



Distribution and Concentration of Neonicotinoid Insecticides on Waterfowl Production Areas in West Central Minnesota

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Abstract

Neonicotinoid insecticides have been reported to occur widely in surface waters, including those of wetlands within the Prairie Pothole Region (PPR). In the US portion of the PPR, the US Fish and Wildlife Service has established Waterfowl Production Areas (WPAs) in an effort to enhance waterfowl production. Most WPAs have an area of protected upland surrounding wetlands that can act as a buffer to reduce the transport of contaminants, including pesticides. We assessed the extent that neonicotinoid insecticides occurred in the ponded water of wetlands within WPAs located along a gradient of agricultural influence throughout west-central Minnesota. Of the five neonicotinoids we tested for, two were not detected. However, at least one of the other three, imidacloprid, clothianidin and thiamethoxam, were detected in 29% of our wetland water samples. Additionally, both the occurrence and total concentrations of neonicotinoids were higher in sites with higher surrounding crop use. Neonicotinoid insecticides, if persistent for long periods of time, have the potential to affect aquatic-invertebrate communities within PPR wetlands. Our research indicates that areas often perceived as protected may still be at risk to neonicotinoid contamination, emphasizing the importance of maintaining effective grassland buffers around wetlands.

Keywords Prairie pothole region · Neonicotinoids · Pesticides · Water quality

Introduction

Anthropogenic stressors to aquatic environments, including inputs from agrochemicals, can have detrimental impacts to these important resources. Wetlands provide areas of high biodiversity and provide vital ecological functions, for example, through groundwater recharge and the provisioning of food resources and habitat for a wide range of fish and wildlife species (Houlahan et al. 2006; Erwin 2009). The increased reliance on chemical fertilizers and pesticides has contributed to a growing concern about potential environmental impacts, including effects on aquatic ecosystems, including wetlands (McLaughlin and Mineau 1995; Stehle and Schulz 2015). In addition to impacting aquatic insects directly, insecticides unintentionally introduced into wetlands could have larger

impacts to the ecosystem, for example, through cascading effects throughout the food web and the disruption of ecological services (Donald et al. 1999).

The Prairie Pothole Region (PPR) encompasses an estimated 777,000 km² area in North America, extending from central Alberta to central Iowa, and, in the United States alone, contains nearly 2.6 million hectares of wetlands (Dahl 2014). This ecologically important region is responsible for 40–60% of the duck production in North America and provides critical habitat for many other wetland dependent species (Guntenspergen et al. 2002). Over the last decade, this area has undergone a significant change in land-use practices, with many farms shifting towards large-scale operations, relying heavily on the use of insecticides to limit crop damage and improve agricultural yields (Meehan et al. 2011).

The increasing reliance on insecticides can be partially attributed to the introduction and rapid adoption of neonicotinoid insecticides. This class of insecticide is one of the most widely used globally and accounts for nearly 26% of the global insecticide market (Sparks 2013). First developed in the 1980s and brought to market in the early 1990s, neonicotinoids are now licensed for use in over 120 countries worldwide. Valued for their versatility and broad-spectrum

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toxicity, neonicotinoids are most commonly used as seed treatments, whose use has increased rapidly in the US and other areas of the world in recent decades (Cox Jr et al. 1998; Jeschke et al. 2011; Douglas and Tooker 2015). Common crops treated with neonicotinoids include corn (*Zea mays* L.), soybean (*Glycine max* L.), and wheat (*Triticum* spp.). Facilitated by the high water-solubility of neonicotinoids, the compounds are systemically taken up into plant tissues, providing protection to the young germinating plant (Simon-Delso et al. 2015). However, studies have shown that less than 20% of the active ingredient may be taken up by the plant, with the rest potentially persisting in surrounding soils (Sur and Stork 2003). With relatively long half-lives in soil that can exceed 1000 days and high water-solubility, there is potential for these compounds to persist in the environment and be transported to surrounding water bodies via groundwater, surface runoff or subsurface tile drainage (Goulson 2013; Van Dijk et al. 2013).

