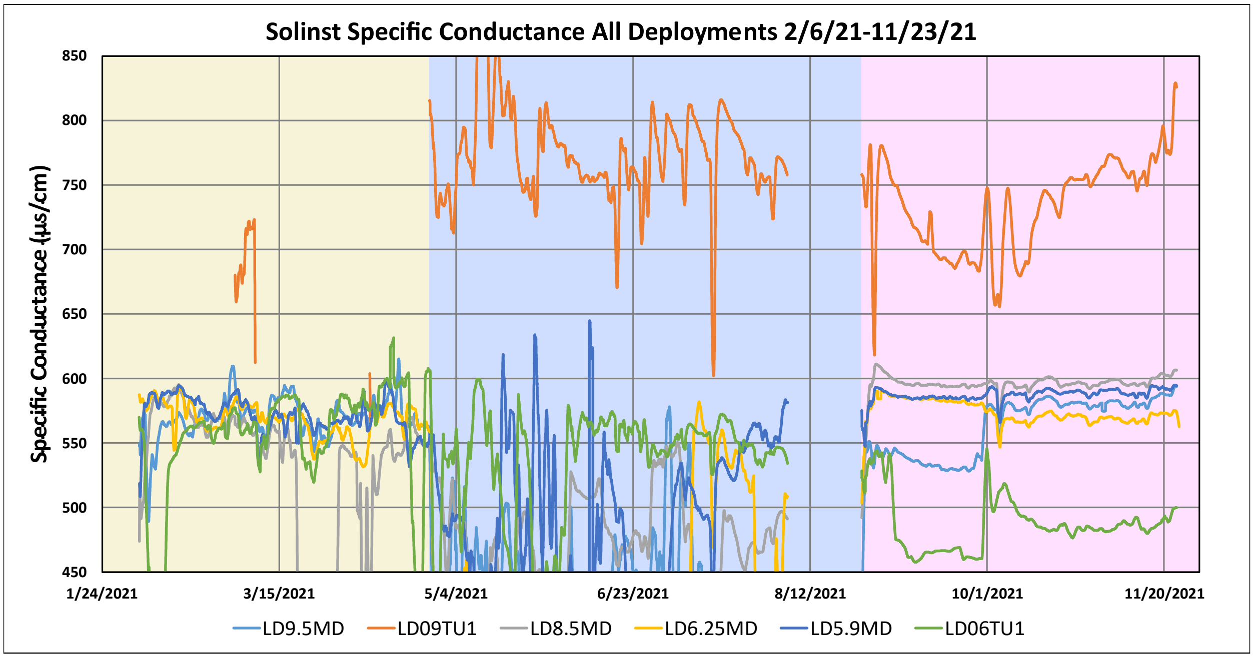



***Figure 45.*** *Rolling 24-hour averaged continuous specific conductance measurements from six Solinst probes installed at Leola Ditch covering all three deployment periods utilizing different installation setups. Yellow background represents the first deployment, blue the second, and pink the third.*

The second deployment period began on 4/26/2021 and ended on 8/5/2021 with all leveloggers placed very near the streambed in a horizontal orientation. Conductivity data collected during this time is unfortunately of very poor quality, showing extremely jumpy and unrealistically low specific conductance values averaging ~150 µS/cm lower than the trend defined by field measurements. To understand what could be causing this issue a bench-scale test was designed in order to replicate conditions likely to be encountered in the streambed environment (**Figure 46**). A single levelogger was placed in a beaker of tapwater and set to real time test mode with measurements of pressure, temperature and conductivity taken every two seconds. The beaker was stirred several times to simulate turbulence, resulting in conductivity spikes of 10-15 µS/cm on some occasions, often resulting in small breaks in the trend of conductivity data. After several minutes a sample of sandy loam soil was added to the beaker to test how bedload sediment might interfere with the transducer. The results perfectly approximated the anomalous data collected during the second deployment period. An initial drop of ~60 µS/cm in conductivity occurred immediately after adding the sediment, followed by another drop in conductivity when the sample was agitated by stirring for a total loss of ~120 µS/cm that remained stable for several minutes afterwards prior to the conclusion of the test.

