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Reduction of neonicotinoid insecticide residues in Prairie wetlands by common wetland plants

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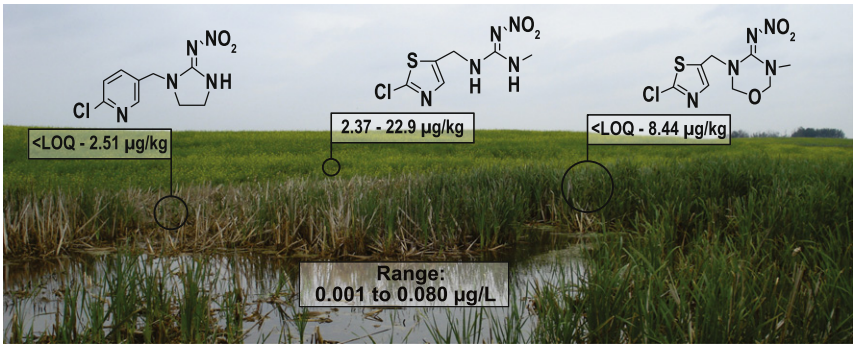
HIGHLIGHTS

- Capability of macrophytes to reduce neonicotinoid transport currently unknown.
- Clothianidin, imidacloprid, and thiamethoxam were detected in macrophyte tissues.
- Unvegetated wetlands had higher frequency and concentrations detected in water.
- Clothianidin concentrations in water reduced as wetland plants grow taller.
- Maintaining wetland vegetation may reduce neonicotinoid water contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

Neonicotinoid insecticides are frequently detected in wetlands during the early to mid-growing period of the Canadian Prairie cropping season. These detections also overlap with the growth of macrophytes that commonly surround agricultural wetlands which we hypothesized may reduce neonicotinoid transport and retention in wetlands. We sampled 20 agricultural wetlands and 11 macrophyte species in central Saskatchewan, Canada, over eight weeks to investigate whether macrophytes were capable of reducing movement of neonicotinoids from cultivated fields and/or reducing concentrations in surface water by accumulating insecticide residues into their tissues. Study wetlands were surrounded by clothianidin-treated canola and selected based on the presence ($n = 10$) or absence ($n = 10$) of a zonal plant community. Neonicotinoids were positively detected in 43% of wetland plants, and quantified in 8% of all plant tissues sampled. Three plant species showed high rates of detection: 78% *Equisetum arvense* (clothianidin, range: <LOQ–2.01 µg/kg), 65% *Alisma triviale* (imidacloprid, range: <LOQ–2.51 µg/kg), and 45% *Typha latifolia* (imidacloprid, range: <LOQ–2.61 µg/kg, thiamethoxam, range: <LOQ–8.44 µg/kg). Overall, unvegetated wetlands had higher detection frequency and water concentrations of clothianidin ($\beta \pm$ S.E.: -0.77 ± 0.26, $P = 0.003$) and thiamethoxam ($\beta \pm$ S.E.: -0.69 ± 0.35, $P = 0.049$) than vegetated wetlands. We assessed the importance of wetland characteristics (e.g. vegetative zone width, emergent plant height, water depth) on neonicotinoid concentrations in Prairie wetlands over time using linear mixed-effects models. Clothianidin concentrations were significantly lower in

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wetlands surrounded by taller plants ($\beta \pm$ S.E.: -0.57 ± 0.12, $P \leq 0.001$). The results of this study suggest that macrophytes can play an important role in mitigating water contamination by accumulating neonicotinoids and possibly slowing transport to wetlands during the growing season.

1. Introduction

Globally, wetlands and their associated macrophyte communities are documented to provide numerous ecosystem services such as regulation of water flows, wildlife support, food, water purification, and CO_2 regulation (Engelhardt and Ritchie, 2001; Bornette and Puijalon, 2011; Clarkson et al., 2013). Macrophytes have the ability to modify both wetland physical structure and environmental conditions (van der Valk, 2012). Physically, the plants slow erosion, reduce sedimentation, filter contaminants and organic inputs, and provide a surface for microorganisms to grow (Vermaat et al., 2000; Rejmankova, 2011). Biologically, they act as refuge and food for numerous wetland-dependent organisms such as amphibians, invertebrates, and water birds.

Standing wetland water is often "buffered" from anthropogenic impacts by the presence of zonal macrophyte communities organized along water depth gradients (Stewart and Kantrud, 1972; Guntenspergen et al., 2002). Macrophytes along this gradient have the capability to remove and/or retain a range of contaminants from entering surface waters (Maillard et al., 2011). Specifically, plants can buffer water from pesticides through sorption or degradation in environments rich in organic matter and biological activity (Carluer et al., 2011) and/or through accumulation by the roots (Gregoire et al., 2009). Buffer zones (<30 m) can reduce concentrations of aqueous and particulate-sorbed pesticides by >50% (Dunn et al., 2011). Recommended buffer widths vary based on target contaminants (Skagen et al., 2008), however few jurisdictions have mandatory buffer zone requirements for wetlands (Mayer et al., 2006). In heavily industrialized agroecosystems (e.g., Prairie Pothole Region), the ability of macrophytes to mitigate or accumulate agrochemicals such as insecticides may greatly reduce the amount of chemical inputs entering surface water systems. For example, broadleaf cattail (*Typha latifolia*) can accumulate metalaxyl (fungicide) and simazine (herbicide; Wilson et al., 2000) and efficiently remove parathion (insecticide) from water and sediment (Amaya-Chávez et al., 2006).

Neonicotinoids are the most widely used class of insecticides (Douglas and Tooker, 2015) whose insecticidal activity is prefaced on their systemic absorption and translocation by crop plants during early growth (Elbert et al., 2008; Simon-Delso et al., 2015). Physicochemical properties such as high water solubility, low octanol/water partition coefficient (K_{ow}), and dissociation constant (pKa) affect the chemical's ability to penetrate plant membranes (Sur and Stork, 2003; Bonmatin et al., 2015). These properties, also make the neonicotinoids persistent in agricultural soils (Goulson, 2013), and readily transported into water through surface and sub-surface runoff (Hladik and Kolpin, 2015; Main et al., 2016). Non-crop plants (e.g., wildflowers) found adjacent to agricultural areas have also been found to contain residues within their tissues ranging from 1 to 86 μg/kg (Stewart et al., 2014; Botías et al., 2015; David et al., 2016). However, studies of neonicotinoid accumulation in non-target vegetation, especially aquatic plants such as wetland macrophytes, remain limited.