With concern for the persistence and potential impact to the environment, there has been increasing interest in examining the fate and distribution of neonicotinoids across the landscape. Recent studies have shown that aquatic systems situated in agricultural regions are susceptible to contamination by neonicotinoid insecticides (Anderson et al. 2013; Main et al. 2014; Hladik et al. 2014). Concentrations of these compounds have been found to occur in rivers, streams, lakes and wetlands receiving surface water from agricultural fields (Struger et al. 2017). Previous work from the prairies of Canada have shown that wetlands devoid of buffer vegetation are of higher probability to become exposed to concentrations of neonicotinoids than wetlands surrounded by perennial vegetation (Main et al. 2015). While wetlands located directly in agricultural fields within the PPR have been shown to contain neonicotinoids (Main et al. 2014; Evsizer and Skopec 2016), it is unclear the level of contamination in more protected areas. The purpose of our study was to describe the distribution and concentration of five common neonicotinoids (imidacloprid, thiamethoxam, clothianidin, acetamiprid and thiacloprid) in wetlands found on Waterfowl Production Areas (WPAs) in west central Minnesota. WPAs managed by the U.S Fish and Wildlife Service (USFWS), provide important waterfowl brood-rearing habitat and serve as protection areas containing a mixture of grassland and wetland habitats. Our study provides additional insight into the fate and distribution of neonicotinoid compounds across the landscape and provides an indication of the water quality of wetlands located in wildlife areas such as WPA's. Documenting the occurrence of neonicotinoids in WPAs of the PPR will ultimately lead to an improved understanding of the fate and potential effects neonicotinoids may have on protected aquatic ecosystems in this and other regions. Such an improved understanding will allow natural resource managers, conservation groups as well as researchers to develop strategies and policies to improve

wetland water quality and wildlife habitat in agriculturally intensive regions.

Methods

Study Area

Our study was conducted within several counties located in the western portion of Minnesota's PPR (Fig. 1). Sampled wetlands were located on WPAs managed by the USFWS Morris Wetland Management District, a unit of the National Wildlife Refuge System. To limit variation in landform geomorphology and precipitation, our study focused on wetlands located within the North Central Glaciated Plains ecological region (ECOMAP 1993).