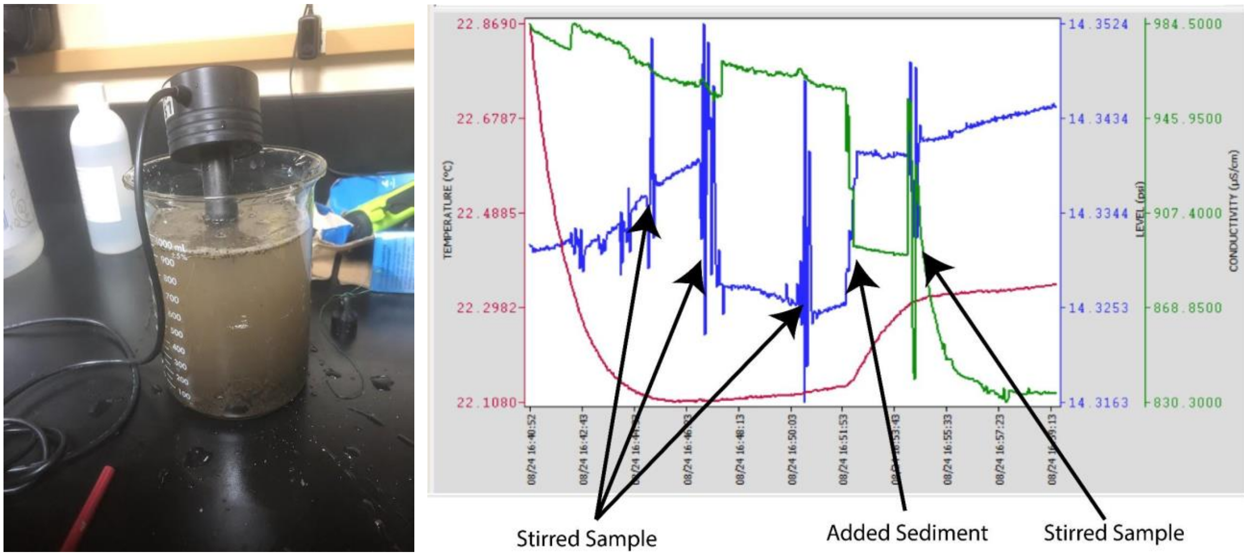



***Figure 46.*** *On left, test setup to determine effect of sediment addition on solinst conductivity probe measurement. On right, graph of several parameters measured by the solinst conductivity probe during the sediment addition test. Red line is temperature, blue line is a measurement of the water level, and green line is conductivity. Note the precipitous drop in conductivity readings after addition of sediment and again after agitating the sample by stirring*

The third and final deployment took place between 8/26/21 and 11/23/21 (**Figure 47**). All leveloggers were encased within protective stilling wells and deployed horizontally near the streambed. The specific conductance data shows an identical diurnal variation to that witnessed during the first deployment period but at a much smaller amplitude (≤ 5 µS/cm). It is unclear if this is due to a slightly smaller variation in daily water temperature or perhaps due to less effect at the higher overall temperatures experienced by the probe in a fall versus winter deployment. When averaged over a 24-hour rolling period the conductivity data is remarkably consistent, especially compared to the previous deployment periods. Four out of six probes recorded conductivity values within ± 25 µS/cm of field measured conductivity, with the probes at LD6TU1 and LD9.5MD off by ~50 µS/cm and ~75 µS/cm respectively during the first part of the deployment from 8/26/21 to 9/29/21. The probe at LD6TU1 became completely buried in sediment during this time and the probe at LD9.5MD may have had an erroneous calibration leading to these discrepancies, both being remedied by cleaning and recalibration on 9/29/21.

After 9/29/21 the data from the probes installed in the main ditch became very consistent, all instruments recording dips in specific conductance at several dates that likely represent real variations in the chemistry of surface water at Leola Ditch. Many closely align with precipitation events recorded by nearby weather stations at Adams-Friendship, Necedah, and Hancock Farms, with incursion of low-conductivity meteoric water a reasonable explanation for the small decreases in conductivity (~10-30 µS/cm) recorded by all probes in the main channel. Both field and lab measurements indicate that specific conductance increases going downstream in Leola Ditch by ~50 µS/cm from LD5.9MD to LD9.5MD (**Figure 29**), something not reflected by the Solinst probe data (**Figure 47**). It appears that although the probes can be made to collect reasonably precise data when deployed under the right conditions, limitations in the calibration process prevent them from accurately capturing the relatively small variation in specific conductance over this limited section of Leola Ditch.