Vegetative buffers can provide a mechanism to slow or reduce contaminant infiltration into surface waters, but diverse plant communities may offer additional benefits for reducing contaminant transport into wetlands (Skagen et al., 2008). Specific native wetland plant species such as *Mentha arvensis* (wild mint) were previously found to be associated with lower neonicotinoid water concentrations (Main et al., 2015). However, it was unclear whether this was due to macrophyte accumulation of the pesticide, plants acting as a physical barrier to transport, plants altering the chemical degradation, or if some species simply indicate the extent of wetland disturbance. As neonicotinoid use and contamination of surface waters continues to grow in both frequency and spatial extent (Main et al., 2014; Douglas and Tooker, 2015; Morrissey et al., 2015), it is important to identify the strength of management strategies to reduce neonicotinoid contamination of surface waters. Therefore, our objective was to determine the ability of specific wetland macrophytes and vegetative buffers in natural Prairie wetlands to alter neonicotinoid water contamination. We propose two non-mutually exclusive hypotheses about macrophyte function in Prairie wetlands: 1) plants are able to accumulate neonicotinoid active ingredients into their tissues; and 2) plants can alter the biotic and abiotic structure of wetlands to reduce neonicotinoid movement or persistence in vegetated wetlands.

2. Methods

2.1. Vegetated and unvegetated wetland site selection

In late spring of 2015, we selected 20 representative agricultural wetlands (10 vegetated; 10 unvegetated) situated in fields under clothianidin-treated canola production (Prosper® Bayer CropScience) near Alvena, Saskatchewan, Canada (52.5167° N, 106.0167° W). Selected wetlands ranged from 0.2 ha to 4.8 ha (median: 0.32 ha), had a starting measured central depth on May 1 between 52 and 100 cm (median: 77 cm), and an average pH of 8.4 ± 0.1. Both vegetated and unvegetated wetlands were managed by the same landowner with field applications, crop (canola), and timing of seeding constant between fields containing the study wetlands. In the same year, however, we noted three canola fields directly adjacent to our selected study fields were seeded using thiamethoxam-treated seeds (Helix Xtra®, Syngenta). No known applications of either acetamiprid or imidacloprid had occurred in the past seven years based on landowner seeding records. All but one of the study wetlands (a vegetated wetland) were classified as semi-permanent (Stewart and Kantrud, 1971), and the presence or absence of a zonal macrophyte community was previously known from wetland assessments conducted in 2012 and 2013 (Main et al., 2015). We defined a non-buffered (i.e., unvegetated) wetland as either completely devoid of littoral vegetation or the majority of wetland vegetation was highly disturbed leaving numerous large openings to the surface water edge. Vegetated wetlands typically contained continuous, concentric rings of vegetation that exhibited the more classical patterns of plant zonation found in undisturbed Prairie wetlands (Guntenspergen et al., 2002). We did not set a minimum vegetation zone width as part of our selection criteria; instead, we focused on visual continuity of vegetation surrounding the wetland of interest. Visual rapid wetland assessments were conducted at each study wetland following methods outlined in Main et al. (2015). During the mid-point of the study, the width of vegetation zones (i.e., buffer) surrounding the vegetated wetland was recorded at four compass points (Spencer et al., 1998; Main et al., 2015) providing an average measurement of the overall buffered area. Percentage of dominant plant species in each wetland zone was also scored during rapid assessments as well as a visual estimate of percent submerged aquatic abundance and/or surface cover per wetland.

2.2. Collection of wetland water and wetland plants

Water samples were collected from both vegetated and unvegetated wetlands at four time periods during our eight-week study: pre-seeding (May 1); post-seeding (May 28); and, twice during the early canola growth period (June 12, June 26). At each wetland, we used two

chemically-cleaned (acetone: hexane washed) 1-L amber glass jars to collect separate subsurface grab samples at a depth of 10–15 cm in the central portion of the pond and near the wetland edge. We collected 40 water samples (wetland central zone and edge) per sampling period from our study agricultural wetlands ($n = 20$) for a total of 160 samples during the eight-week study. Bottles were sealed with Teflon-lined caps, stored in the dark during transport, and refrigerated for three to six weeks at 4 °C until analysis. In addition, we further recorded wetland water depth measurements near the edge and in the central portion of the study ponds. We used a handheld digital water quality meter (YSI Professional Plus) to record standard water parameters (temperature, dissolved oxygen, conductivity, salinity, pH) at each site and calculated wetland area, and elevation using a Garmin (Montana 650) GPS.

We collected samples of multiple plant species from all ten vegetated wetlands. Macrophytes were collected from vegetated wetlands beginning two weeks after seeding and then every two weeks until the end of the eight-week study. Where possible, we sought to collect a minimum of three different plant species of commonly identified Prairie macrophytes (Lahring, 2003) found in – or extending into – the wet meadow or shallow marsh zones at multiple locations surrounding each wetland. We used the wet meadow edge to delineate the biological extent of the wetland (Kantrud and Newton, 1996), and to identify the plants most likely to initially intercept insecticide residues in run-off. To act as a positive control, surrounding treated canola crop plants (~10 per wetland) were also sampled at each of the ten vegetated wetlands. We consistently sampled three common macrophytes: *Alisma triviale* ($n = 20$), *Equisetum arvense* ($n = 23$), and *Typha latifolia* ($n = 31$) from almost all vegetated wetlands and opportunistically collected other emergent and submergent species as they were available throughout the season (Table 1). All emergent plant samples, by species, were approximately the same height during collection and taken from the same zone and general position of the wetland throughout the study. We removed the entire plant by the roots, gently field washed the specimen to remove bulk soil or sediment, and then pooled approximately five plants of each species collected to form a composite sample. Composite samples were placed in polyethylene bags and stored in a cooler to be transported back to the lab. We recorded sample plant height, number of leaves, and wet weight for each composite sample. Plant shoots and roots were thoroughly rinsed with tap water before being placed in polyethylene bags and stored frozen at −40 °C for four to seven months prior to analysis.