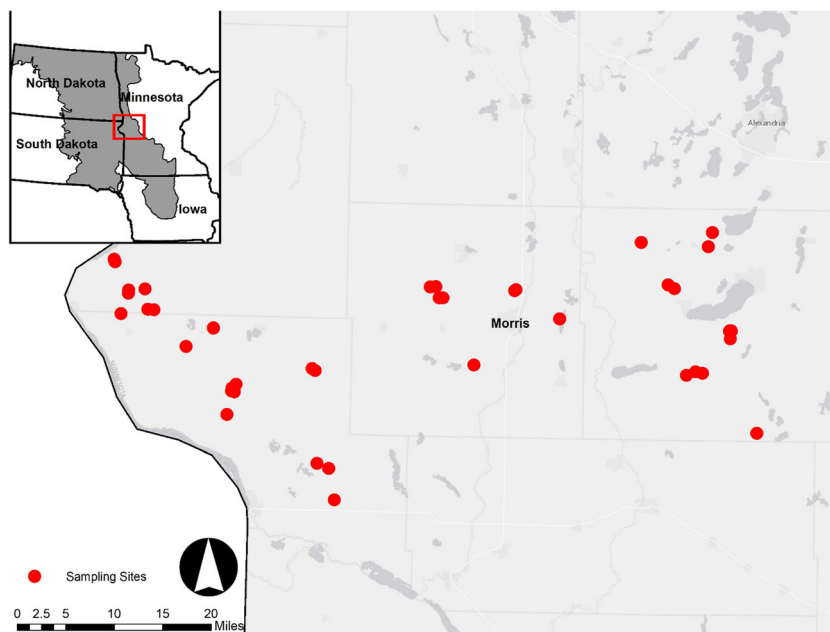
Site Selection

In 2017, 40 wetlands within the Morris Wetland Management District that spanned a gradient of intensity of agricultural use were randomly selected and sampled for neonicotinoids. Pondered-water permanence was determined using vegetation indicators as described by Stewart and Kantrud (1971). Wetlands were selected from all seasonally ponded and semipermanently ponded wetlands in a WPA that were between 0.8 and 10 ha in size. In this region, corn and soybean are the dominant commercial crops, the production of which typically involves the use of neonicotinoid pesticides, primarily as seed treatments (Hladik et al. 2014; Douglas and Tooker 2015). To estimate a wetland's susceptibility to potential neonicotinoid contamination, we compiled data on crop cover from 2012 to 2015 from the US Department of Agriculture (USDA) National Agricultural Statistics Service (NASS)'s Cropland Data Layer (USDA 2012, 2013, 2014, 2015) and generated a 500-m buffer around each basin using GIS software (ArcGIS 10.4). Compiled land-use data were calculated through the Geospatial Modeling Environment (GME) which uses the open-source statistical software R and ArcGIS to sum the individual raster cells of each land-use type (Beyer 2015; R Core Team 2018). Output from the software provided estimates of the percent cropland within each 500-m buffered area, which was used to generate a low (<25%), moderate (25–75%) and high (>75%) rating of surrounding cropping intensity for each wetland.

Field Methods

Water samples were collected from each wetland on three separate occasions so that sample timing matched important agricultural activities taking place on the landscape. Since previous studies (Hladik et al. 2014; Main et al. 2014) have shown that neonicotinoid levels tend to be the highest during

Fig. 1 Location of wetland sites in west-central Minnesota sampled for neonicotinoids in spring and early summer of 2017



the early spring and summer months, our three sampling events were targeted to occur these times. Our first sampling took place in April, between snowmelt and planting activities to account for the potential runoff of neonicotinoids in snowmelt (Main et al. 2017). Following updates from the Minnesota Crop Progress and Condition report (USDA 2017), we conducted our second sampling event near the end of May when 94% of corn and 74% of the soybean crop had been successfully planted. Our final sampling event occurred during the early part of the growing season, in the month of June. Water samples were pesticide analyses were collected at ~10-cm water depth at each sampling location beyond emergent vegetation. Samples were stored at 4 °C in the field and subsequently frozen at the lab until analysis.

Chemical Analysis

Wetland water samples were analyzed for neonicotinoids at the Mississippi State Chemical Laboratory, Mississippi State University (Starkville, Mississippi) by liquid chromatography coupled with tandem mass spectrometry detection (LC/MS/MS). Quantifications were performed using external calibration standards using certified standard reference material. Samples were analyzed for five neonicotinoid compounds: imidacloprid, thiamethoxam, clothianidin, acetamiprid and thiacloprid. The detection limit for all neonicotinoids was 0.002 ppb. Means concentrations of each compound were calculated using data only from wetlands when compounds were detected, i.e., non-detections were not included.

Results

Chemical analyses of the collected water samples indicated widespread distribution of neonicotinoids within the wetland management district. Of the five neonicotinoid compounds we tested for, three (clothianidin, thiamethoxam, imidacloprid) were detected. Overall 50% (20/40) of the wetlands that were sampled for neonicotinoids tested positive for at least one compound throughout the multiple sampling events. Of the 120 wetland water samples collected and analyzed during our study, at least one compound was detected in 35 (29%). Of these 35, 12 (34%) contained two or all three neonicotinoid compounds. The mean total neonicotinoid concentration of samples with detectable concentrations was 14.7 ng/L and the maximum concentration was 60 ng/L (Table 1). Only clothianidin was present during each of the three sampling events while the other two were detected in only two of the sampling periods (Table 2). Overall, concentrations of the three detected compounds were relatively similar throughout the multiple sampling events (Fig. 2). Clothianidin was the most commonly occurring neonicotinoid in water samples (present in 24% of our samples) but had the lowest mean and maximum concentration (mean: 8.6 ng/L; maximum: 37 ng/L) compared to the other detected compounds, imidacloprid (mean: 13.1 ng/L; maximum 38 ng/L) and thiamethoxam (mean: 10.6 ng/L; maximum: 60 ng/L).