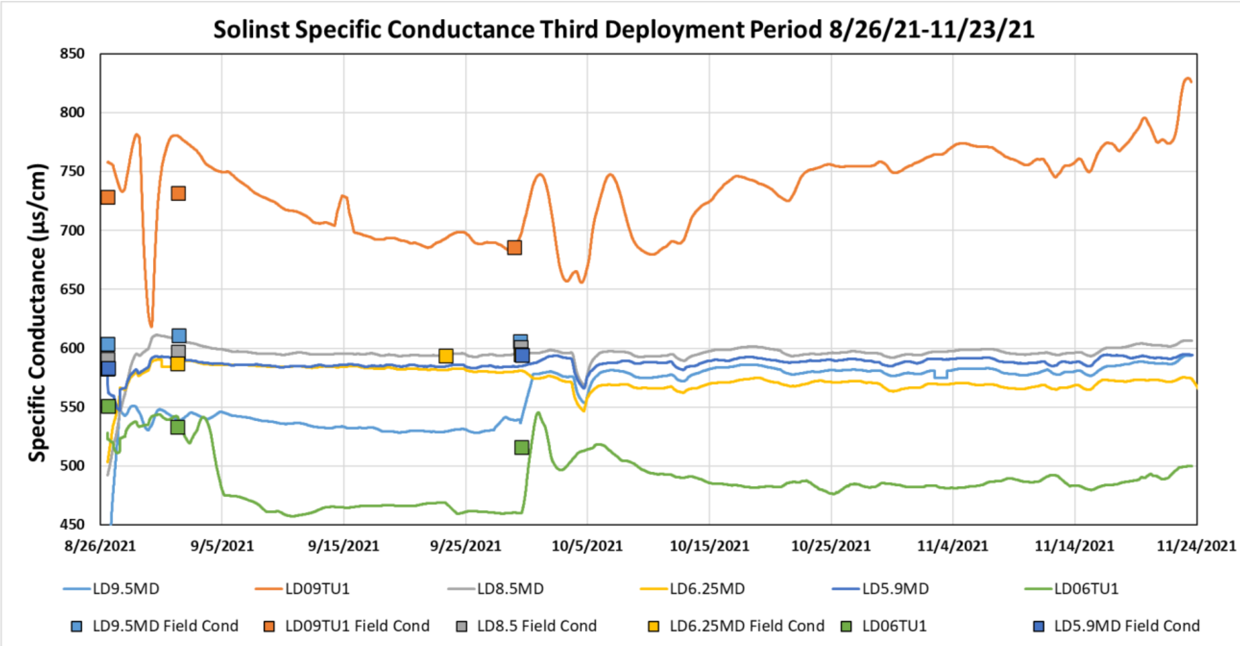



***Figure 47.*** *Rolling 24-hour averaged continuous specific conductance data from six Solinst probes installed at Leola Ditch during the third deployment period. The probes were deployed horizontally near the streambed and placed within protective stilling wells. Square symbols the same color as the continuous data represent field measurements of specific conductance taken at the same stations the probes were installed.*

The purpose behind installation of Solinst conductivity probes at Leola Ditch was to capture fluctuations in specific conductance of the surface water which could be correlated to input from specific water sources that contain different concentrations of neonicotinoids. What this experience shows is that the Solinst probes can only capture consistent data when they are installed in a manner preventing sediment from interfering with the conductivity sensor, and even then they are not able to accurately capture relatively small variations in specific conductance <50 µS/cm as would be required for a detailed survey over a relatively small section of a surface water system. Additionally, an initial hypothesis was that specific conductance may be able to be used as a direct proxy for nitrate concentration and by association neonicotinoid concentration. However, an analysis of major ions in surface water and groundwater indicates that the primary driving factor behind variation in specific conductance in the Leola Ditch system are the soluble products of carbonate minerals (**Figure 30**). For future studies Solinst conductivity probes may be useful in systems where mixing between different water sources with distinct specific conductances takes place at a scale not seen at Leola Ditch, where relatively small tributaries with <1 cfs of flow intersect the main channel and are not seen to significantly impact its chemistry.