2.3. Neonicotinoid chemical analysis: wetland water and plant tissues

Wetland water and plant tissue samples were analyzed at the National Hydrology Research Centre, Environment and Climate Change Canada, Saskatoon, SK. Water samples were analyzed using methods previously published in Main et al. (2014). Mean limits of quantification (LOQ) in wetland water were as follows: acetamiprid, 0.002 μg/L; clothianidin, 0.005 μg/L; imidacloprid, 0.003 μg/L; and, thiamethoxam, 0.008 μg/L. Mean recoveries of wetland water ($n = 5$) fortified at 0.050 μg/L were as follows (mean ± SD): acetamiprid (96.6 ± 12.2%); clothianidin (65.6 ± 11.6%); imidacloprid (85.9 ± 11.1%); and, thiamethoxam (77.6 ± 16.1%). All neonicotinoid concentrations were recovery corrected to the mean recovery; all laboratory and field blanks were below detection. Analytical standards of thiamethoxam, clothianidin, imidacloprid and acetamiprid were purchased from Sigma-Aldrich, Chem Service (West Chester, PA, USA). Two internal standards (ISTD), Thiamethoxam-d_3 and imidacloprid-d_4, were purchased from CDN Isotopes (Pointe-Claire, Quebec, CA).

Plant tissue samples were analyzed using a multi-step QuEChERS method (Sigma-Aldrich, n.d.) modified for each macrophyte. Samples were prepared in the lab by washing a third time, rinsing any residual soil or sediment off the plant/roots using tap water followed by an additional rinse of Milli-Q water. Tissue samples, a composite of both roots and shoots, were cut into ~2.5 cm pieces, then placed in storage bags, and re-frozen at −20 °C. Frozen plant samples (~100 g) were homogenized in a food processor with dry ice added if the sample was too wet to grind. A subsample of this tissue was used to calculate percent moisture for each sample. QA/QC tissue samples for each plant species were previously homogenized. 10 g of the homogenate was added to a 50 mL centrifuge tube and spiked with target analytes (60 ppb). The target analytes spiked into the QA/QC samples were the same suite of neonicotinoid active ingredients being analyzed for. Again, we weighed ~10 g of sample into a 50-mL centrifuge tube and noted the weight for final calculations. 15 mL of acetonitrile (ACN) was added to the tube then shaken intensively for ~1 min. Four grams of anhydrous $MgSO_4$ and 1 g of NaCl were added for the liquid/liquid partition and again shaken intensively for ~1 min. Tubes were centrifuged at 4500 rpm for 7 min followed by a 6 mL aliquot of supernatant being decanted into a 15 mL cleanup tube (Thermo Scientific product number 60105–205 (900 mg $MgSO_4$/300 mg PSA/150 mg GCB)) and shaken intensively for ~1 min. Tubes were then centrifuged at 5000 rpm for 2 min. We then placed a 3 mL aliquot of this final supernatant into a test tube. A stream of nitrogen was used to facilitate drying of the sample. Samples were then reconstituted by adding 100 μL ACN and 400 μL Milli-Q water using a vortex mixer, sonicated for ~1 min and transferred to a total recovery vial. 10 μL of a solution containing 2.5 ppm of each ISTD were added to each sample and mixed using a vortex mixer. Final extracts were analyzed directly through use of LS-MS/MS. The plant sample moisture content was determined after allowing wet samples to dry for approximately 48 h.

Due to the diversity of plant species collected as part of our field study and the complex matrices of their tissues, limits of detection

Table 1
Summary of positive detections and concentration ranges (μg/kg) of individual neonicotinoids in wetland plant tissues collected from vegetated wetlands surrounded by clothianidin-treated canola fields in Saskatchewan, Canada.

Plant species	*n* wetlands	*n* plant samples (composite)	*n* samples < LOQ > LOD[a]	*n* samples > LOQ	% positive detections	Clothianidin (range: μg/kg)	Thiamethoxam (range: μg/kg)	Imidacloprid (range: μg/kg)
Algae spp.[b]	2	4	2	0	50	<LOD	<LOD	<LOD - Tr
Alisma triviale	7	20	12	1	65	<LOD - Tr	<LOD - Tr	<LOD - 2.51
Beckmannia syzigachne	1	1	0	0	0	<LOD	<LOD	<LOD
Equisetum arvense	8	23	14	4	78	<LOD - 2.01	<LOD - Tr	<LOD - Tr
Hordeum jubatum	1	1	0	0	0	<LOD	<LOD	<LOD
Mentha arvensis	1	1	0	0	0	<LOD	<LOD	<LOD
Phalaris arundinacea	1	1	0	0	0	<LOD	<LOD	<LOD
Potamogeton pusillus	3	8	2	0	25	<LOD	<LOD	<LOD - Tr
Potamogeton richardsonii	1	2	0	0	0	<LOD	<LOD	<LOD
Sparganium eurycarpum	2	5	1	0	20	<LOD - Tr	<LOD - Tr	<LOQ
Typha latifolia	10	31	11	3	45	<LOD - Tr	<LOD - 8.44	<LOD - 2.61
CLO-treated canola	10	10	0	10	100	2.37 - 22.9	<LOD - 12.34	<LOD

[a] Trace (Tr) indicates a positive detection (<LOQ >LOD) < LOD = below limit of detection. LOD and LOQ are the same as Table 2.
[b] Algae spp. collected were free floating on the surface of some wetlands.

Table 2
Summary of limits of detection (LOD) and limits of quantification (LOQ) for wetland water and for plant species (wet weight) collected from vegetated wetlands. Concentrations are reported in micrograms per liter (μg/L) in water and micrograms per kilogram (μg/kg) for plant tissues.

		Clothianidin (μg/kg)		Thiamethoxam (μg/kg)		Imidacloprid (μg/kg)	
Sample Type	% Moisture	LOD	LOQ	LOD	LOQ	LOD	LOQ
Wetland water (μg/L)	-	0.002	0.005	0.002	0.008	0.001	0.003
Typha latifolia	86	0.20	0.50	0.50	1.50	0.20	0.60
Alisma triviale	89	0.50	1.50	0.40	1.30	0.40	1.20
Equisetum arvense	79	0.22	0.67	0.45	1.35	0.29	0.87
Hordeum jubatum	62	0.58	1.73	0.13	0.40	0.46	1.37
Beckmannia syzigachne	83	0.25	0.76	0.54	1.61	0.23	0.69
Sparganium eurycarpum	91	0.26	0.79	0.38	1.14	0.20	0.59
Potamogeton pusillus	87	0.14	0.42	0.39	1.18	0.25	0.75
Algae spp.	n/a	0.17	0.50	0.40	1.20	0.27	0.80
Potamogeton richardsonii	89	0.20	0.61	0.47	1.42	0.19	0.56
Phalaris arundinacea	73	0.20	0.70	0.60	1.70	0.20	0.60
Mentha arvensis	81	0.66	1.99	0.10	0.31	0.55	1.65
Canola	87	0.35	1.04	0.55	1.64	0.47	1.41

and quantification varied by plant species as listed in Table 2. To evaluate the number of neonicotinoid detections in plant tissues, we combined all trace detections (<LOQ >LOD) regardless of specific active ingredient and report these as positive detections. However, due to low concentrations and frequent zero values, we were unable to specifically examine changes in residue concentrations over time.