Sample timing and the amount of cultivated crop near each basin played a role in the number of detections and the concentration levels found within a water sample (Table 2). Data collected during the separate sampling events indicate the detection frequency of neonicotinoids was highest during our post planting survey event (Fig. 3.) Roughly 49% (17/35) of

Table 1 Summary of detection, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients in water from prairie wetlands of west-central Minnesota. Total of 120 samples across three sampling events

Compound	Samples Detected	Detection Freq. (%)	mean (ng/L)	max (ng/L)
Total Neonicotinoid (ng/L) ^a	35	29	14.7	60.0
Imidacloprid (ng/L)	8	7	13.1	38.0
Thiamethoxam (ng/L)	15	13	10.6	60.0
Clothianidin (ng/L)	29	24	8.6	37.0

^a Total neonicotinoid concentrations are the sum of all three active ingredients

the positive detections were from this time, with the planting phase accounting for another 46% (16/35) of the positive detections. Pre-planting detections were very low, with a detection frequency of only 5% (2/35). Total neonicotinoid concentrations also followed a similar pattern. Peak concentrations of neonicotinoids were highest during the post-planting and planting phase with mean concentrations of 15.6 and 15.3 ng/L, respectively. The mean concentration of 2.0 ng/L found during the pre-planting phase was much lower.

Land use within the buffer area also influenced the occurrence and concentration of neonicotinoids in the samples. Areas classified with high cropping intensity had the greatest detection frequency with a 70 and 80% detection rate during the planting and post planting phases (Fig. 3b). These areas also were found to have our highest concentration levels with mean values of 15.9 and 17.8 ng/L, also in relation to the planting and post-planting survey events. Areas under moderate cropping intensity still had a number of detections both during the planting and post-planting phases, with a detection

frequency of 40%, throughout both time periods. Mean concentrations during the planting and post-planting surveys were similar to the levels found within the high-intensity regions with mean concentrations of 16.5 and 15.1 ng/L. Overall, we identified a positive correlation between land use and total neonicotinoid concentrations within our study wetlands, $r_s = .39$, $p = 0.02$ (Fig. 4).

Discussion

To our knowledge, this is the first study that specifically assessed the occurrence of neonicotinoid insecticides on federally managed and protected WPAs in west central Minnesota. Detected levels of neonicotinoids in rivers, streams and drainage ditch systems (Starnes and Goh 2012; Hladik and Kolpin 2016; Struger et al. 2017), and in a series of studies focusing solely on surface waters of wetlands in Canada's PPR (Main et al. 2014, 2015, 2016) have shown

Table 2 Summary of detection, arithmetic means and maximum concentrations of total neonicotinoids and active ingredients throughout different sampling periods in water from prairie wetlands of west-central Minnesota

Sample Timing 2017			Detection (%)	Total Neonic. (ng/L) ^a		Imidacloprid (ng/L)		Thiamethoxam (ng/L)		Clothianidin (ng/L)	
	Crop Presence	Wetlands (n)		Mean	Max	Mean	Max	Mean	Max	Mean	Max
Pre-Planting	Low	10	0	ND ^b	ND	ND	ND	ND	ND	ND	ND
	Mid	20	5	2.0 ^c	2.0	ND	ND	ND	ND	2.0	2.0
	High	10	10	2.0 ^c	2.0	ND	ND	ND	ND	2.0	2.0
Planting > 80%	Low	10	10	2.0 ^c	2.0	2.0	2.0	ND	ND	ND	ND
	Mid	20	40	16.5	58.0	23.0	38.0	4.4	6.0	8.2	17.0
	High	10	70	15.9	60.0	ND	ND	24.0	60.0	6.5	12.0
Post-Planting	Low	10	10	2.0 ^c	2.0	ND	ND	ND	ND	2.0	2.0
	Mid	20	40	15.1	45.0	13.0	17.0	5.7	10.0	11.1	26.0
	High	10	80	17.8	41.0	4.0	4.0	12.0	20.0	10.8	37.0
Overall		120	29	8.1	60.0	4.7	38.0	5.1	60.0	4.7	37.0