# CONCLUSIONS AND RECOMMENDATIONS

## Human and Animal Health

At the present time there are no established drinking water standards at either national or state level for any of the neonicotinoid insecticides (e.g., clothianidin, imidacloprid or thiamethoxam). The Wisconsin Department of Agriculture, Trade and Consumer Protection has requested assistance and guidance from Wisconsin Department of Health Services (DHS) and DNR to review the current distribution and associated concentrations of these compounds in surface and groundwater of the state. The WI DHS has both the authority and responsibility to review these data and propose rules for new standards under chapter 160 of the Wisconsin State Statute. Much of the contemporary human health data centers on the effects of imidacloprid, and research suggests chronic exposures could increase the prevalence of high fat diet (HFD)-induced liver steatosis, using mice as a model system.

Earlier studies have assessed the environmental levels of imidacloprid, and most of these have detected residues of imidacloprid in many environmental sites including soil, water, sediment, as well as in food which has been treated with the active ingredient. Additional studies have consistently reported detections of imidacloprid in both human and other non-target vertebrate species. Outside of these detection studies, however, little definitive information exists to describe the potential adverse health effects associated with chronic exposures to field realistic doses of imidacloprid. Moreover, studies revealing the definitive associations existing between low dose exposures to imidacloprid and its adverse health effect outcomes in human populations are highly limited. The US EPA publishes a list of Human Health Benchmarks for Pesticides (HHBP), in the absence of any established federal standards. They are developed based on an assumed lifetime exposure scenario to assist in considering whether the detection of a pesticide in drinking water may pose a significant human health risk (U.S. EPA, January 2017). Detected concentrations of neonicotinoids in both stream (surface) and groundwater samples are well below the chronic current HHBP values derived for each of the insecticides: clothianidin (630 µg/L), imidacloprid (360 µg/L), and thiamethoxam (77 µg/L).

## Non-Target Impacts

The US EPA lists the aquatic life benchmarks for invertebrates in freshwater for each of the 3 commonly encountered neonicotinoid insecticides. To establish these estimates, the US EPA works closely with the U.S. Geological Survey to identify aquatic ecotoxicity values from risk assessments developed by EPA for individual pesticides during the earlier re-registration eligibility decision (RED) and review. Comparing measured concentrations of the neonicotinoid pesticides in water with an estimated Aquatic Life Benchmark (ALB) is then important for interpreting monitoring data and in identifying and prioritizing sites and pesticides that may require further investigation. These ALB estimates represent concentrations below which pesticides are not expected to represent a risk of concern for aquatic life. Important to note, the US EPA developed the ALBs independent of any legal protection standard or requirement under the Endangered Species Act (ESA); therefore, the ALBs should be viewed as advisory, and do not necessarily equate to any particular finding under the ESA.

Estimated ALBs have been established for only a portion of the neonicotinoids included in the current study. The neonicotinoid ‘imidacloprid’ has estimated acute (0.385 µg/L) and chronic (0.01 µg/L) ALB levels.

For thiamethoxam, EPA lists only an acute benchmark for invertebrates at 17.5 µg/L. With specific regard to total neonicotinoids present in surface water environments, a recent and growing body of research is forming on the acute and chronic effects of neonicotinoids on aquatic invertebrates. Several researchers recently reviewed some of the most recent aquatic toxicity studies performed in surface waters from 29 studies in nine countries worldwide along with published data on their acute and chronic toxicities to 49 species of aquatic insects and crustaceans, including 12 invertebrate orders (Morrissey, et al., 2015). These reviewers recommended that to avoid lasting effects on aquatic invertebrate communities, ecological thresholds for cumulative concentrations of neonicotinoids needs to remain below 0.2 µg/L for short-term acute, or 0.035 µg/L for long-term chronic exposures. Figure 23 shows these recommended cumulative thresholds relative to the results of samples from Tenmile and Carter Creeks. The figure shows that the recommended acute threshold was reached or exceeded in five samples collected from Tenmile Creek. The recommended chronic threshold was exceeded in all samples for which there were detections, in both streams. It is important to note that the 0.035 µg/L recommended chronic threshold for cumulative neonicotinoids is less than the BLS reporting limits of 0.05 µg/L for imidacloprid and 0.067 µg/L for both clothianidin and thiamethoxam.

## Groundwater modeling and watershed monitoring approaches

This study demonstrated how groundwater models can aid in the delineation of groundwater contributing areas to surface water features and provide an approach for evaluating relationships between land-use activities and water quality. In this study, evaluations were performed for a series of repeat stream-monitoring locations, with corresponding delineated groundwater contributing areas of anywhere from 1 to over 50 square miles across the Central Sands region. Similarly, this same approach could be applied by researchers and regional stakeholders as a means of interrogating groundwater and surface water dynamics, water quality conditions, and land-use activity at a variety of scales.