2.4. Statistical analyses

Imidacloprid Toxic Equivalences (TEQs) were calculated following methods outlined in Cavallaro et al. (2016). Mean water concentrations and detections for all individual neonicotinoids are also presented (Table 3). TEQs are an improved method to represent the toxicity of the total mixture of different neonicotinoids found in water samples by multiplying the concentration of each compound by its toxic equivalency factor (i.e., imidacloprid TEF = 1; Cavallaro et al., 2016). The calculated TEQs then represent a chronic toxic potential of the water samples to sensitive non-target aquatic insect larvae.

2.4.1. Effect of vegetation and wetland characteristics on aqueous insecticide concentrations

Using a linear model, we tested whether neonicotinoid concentrations were significantly different in vegetated versus unvegetated wetlands over the study period. We then analyzed the effects of wetland characteristics and vegetative plant measures on individual neonicotinoid concentrations (clothianidin, thiamethoxam only as these were the most commonly found) in wetlands using a linear mixed-effects model in package "nlme" (Pinheiro et al., 2014) in R 3.1.1 (R Core Team, 2014). Model fixed effects included surrounding vegetation presence or absence (wetland type), submerged aquatic plant abundance (%), buffer zone width (m), mean emergent plant height (cm), wetland area (ha), central wetland depth (cm), dominant shallow marsh vegetation, and time (sampling week), while wetland ID was included as a random effect to account for repeated measures. We used the mean water concentration in each individual wetland per sampling period as our response variable as concentrations collected between the edge and center were not statistically different (see results). All predictor variables were converted to z scores to standardize and screened for correlations ($r > 0.7$; Dormann et al., 2013). Active ingredient concentrations were log-transformed prior to analysis. Model fit was assessed through a combination of visual inspection of residuals and a Shapiro-Wilk test to assess the fit of residuals to a normal distribution (Michel, 2014).

Using the dredge function in package "MuMIn" (Bartoń, 2016), we conducted subset model selection where models are fitted through repeated evaluation from the fully-parameterized (global) model. This produces a subset of the most parsimonious models based on corrected Akaike's Information Criterion (AICc) values for small sample sizes which decreases the probability of selecting models which have too many parameters (Burnham and Anderson, 2002). However, as many of the top models showed minor differences in their information criterion ($AIC_c < 2$), we used a model averaging approach to determine the most stable models with robust parameter estimates (Arnold, 2010; Grueber et al., 2011). The top model set was then averaged using the zero method where a parameter estimate of zero is substituted into models where a given parameter is absent (Burnham and Anderson, 2002; Grueber et al., 2011). The zero method reduces the effect size of

Table 3
Summary of percent detections, arithmetic means (±SE), and maximum concentrations of neonicotinoids (clothianidin, imidacloprid, thiamethoxam) in water from wetlands situated in clothianidin-treated canola fields. Percent detections are calculated from values >LOQ only. Concentration values are reported in μg/L and presented as overall summaries by week for each wetland type: unvegetated or vegetated. Toxic equivalency (TEQ- Imidacloprid) (see Cavallaro et al., 2016) is calculated to represent the toxic potential of the water samples to sensitive aquatic insect larvae from combined neonicotinoid exposure relative to imidacloprid.

		Detection (%)			Clothianidin (μg/L)		Thiamethoxam (μg/L)		Imidacloprid (μg/L)		TEQ - Imidacloprid (μg/L)	
Wetland type	Week	CLO	THX	IMI	Mean ± S.E.	Max.	Mean ± S.E.	Max.	Mean ± S.E.	Max.	Mean ± S.E.	Max.
Unvegetated	1	90	30	ND	0.016 ± 0.003	0.035	0.005 ± 0.003	0.026	ND	ND	0.025 ± 0.005	0.057
$n = 10$	4	90	30	30	0.011 ± 0.003	0.029	0.002 ± 0.001	0.006	0.001 ± 0.001	0.005	0.028 ± 0.005	0.054
	6	80	40	10	0.018 ± 0.004	0.037	0.080 ± 0.049	0.425	0.000 ± 0.000	0.003	0.030 ± 0.007	0.064
	8	80	20	ND	0.008 ± 0.003	0.035	0.005 ± 0.004	0.040	ND	ND	0.013 ± 0.005	0.056
Vegetated	1	90	20	10	0.013 ± 0.002	0.022	0.001 ± 0.001	0.007	0.000 ± 0.000	0.004	0.021 ± 0.003	0.036
$n = 10$	4	50	20	ND	0.003 ± 0.001	0.011	0.001 ± 0.001	0.006	ND	ND	0.005 ± 0.002	0.018
	6	50	10	40	0.006 ± 0.002	0.013	0.004 ± 0.004	0.041	0.002 ± 0.001	0.007	0.012 ± 0.003	0.023
	8	50	10	20	0.004 ± 0.002	0.013	0.005 ± 0.005	0.047	0.001 ± 0.000	0.003	0.007 ± 0.002	0.021

ND = no detection.

weak effect predictors and/or decreases the effect size and errors of predictors appearing in models with small model weights (Grueber et al., 2011).

2.4.2. Differences in neonicotinoid concentration among wetland plant species

We assessed differences in concentrations for each of the three neonicotinoid active ingredients found in plant tissues using a linear model in R. Because of zero inflation due to non-detections, a fixed value equivalent to half of the LOD was added to all data points and then all concentration data were log-transformed to identify differences in active ingredient concentrations between plant species. Post-hoc testing among plant types for each individual active ingredient were examined using pair-wise comparisons in package "multcomp" (Hothorn et al., 2013).

3. Results

3.1. Relationship between vegetation measures and neonicotinoid concentrations in wetland water

Clothianidin was detected in 63% (101/160) of all wetland water samples, whereas, thiamethoxam was detected in 17% (27/160) and imidacloprid in 7% (11/160) of the same set of water samples. Acetamiprid was not detected in any water sample. Although we were interested in determining if concentrations differed between the two sampling areas of the wetland, edge concentrations were not statistically different from samples collected in the center of the study ponds ($\beta \pm$ S.E.: 0.26 ± 0.28, $P = 0.35$). Neonicotinoids were more frequently detected in unvegetated wetlands (88%) than in vegetated wetlands (75%). However, mean neonicotinoid concentrations in water in both unvegetated and vegetated wetlands remained relatively low throughout the study (Table 3; Fig. 1). Clothianidin and thiamethoxam concentrations in water were significantly higher in unvegetated than vegetated wetlands (CLO: $\beta \pm$ S.E.: -0.77 ± 0.26, $P = 0.003$; THX: $\beta \pm$ S.E.: -0.69 ± 0.35, $P = 0.049$). However, thiamethoxam mean and maximum concentrations were an order of magnitude higher in unvegetated wetlands during Week 6 (Table 3).