Pre-planting surveys occurred before any type of work in field, planting surveys occurred when 80% of the corn and soybean crop had been planting and post-planting surveys occurred following all planting activities. In addition to the three neonicotinoids listed, acetamiprid and thiacloprid were also tested for, but were not detected in any water samples

^a Total neonicotinoid concentrations are the sum of all three active ingredients detected in water samples

^b ND: not detected; reporting limit 2 ng/L for all active ingredients

^c Single detection found at this cropping intensity

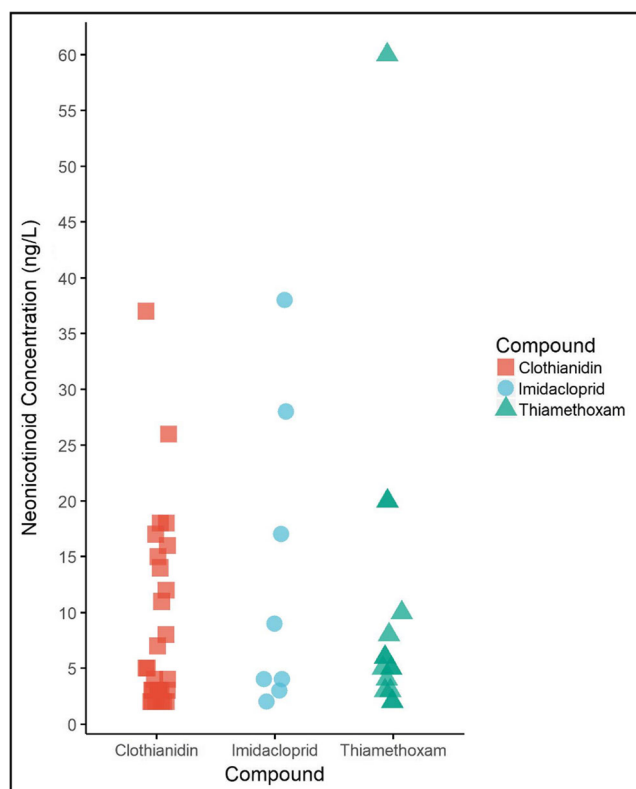


Fig. 2 Distribution and concentrations of neonicotinoid concentrations detected from water sampling of prairie wetlands conducted over three sampling periods in the spring and summer of 2017

these insecticides to be frequently detected, but highly variable from region to region. While the majority of the research sites studied by Main et al. (2014, 2015, 2016) were located directly in agricultural fields, our research sites were located within WPAs, but our results indicate that wetlands found on protected habitats such as WPA's are not immune to contamination by neonicotinoids. Overall 29% of our water samples had detectable levels of neonicotinoid pesticides. In addition, half of the wetlands tested positive for a least one compound throughout the three sampling events.

Although the sites sampled during our study spanned a gradient of influence from surrounding agricultural activities, the study locations were situated in protected areas, with at least a portion of their upland habitat consisting of uncultivated grassland vegetation. As our sites were less disturbed, consequently the neonicotinoid concentrations we report in this study are lower than previous reported values in agriculturally dominated wetlands within the PPR and other aquatic ecosystems that have been surveyed for neonicotinoids previously in central North America (Main et al. 2014; Hladik and Koplin 2016). Main et al. (2014) found concentrations of four neonicotinoid compounds in wetlands within cropped fields to have a mean concentration of 91.7 ng/L and a maximum concentration as high as 3110 ng/L, compared to our findings of an average and maximum total neonicotinoid concentration