For example, within the Fourteenmile Creek watershed (HUC 10 = 0707000306), particle-tracking output generated from the Kraft and Mechenich model (2010) illustrates several interesting relationships between groundwater and surface water features (**Figure 48**). Sections of Fourteenmile Creek that are simulated as "gaining" (i.e., stream gains water from inflow of groundwater through the streambed), are considered to receive baseflow from the corresponding groundwater contributing area. These sections are indicated by the yellow model cells with corresponding contributing areas shown from yellow to blue. By contrast, other areas that are simulated as "stable" (i.e., stream neither gains nor loses water) or "losing" (i.e., stream loses water to groundwater by outflow through the streambed) are not considered to receive baseflow and do not have a corresponding groundwater contributing area (**Figure 48**). These areas are shown as grey cells with no contributing areas.

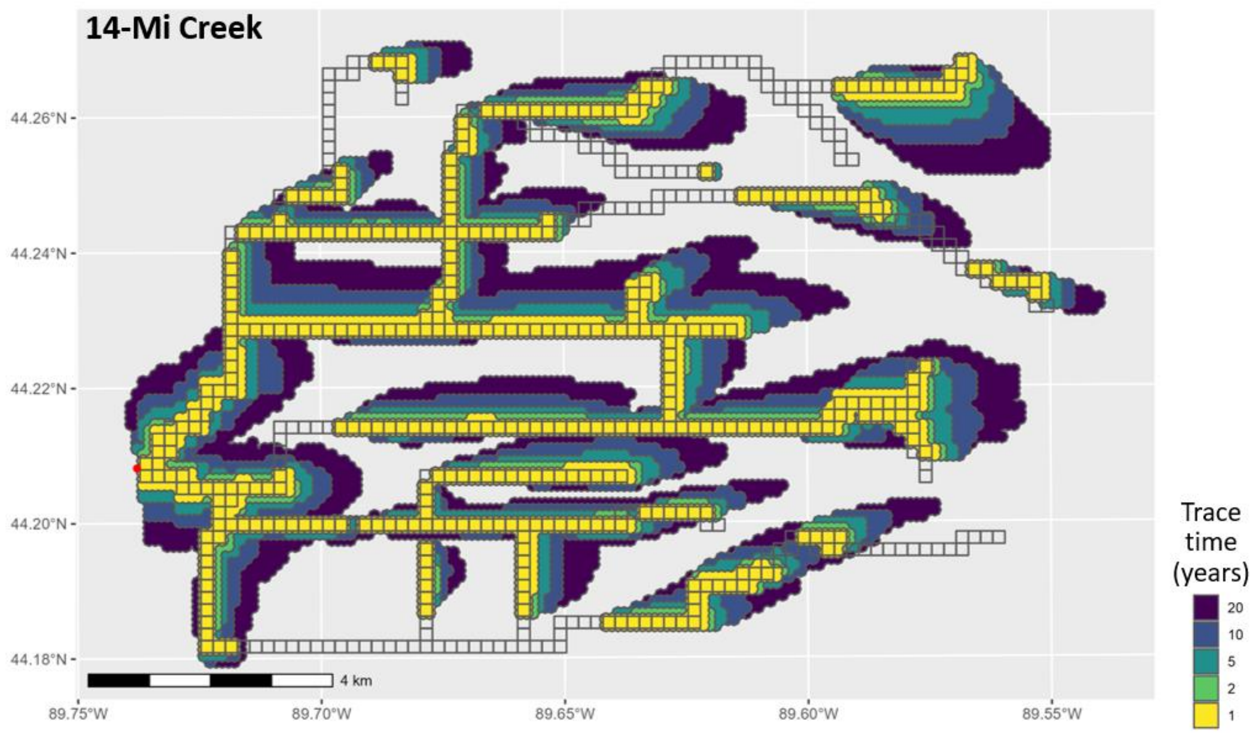



***Figure 48**. 20-year time-of-travel groundwater contributing areas as delineated using the Kraft and Mechenich model (2010) for the Central Sands Region. Modeled surface water features (e.g., creeks and ditches) are shown by small squares, each representing a 200m x 200m (656 x 656 ft) model cell. At this scale, model output identifying gaining and losing sections of streams can provide insights into local groundwater-surface water dynamics.*