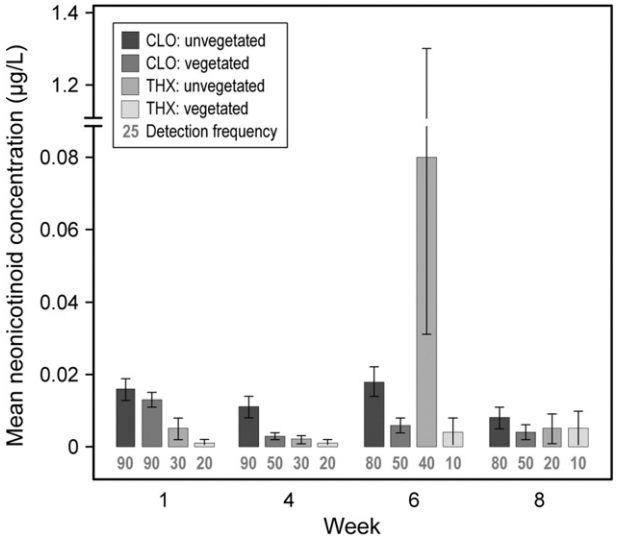

Fig. 1. Mean neonicotinoid concentrations (clothianidin, thiamethoxam; µg/L) measured in vegetated and unvegetated wetlands surrounded by clothianidin-treated canola in Saskatchewan, Canada. Bars represent means (±SE) for each wetland type and individual active ingredient over the 8-week study period. Frequency of detection (%) is listed under each representative bar on the graph.

3.1.1. Factors influencing clothianidin concentrations in water

Model weights (AIC_{wt}) are evidence of a particular model being the best-supported model. Our top model ($AIC_{wt} = 0.23$) evaluating basic wetland and vegetation measures on aqueous clothianidin concentrations included only emergent plant height and central water depth (Table 4). Wetland area, dominant shallow marsh species, wetland area, and the percentage of wetland open water containing submerged aquatic plants were the only variables excluded from all top models. Of included predictor variables, only emergent plant height had a high relative importance ($AIC_w = 1$; Table 5). Clothianidin concentrations were lower in wetlands surrounded by taller emergent plants ($\beta = -0.57 \pm 0.12$, $z = 4.72$, $P \leq 0.001$) regardless of vegetation zone width.

3.1.2. Factors influencing thiamethoxam and imidacloprid concentrations in water

Our best model evaluating wetland measures on thiamethoxam concentrations in water ($AIC_{wt} = 0.20$) included one variable, vegetation disturbance ($AIC_w = 0.54$). All other predictors had a relative importance <0.18 and none were significant (Table 4). The fact that wetlands were largely influenced by clothianidin-treated canola in tandem with infrequent detections of thiamethoxam over the course of the study may have decreased the ability of these models to more effectively explain variation in thiamethoxam concentrations. Surprisingly, imidacloprid was detected in 7% of water samples despite no known applications of this compound in recent years. Given the limited imidacloprid data, models would not converge due to the low detection frequency and the lack of variation in imidacloprid concentrations.

3.2. Neonicotinoids in wetland plants

Of the 11 wetland plant and algae species collected from the 10 vegetated wetlands, six species contained positive neonicotinoid detections defined as >LOD (Table 1). The three most common and abundant species collected were *Equisetum arvense*, *Alisma triviale*, and *Typha latifolia*. We detected the first active ingredient in non-target plant tissues a week after seeding had ended (Week 4: May 29) and in both subsequent plant collection dates within the eight-week study (Week 6: June 12, Week 8: June 26). Positive frequencies of detections by species ranged from 20% for *Sparganium eurycarpum* to 78% for *Equisetum arvense* (Fig. 2). Neonicotinoid residues >LOQ were detected in 8% of all macrophytes collected, but only in three representative species: *Equisetum*, *Alisma* and *Typha*. In all wetland plants collected, *T. latifolia* contained the highest quantifiable thiamethoxam concentration of 8.44 µg/kg. By comparison, all treated canola plants collected from the agricultural field surrounding our study wetlands had quantifiable clothianidin concentrations in plant tissues ranging from 2.37 to 22.9 µg/kg (Table 1).

In comparing the neonicotinoid concentrations across plant species (Fig. 3), canola crop plants collected from the same study fields had the highest neonicotinoid concentrations of all plants sampled followed by the 3 most common wetland plants: *Typha* > *Alisma* > *Equisetum*. Canola plants were significantly higher in clothianidin than *Typha* ($\beta = 4.02 \pm 0.25$, $t = 16.1$, $P \leq 0.001$), *Alisma* ($\beta = 3.73 \pm 0.27$, $t = 14$, $P \leq 0.001$), and *Equisetum* ($\beta = 3.05 \pm 0.26$, $t = 11.7$, $P \leq 0.001$). Canola was also significantly higher in thiamethoxam than *Alisma* ($\beta = 1.30 \pm 0.27$, $t = 4.77$, $P \leq 0.001$), *Equisetum* ($\beta = 1.29 \pm 0.27$, $t = 4.84$, $P \leq 0.001$) and *Typha* ($\beta = 0.99 \pm 0.26$, $t = 3.87$, $P = 0.001$) but did not differ among wetland plant species.

4. Discussion

Wetland macrophytes have a proven ability to reduce contaminants in aquatic ecosystems and act as indicators and/or drivers of both neonicotinoid detections and concentrations in Canadian Prairie wetlands (Main et al., 2015). Macrophytes are capable of improving wetland water quality through accumulation of pesticides, attenuating

Table 4
The top approximating models (i.e., $\Delta AIC_c < 2$ Akaike's Information Criterion [AICc] corrected for small sample size) which were included as part of the overall model averaging approach evaluating wetland measures on clothianidin (CLO) and thiamethoxam (THX) water concentrations collected over 8 weeks during summer of 2015.