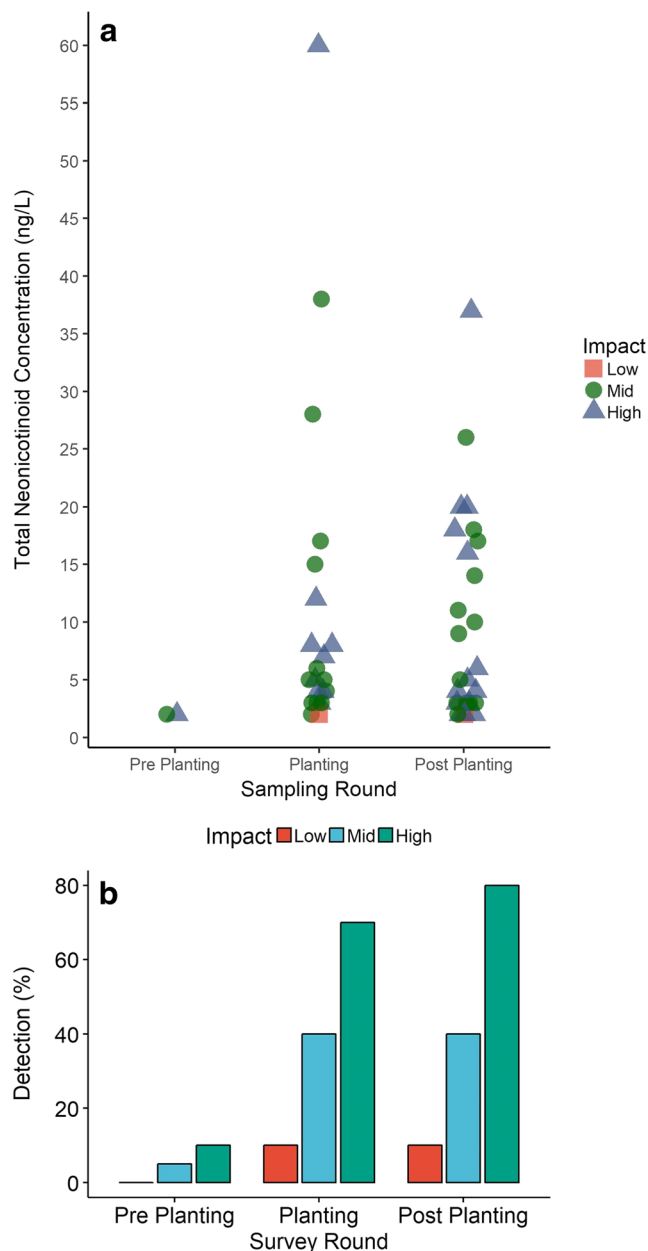
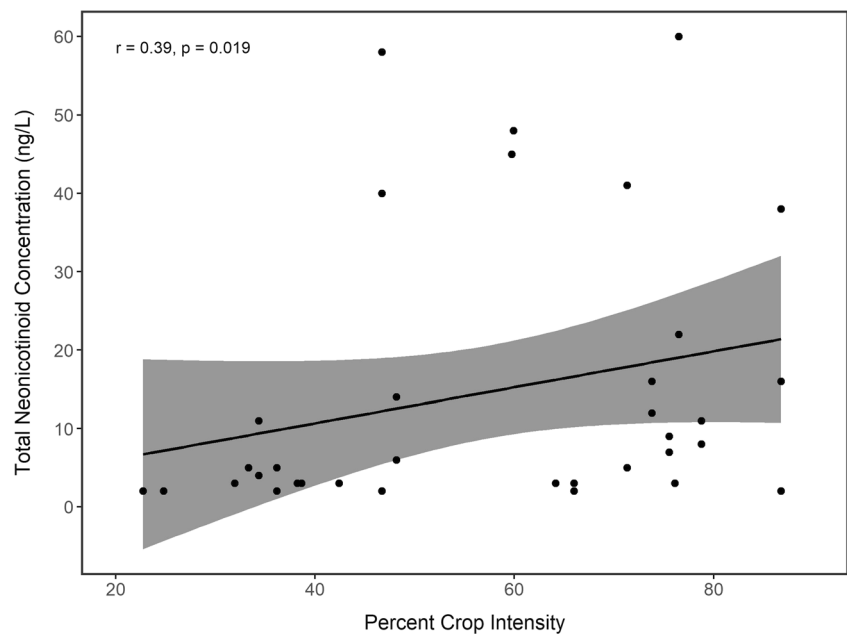