While this model output is complex, it also provides a means to 1) field validate the presence of gaining and losing stream sections with a watershed, 2) improve our conceptual models of how groundwater and surface water interact, and 3) help practitioners design better monitoring of streamflow, stream water quality, groundwater quality, and overall watershed health. As demonstrated in this study, our experiences along Leola Ditch confirmed many of the gaining/losing dynamics simulated by the model. Water quality and hydrogeological data collected for this study along Leola Ditch also showed that along gaining sections of streams, such as the upper 3-4 miles of Leola Ditch, the baseline concentrations for nitrate and neonicotinoids in surface water remained consistent across multiple seasonal sampling campaigns (**Figure 31**). While these observations were made over a relatively short time period, from fall 2020 through spring 2022, they were collected during dry, wet, and snowmelt conditions and suggest that nitrate and neonicotinoid concentrations in surface water may represent time-weighted, spatially averaged concentrations of groundwater from across the entire contributing area, which gradually discharge to surface water over time. The stream, in this case, integrates groundwater quality across the contributing area and may provide a convenient way to track groundwater quality over time. If true, long-term synoptic surveys of stream water quality (e.g., annual surveys over decades) could provide an approach for tracking changes in the overall quality of groundwater within the corresponding contributing areas. Taken together these techniques could help watershed managers looking to monitor long-term stream and groundwater quality and water resource technicians looking to optimize the design of watershed-scale sampling and monitoring programs.

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# APPENDIX A. AWARDS, PUBLICATIONS, REPORTS, PATENTS, PRESENTATIONS, STUDENTS, IMPACT

## Presentations

- Parsen, M, B Bradford, R Groves, D Hart, W Fitzpatrick, M Lipke (Sep 22, 2021). *Neonicotinoid contaminants in Wisconsin groundwater: relationships to landscape cropping systems*. DATCP Bureau of Agrichemical Management, Madison, WI (virtual presentation).
- Lipke, M (Mar 11, 2022). *Temporal and spatial dynamics and predictors of neonicotinoid contamination in groundwater fed streams in Central Wisconsin*. American Water Resources Association, Wisconsin Chapter, Spring 2022 Meeting (virtual presentation).
- Parsen, M, B Bradford, D Feinstein, R Groves (Mar 11, 2022). *A model-based approach to delineate stream contributing areas and evaluate relationships between land use and in-stream neonicotinoid concentrations within the Central Sands Region of Wisconsin*. American Water Resources Association, Wisconsin Chapter, Spring 2022 Meeting (virtual presentation).
- Hart, D, M Parsen, M Lipke, W Fitzpatrick, B Bradford, R Groves (Apr 22, 2022). *Variations in groundwater/surface water interactions along Leola Ditch in Wisconsin's Central Sands.* American Water Resources Association, Spring 2022 Meeting (virtual presentation).
- Parsen, M, B Bradford, D Feinstein, R Groves, D Hart, W Fitzpatrick, M Lipke (Sep 12, 2022) *Delineating stream contributing areas and evaluating relationships between land use and in-stream neonicotinoid concentrations within the Central Sands Region of Wisconsin*. Fourteenmile Creek Watershed Alliance Monthly Meeting, Nakoosa, WI.
- Parsen, M, B Bradford, R Groves, D Hart, W Fitzpatrick, M Lipke (Sep 27, 2022). *Neonicotinoid contaminants in Wisconsin groundwater: relationships to landscape cropping systems*. DATCP Bureau of Agrichemical Management, Madison, WI.
- Parsen, M, B Bradford, D Feinstein, R Groves, D Hart, W Fitzpatrick, M Lipke (Nov 7, 2022) *Stream contributing areas and water quality: Focus on the Leola Ditch Subwatershed (14-Mile Creek Watershed)*. Fourteenmile Creek Nine-Key Element Plan Working Group Presentation, Stevens Point, WI.
- Parsen, M, R Groves, B Bradford, D Feinstein, D Hart, W Fitzpatrick, M Lipke (Jan 11, 2023). *Patterns of pesticide contaminants in Wisconsin's Central Sands*. Wisconsin Agribusiness Classic, Madison, WI.
- Parsen, M (Feb 8, 2023). *Stream contributing areas and water quality in the Central Sands Region: Focus on the 14-Mile Creek Watershed.* Wisconsin Potato and Vegetable Growers Association & UW Extension Grower Education Conference, Stevens Point, WI.

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