Top model set: CLO[a]	AIC_c	ΔAIC_c	Model weight (AIC_{wt})
Emergent plant height + water depth	211.24	0.00	0.23
Emergent plant height + week	211.85	0.61	0.17
Emergent plant height + water depth + buffer width	212.52	1.28	0.12
Emergent plant height + week + buffer width	212.92	1.68	0.10
Null	240.40	29.2	<0.001
Top model set: THX[a]	**AIC_c**	**ΔAIC_c**	**Model weight (AIC_{wt})**
Vegetation disturbance	286.19	0.00	0.20
Intercept only	286.58	0.38	0.17
Vegetation disturbance + water depth	287.41	1.22	0.11
Wetland type	287.59	1.40	0.10
Null	295.20	9.01	<0.001

[a] Explanatory variables in the global model included: surrounding vegetation presence or absence (wetland type), submerged aquatic plant abundance (%), buffer zone width (m), mean emergent plant height (cm), wetland area (ha), central wetland depth (cm), dominant shallow marsh vegetation, and time (sampling week). The overall corrected criterion scores (AIC_c), change in AICc (ΔAIC_c), and model weights (AIC_{wt}) are presented.

pesticide movement, and influencing retention time needed for chemical degradation (Stehle et al., 2011). In this study, we found that wetlands surrounded by intact zonal vegetation communities were more likely to have lower neonicotinoid concentrations than unvegetated wetlands. Indeed, neonicotinoid concentrations were negatively correlated with emergent plant height such that regardless of vegetation zone width (i.e., buffer), concentrations were lower in wetlands surrounded by taller plants. This suggests a potential mitigation effect through both transport and accumulation during the agricultural growing period. Additionally, some common Prairie wetland species (e.g., *Typha*, *Alisma*, *Equisetum*) accumulated neonicotinoids under field conditions. Collectively, this provides new evidence that intact vegetated wetlands have the potential to significantly reduce neonicotinoid transport and retention in water bodies such as Prairie wetlands.

4.1. Neonicotinoid accumulation in wetland plants

We found evidence of trace positive detections (>LOD < LOQ) in 43% of our wetland plant samples and quantifiable concentrations in 8%. This demonstrates the ability of some common Prairie wetland plants to accumulate neonicotinoids. By comparison, <4% of pesticide residues (including insecticides (e.g., dimethoate)) were accumulated through either uptake or sorption by plants in constructed wetlands (Elsaesser et al., 2011). Plant retention of pesticides can be highly variable with between 10% (metolachlor: herbicide) and 76% (cyfluthrin: insecticide) of mass associated with vegetation in constructed wetlands (Vymazal and Březinová, 2015). In the present study, *Typha latifolia* contained the highest quantifiable concentration of 8.44 μg/kg (thiamethoxam) in spite of *Equisetum* samples having both more frequent trace (78%) and quantifiable residues (max = 2.01 μg/kg). All three macrophyte species that accumulated measurable active ingredients are tolerant of anthropogenic disturbance, are longer-lived perennials, and spread rapidly through dense rhizomes where they can colonize quickly. This may explain their ability to accumulate insecticide residues as pesticide sorption can be specific to certain types of plants, their physical characteristics, water transpiration rates, and their ability to develop greater surface area below water (Li et al., 2005; Elsaesser et al., 2011). *Typha* spp. can effectively reduce experimental inflows of other insecticides (e.g., diazinon, permethrin; Moore et al., 2013). Though we are unaware of any data existing on insecticide accumulation by *A. triviale*, a study of 2.4-D effects on water plantains (*Alisma*) found that plants in five-leaf and scape elongation stages retained more compound

Table 5
Model-averaged results from the global model to explain the variation in neonicotinoid concentrations (clothianidin, thiamethoxam) in water collected from vegetated wetlands surrounded by clothianidin-treated canola fields in Saskatchewan, Canada.

Model parameters: clothianidin[a]	β	SE[b]	*z*	*P*	AIC_w
Intercept	−5.07	0.21	23.3	<0.001	–
Emergent plant height	−0.57	0.12	4.72	<0.001	1.00
Water depth	0.18	0.18	1.01	0.31	0.63
Time (sampling week)	−0.06	0.09	0.60	0.55	0.37
Zone width	−0.01	0.03	0.38	0.70	0.22
Wetland type	−0.03	0.14	0.18	0.85	0.09
Vegetation disturbance (%)	0.00	0.00	0.00	0.87	0.08
Model parameters: thiamethoxam[a]					
Intercept	−6.82	0.59	11.3	<0.001	–
Vegetation disturbance (%)	0.01	0.01	0.72	0.47	0.54
Water depth	0.02	0.09	0.25	0.80	0.11
Wetland type (vegetated or unvegetated)	0.00	0.41	0.01	0.99	0.18
Emergent plant height	−0.01	0.01	0.21	0.83	0.08
Time (sampling week)	−0.01	0.04	0.15	0.88	0.07

[a] Retained predictors differed by active ingredient. Six predictors were retained in both of the competing clothianidin and thiamethoxam top model set (see Table 4).
[b] SE = unconditional standard errors; AICw = variable importance weights.

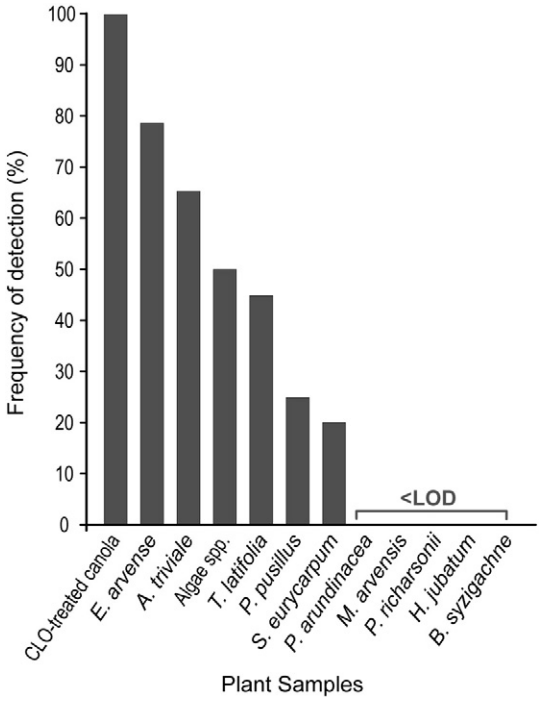

Fig. 2. Percentage positive detections (<LOQ > LOD) of neonicotinoid active ingredients (clothianidin, imidacloprid, thiamethoxam) in canola and wetland plant tissues from samples collected from vegetated wetlands situated in canola fields of Saskatchewan, Canada. <LOD = below limit of detection.