Fig. 3 Summary of (a) detection concentrations and (b) detection frequencies of total neonicotinoids in relation to planting activity and agricultural intensity from water samples collected from wetlands during the spring and summer of 2017. Low (<25%), moderate (25–75%) and high (>75%) categories represent cropped area within a 500-m buffer surrounding each wetland (based on crop cover data from 2012 to 2015). Shapes represent individual sites for which neonicotinoid compounds were detected

of 14.7 ng/L and 60 ng/L, respectively. Drained wetlands in the PPR of Iowa also showed detectable levels of neonicotinoids (Evelsizer and Skopec 2016) at levels exceeding both our study and the study conducted by Main et al. (2014). Since the wetlands studied by Evelsizer and Skopec (2016) were no longer functioning as intact wetlands and subject to traditional farming practices, it was not unexpected to find levels of concentrations an order of magnitude greater

Fig. 4 Relationship between the detected total neonicotinoid concentrations and the amount of cultivated crops within the 500-m buffer area around wetlands



than values reported here. The difference between our results and those of others studying wetlands in crop fields suggest that catchments containing a significant portion of perennial cover surrounding the wetland may result in lower concentrations of pesticide contaminants. While our study area contains many WPAs spread across the region, a large majority of the area consists of field crop agriculture dominated by corn, soybean, wheat, and sugar beets. The intensity of agricultural use within the surrounding watershed can have a major influence on the occurrence of neonicotinoids, and, in comparison to our low-intensity sites, we observed high detections and concentration of neonicotinoids in areas receiving moderate (25–75%) to high (<75%) cropping intensity within our 500-m buffer distance to the wetland (Fig. 4). Other studies of wetlands have found similar results, with an increased presence of contaminants between buffered and non-buffered wetlands (Osborne and Kovacic 1993; Riens et al. 2013).

As observed in other studies of neonicotinoid distribution across North America (Hladik et al. 2014; Evelsizer and Skopec 2016), the three most common active ingredients; imidacloprid, clothianidin and thiamethoxam were the compounds detected during our survey. Transport of neonicotinoids into wetlands via snowmelt has been shown to be a major driver of detectable concentrations of active compounds in wetland surface waters prior to spring planting activities (Main et al. 2016). However, this was not particularly evident at our sites with only 2 of the 40 basins having a detectable concentration prior to planting. This may have been due to the early loss of snow from the landscape during the spring of 2017, while wetlands were still frozen, resulting in less transport of pesticides during spring thaw. As the ground was still frozen when the majority of snow melted, meltwater

may not have percolated through soil and neonicotinoids present in the soil from surrounding agricultural activities may not have been readily transported into our wetlands during snowmelt. Our second and third samplings, which followed periods of precipitation, resulted in our highest observed detections and concentration of neonicotinoids. Precipitation events coinciding with planting activities during the spring and early summer has also been a common mechanism for the transport of neonicotinoids to nearby surface waters with previous studies observing a similar pattern (Hladik et al. 2014; Struger et al. 2017). Struger et al. (2017) found a positive correlation between active ingredients and the sampling day following rainfall events, highlighting the importance of sample timing in an effort to assess peak runoff events. Additional research efforts should be focused on developing a better understanding of how persistent these chemicals are, and to what extent they remain in wetlands over the growing season.

Agricultural drainage was evident at several of our survey sites, which could potentially help explain the transport of neonicotinoids onto some of the WPAs. While surface-water runoff can be a major driver, sub-surface tile drainage can also contribute to the delivery of neonicotinoids to nearby wetlands, especially if the drain outlet discharges directly into the wetland or nearby ditch leading to the wetland. Neonicotinoid use throughout the region is primarily in the form of seed treatments, and, when subjected to seasonal rains in the spring and early summer, compounds have been shown to directly move into tile systems providing a preferential flow of neonicotinoid contaminated water to nearby sites (Wettstein et al. 2016; Chrétien et al. 2017). Small streams and agricultural ditch systems can provide another exposure route of neonicotinoid contaminated water (Starnes and Goh 2012;

Struger et al. 2017). Many of these small systems, which can be primarily fed by agricultural runoff, can route water throughout an area and interconnect wetlands across the landscape. These streams and drains can empty or travel through WPAs subjecting organisms to repeated pulses of multiple active ingredients that can result in cumulative impacts on organisms (Maloney et al. 2017). Throughout our study, we observed several of our study sites containing mixtures of neonicotinoids, with 34% of our detected samples having at least two compounds.