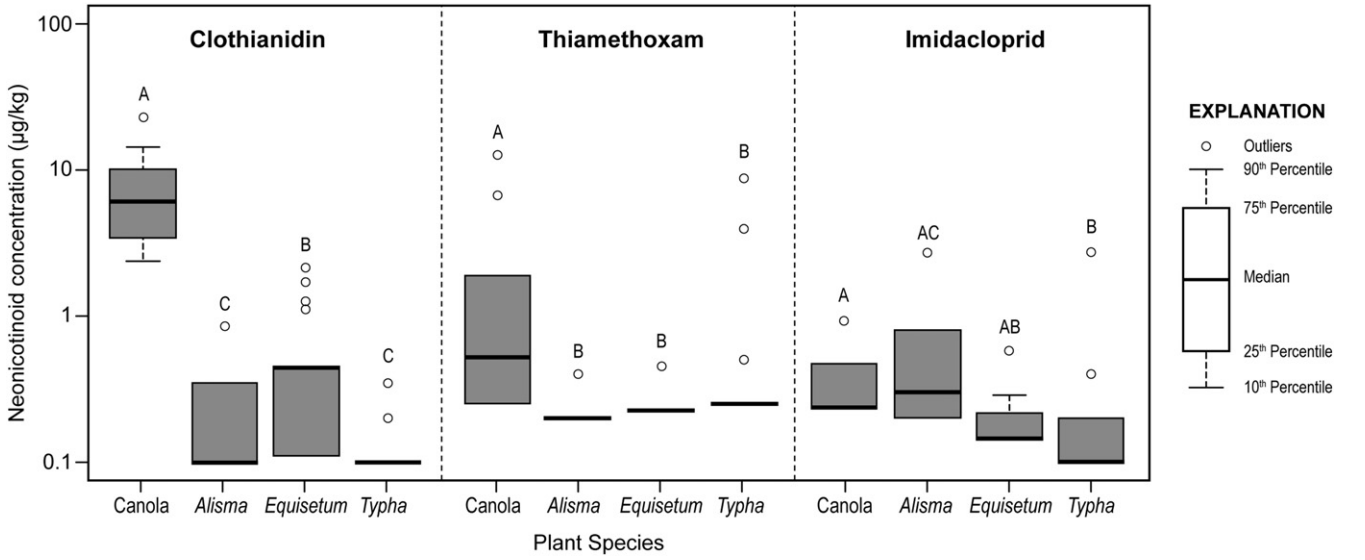

Fig. 3. Log-scale boxplot comparison of differences between neonicotinoid active ingredient concentrations (µg/kg) detected in positive controls (canola) and other common wetland plant species collected from 10 study Prairie wetlands in agricultural fields treated with clothianidin-treated canola seed. Outliers are plotted as open circles. Boxplots sharing the same letter (i.e., A, B) by active ingredient indicate no statistical difference in means.

than plants in early flowering stages (Ransom et al., 1983). Of all species we collected, only *E. arvense* and *T. latifolia* had more than one quantifiable residue. Although farmers consider it a noxious weed (Soltani et al., 2015), *E. arvense* may present a potential source of neonicotinoid accumulation and/or mitigation.

It is unclear as to why thiamethoxam and imidacloprid residues were detected in both water and plant tissues during this study as all wetlands were situated in clothianidin-treated canola fields. However, the availability of other active ingredients for accumulation may also have been the result of proximity to fields using other seed-treatments (i.e., thiamethoxam), previous landowner crop rotations, or persistence in agricultural soils (Jones et al., 2014; Hilton et al., 2015; Main et al., 2016). This confirms previous findings that wetland contamination can extend beyond the annual treatment of the field (Main et al., 2014) particularly during wetter years from solubilisation and transport of active ingredients persisting in agricultural soils and/or wetland sediments.

Comparisons of neonicotinoid data in wetland plants are limited. In Suriname, *Eleocharis mutata* removed up to 100% of imidacloprid with 86% removal by *Nymphaea amazonum* in mesocosms experimentally exposed to target concentrations (60 µg/L) over 9 days (Mahabali and Spanoghe, 2014). The highest application rate (1000 µg/L) had an average removal efficiency of 72% for the same period with up to 78.9% of the applied imidacloprid retained in plant tissues and roots of *N. amazonum* (Mahabali and Spanoghe, 2014).

Neonicotinoids can accumulate in plants found near treated fields. Clothianidin concentrations up to 4 µg/kg in milkweed plants were detected in South Dakota (Pecenka and Lundgren, 2015). In field margin dandelions, detections ranged between 2.9 µg/kg (clothianidin) and 9.4 µg/kg (thiamethoxam) near seed-treated corn fields (Krupke et al., 2012). In a 2015 study of wildflowers surrounding seed-treated oilseed rape fields, 11.6% (imidacloprid) to 58.1% (thiamethoxam) of plant pollen samples contained a neonicotinoid active ingredient with concentrations up to 86 µg/kg (Botías et al., 2015). Similarly, wild plant foliage collected from margins neighbouring seed-treated fields contained neonicotinoid residues ranging from ≤0.02 to 106.2 µg/kg with concentrations detected in oilseed rape foliage ranging from 1.4 to 11 µg/kg (Botías et al., 2016). Even though our plant tissue concentration values fall within the range listed above, the difference in terrestrial versus aquatic plant species and use of whole plants versus plant parts precludes a direct comparison of results. Additionally, the analytical limits of detection (0.12–0.16 µg/kg) and quantification (0.36–0.48 µg/kg) in other studies (e.g., Botías et al., 2015) were lower than our study (LOD: 0.10–0.55 µg/kg, LOQ: 0.31–1.99 µg/kg). Regardless, there is a consistency that non-crop terrestrial and wetland plants near agricultural production have the ability to accumulate these insecticides.

4.2. Effectiveness of wetland plants to mitigate neonicotinoid water contamination

In our study, higher neonicotinoid concentrations were detected in wetlands lacking intact vegetation communities and/or those with highly disturbed vegetation zones. In addition, plant height – indicative of the rapid spring growth period – was correlated with reductions in neonicotinoids that only occurred in the vegetated wetlands. Vegetated wetlands are known to be more effective at pesticide removal (Moore et al., 2002; Rose et al., 2006; Gregoire et al., 2009) and/or mitigation (Bennett, 2005; Riens et al., 2013) than unvegetated wetlands. To date, studies have focused on the size of the vegetation community, zone width, or "buffer" to reduce aqueous concentrations of pesticides (Dunn et al., 2011; Zhang et al., 2010). In contrast, both here and in a previous analysis, we found that zone (buffer) width was less important in explaining neonicotinoid concentrations in Prairie wetlands whereas the presence of certain plant species in the wet meadow and shallow marsh zones were strong predictors (Main et al., 2015).

Wetlands surrounded by taller plants were more likely to have lower neonicotinoid concentrations regardless of measured buffer width. We speculate that plant height may be acting as a surrogate for plant age and growth stage where older, larger plants, have more developed root systems and leaf structures leading to increases in overall plant density and biomass. The extensive rhizomatic root system of plants such as *Typha latifolia* may play a role in attenuating movement of neonicotinoids into wetlands and possibly provide a greater root surface area for neonicotinoid accumulation. In agricultural soils, soil pore space contains water with nutrients and may further contain contaminants (i.e., insecticides) available for root-uptake (Chiou et al., 2001). Clothianidin persists in soil pore water of agricultural fields under corn production (Whiting et al., 2014). Potential sorption of neonicotinoids to plant tissues is dependent on the root concentration factor (RCF) or the ratio between concentration in root (g/g) and concentration in solution (mg/L; Bonmatin et al., 2015). Depending on the species and fluctuations in the wetland hydroperiod, macrophytes can

develop more extensive below- or above-ground biomass (Miller and Zedler, 2003) leading to improved accumulation from agricultural soils. Older plants may also transport more residues into their tissues as well as indirectly slow surficial flows due to increased plant density (Stehle et al., 2011). Experimental data of aquatic plant accumulation of insecticides indicated that compared to roots, measurable amounts of imidacloprid were higher in shoots and leaves regardless of plant type (Mahabali and Spanoghe, 2014). Macrophytes such as cattails (i.e., *Typha*) also contain a higher lignin content as they age where older tissues retain chemicals more effectively than younger stems (Bonmatin et al., 2015). As we did not measure specific concentrations in roots versus shoots, overall macrophyte biomass (above- or below-ground), nor plant density, more experimental studies are needed to better understand the specific mechanism of uptake by wetland macrophytes.

Although macrophytes may provide a mechanism for neonicotinoid mitigation by attenuating and/or removing these insecticides from being transported into Prairie wetlands, we cannot rule out that wetland vegetation may instead be (1) altering the abiotic environment (van der Valk, 2012) or (2) acting as a proxy for more complex interactions such as the effect of disturbance. Effects of abiotic variables such as wetland depth, pH, and soil-water interactions may need further experimental examination as they have the ability to influence pesticide degradation (Wheeler, 2002). Though there is disagreement about whether emergent macrophytes can influence pH changes (Wetzel, 2001), some species, such as the sedge *Eriophorum angustifolium* have been shown to modify pH via root system basification (Javed et al., 2013). Conversely, canopies of submerged aquatic plants and algae are more likely to influence overall pH changes in waterbodies through respiration and modification of the CO_2/bicarbonate equilibrium (Barko et al., 1988; Frodge et al., 1990). Although density of aquatic plants was not retained in our models, species such as *Myriophyllum spicatum* (Eurasian watermilfoil) can indirectly increase the rate of photolysis and hydrolysis of thiamethoxam in microcosms under both laboratory and outdoor conditions (Traisup, 2012). Additionally, macrophyte presence and height may be a surrogate of levels of cropping intensity and disturbance in agricultural wetlands. Prairie wetlands with >45% of zonal plant communities disturbed (or removed) were associated with increased neonicotinoid detections in water (Main et al., 2015). The presence of certain shallow marsh species (*Equisetum* sp.) associated with high water concentrations (Main et al., 2015) could also be more indicative of the ability of these plants to thrive in disturbed environments (Stewart and Kantrud, 1972). Ultimately, a growing body of work indicates the importance of both biotic and abiotic modifying factors of neonicotinoid fate and persistence in wetlands (Anderson et al., 2015).

4.3. Macrophyte herbivory

To our knowledge, the potential for macrophyte-accumulated neonicotinoid residues to affect vertebrate and/or invertebrate wetland herbivores remains unexplored in the literature. A range of species may consume contaminated macrophytes in Prairie wetlands including larger semi-aquatic mammals such as muskrats (*Ondatra zibethicus*), that frequently ingest *Typha* stands (Virgl and Messier, 1992). Smaller grazing and shredding aquatic invertebrates such as trichoptera and specialized dipterans (Newman, 1991), terrestrial lepidopterans, and coleopterans (Penko and Pratt, 1987) may also consume macrophyte material. Similarly, wetland aquatic macroinvertebrates may be exposed to neonicotinoids following leaf decomposition and/or macrophyte senescence. For example, shredders such as *Gammarus pulex*, consuming imidacloprid-containing fallen tree leaves experienced impaired feeding (Kreutzweiser et al., 2009; Agatz et al., 2014). This pathway may further indirectly reduce wetland ecosystem services such as nutrient-cycling, food-web energy flows (Covich et al., 1999), and leaf-litter breakdown (Kreutzweiser et al., 2009). Macrophytes in agricultural wetlands accumulate large amounts of nutrients which are released during die-back in winter (Kröger et al., 2007); however, it is unclear if the same phenomenon would occur with accumulated neonicotinoids. Our current research did not examine the fate of active ingredients once they are accumulated in non-target plants. Future studies should evaluate the potential of neonicotinoid release back to aquatic systems during plant senescence or exposure of invertebrate and vertebrate species to neonicotinoids through wetland plant decomposition, pollination and/or herbivory (Chagnon et al., 2015).

5. Conclusions

Plants are known to directly influence the transformation and transport of insecticides (Gao et al., 2000). The number of positive neonicotinoid detections in our sample plants, higher neonicotinoid water concentrations in unvegetated wetlands, and the reductions in concentrations with plant growth confirm that common macrophytes in Prairie wetlands have the potential to significantly reduce, directly or indirectly, annual inflow of neonicotinoids. However, our ability to quantify actual residues in all plants sampled may have been constrained by our limits of analytical quantification. Wetland plants may also act as surrogates for more complex biotic (i.e., plant community responses to disturbance) or abiotic (e.g., soil-water interactions or pH) interactions affecting persistence. Therefore, experimental studies addressing the mechanisms by which macrophytes interact with neonicotinoids are needed. Ultimately, this is the first study to provide evidence of macrophyte accumulation of neonicotinoids and their potential to influence contamination of ecologically important wetlands.

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