In addition to the frequent occurrence of neonicotinoid compounds within protected WPAs, comparison of concentration data from our three survey periods to published aquatic benchmark values indicate that wetlands found on WPAs can contain concentrations that exceed the suggested chronic toxicity benchmark set for imidacloprid. The current benchmarks set by the U.S. Environmental Protection Agency are 385 and 10 ng/L (USEPA 2018) for acute and chronic toxicity with similar thresholds of 200 and 8.3 ng/L, respectively, set by the European Water Framework Directive (Smit et al. 2015). Additional research is needed to evaluate whether these pesticides are having significant impacts on these protected wetlands.

Neonicotinoids are thought to be linked to the declines of a variety of organisms, with much attention on bees and other native pollinator species (Krupke et al. 2012; Hallmann et al. 2014; Hladik et al. 2016). However, in wetlands and other surface waters experiencing contamination by neonicotinoids, non-target organisms such as aquatic insect species also have the potential to be exposed to both acute and chronic concentrations, affecting emergence success and sex ratios (Cavallaro et al. 2017). Though, it should be noted that many of the studies testing impacts to aquatic insects were conducted *ex situ*, with a limited amount of field studies measuring the chronic and acute impact in natural field conditions. The waterfowl species that use WPAs rely heavily on the availability of aquatic insects during times of breeding and migratory activities (Danell and Sjöberg 1977). Long-term exposure of neonicotinoid concentrations exceeding 35 ng/L have been shown to have chronic effects on aquatic invertebrate populations that are sensitive to pesticide contamination (Morrissey et al. 2015). While only 7 of the 40 wetlands we sampled were found to contain concentrations above this critical value, it does provide evidence that areas considered protected can still be impacted by the transport of neonicotinoids. Our research, as well as that of others (e.g., Main et al. 2015), suggests maintaining buffers of grassland habitat can be an effective way to reduce neonicotinoid concentrations in wetlands of the PPR, but the design and effectiveness of buffers may vary.

Conclusion

Our sampling of prairie-pothole wetlands throughout western Minnesota identified the presence of three of the most commonly used neonicotinoids in the region (USGS 2014). Out of the five active ingredients tested, clothianidin, imidacloprid and thiamethoxam were found to occur in sampled wetlands during the 2017 sampling season. The results found in this study corroborate other studies conducted in similar regions of North America, confirm the widespread distribution of these compounds within the environment (Starner and Goh 2012; Main et al. 2014; Hladik and Koplín 2016), and extend their identified distribution to areas often considered to be protected. As expected, land-use intensity was positively correlated with detected concentrations of these pesticides at our study sites, with higher concentrations of neonicotinoids found in areas with a higher percentage of the surrounding watershed used for crop production, suggesting that sites with large intact buffers may provide protection from agricultural chemicals such as neonicotinoids.

Not only did several of our wetlands exceed the guidelines for individual chronic pesticide thresholds, a number of our sites also tested positive for multiple active ingredients. However, little is known about the potential toxicity of mixtures of neonicotinoids on aquatic organisms, with recent research (Maloney et al. 2017) indicating that simple additivity is not adequate to determine toxicity. While we did not test for other commonly used agrochemicals, others have shown that chemicals such as fertilizers, herbicides and other insecticides can also be common in wetlands surrounded by agricultural production (Riens et al. 2013; Eveltizer and Skopec 2016). Evaluating such effects to aquatic organisms can be a challenge to scientists. However, we suggest that further research continue to examine the fate of agrochemicals in prairie-wetland ecosystems and develop an understanding of their impacts to aquatic ecosystems.

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