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Julia K. Lindgren

Tiffany L. Messer

Daniel N. Miller

Daniel D. Snow

Thomas G. Franti

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Neonicotinoid pesticide and nitrate mixture removal and persistence in floating treatment wetlands

Julia K. Lindgren[1] | **Tiffany L. Messer**[2] | **Daniel N. Miller**[3] | **Daniel D. Snow**[4,5] | **Thomas G. Franti**[1]

[1]Biological Systems Engineering Dep., East Campus, Univ. of Nebraska-Lincoln, 5223 L.W. Chase Hall P.O. Box 830726, Lincoln, NE 68583-0726, USA

[2]Biosystems and Agricultural Engineering Dep., Univ. of Kentucky, 128 Barnhardt, Lexington, KY 40506, USA

[3]USDA-ARS, Agroecosystem Management Research Unit, 251 Filley Hall, UNL East Campus, Lincoln, NE 68583, USA

[4]School of Natural Resources, Univ. of Nebraska-Lincoln, East Campus, Lincoln, NE, USA

[5]Water Sciences Laboratory, Univ. of Nebraska-Lincoln, East Campus, 1840 N. 37th Street, Lincoln, NE 68583-0844, USA

Correspondence
Tiffany L. Messer, Biosystems and Agricultural Engineering Dep., Univ. of Kentucky, 128 Barnhardt Lexington, KY 40506, USA.
Email: tiffany.messer@uky.edu

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Abstract

Mesocosm and microcosm experiments were conducted to explore the applicability of floating treatment wetlands (FTWs), an ecologically based management technology, to remove neonicotinoid insecticides and nitrate from surface water. The mesocosm experiment evaluated three treatments in triplicate over a 21-d period. Floating treatment wetland mesocosms completely removed nitrate-N over the course of the experiment even when neonicotinoid insecticides were present. At the completion of the experiment, 79.6% of imidacloprid and degradation byproducts and 68.3% of thiamethoxam and degradation byproducts were accounted for in the water column. Approximately 3% of imidacloprid and degradation byproducts and 5.0% of thiamethoxam and degradation byproducts were observed in above-surface biomass, while ~24% of imidacloprid and degradation byproducts, particularly desnitro imidacloprid, and <0.1% of thiamethoxam and degradation byproducts were found in the below surface biomass. Further, 1 yr after the experiments, imidacloprid, thiamethoxam, and degradation byproducts persisted in biomass but at lower concentrations in both the above- and below-surface biomass. Comparing the microbial communities of mature FTWs grown in the presence and absence of neonicotinoids, water column samples had similar low abundances of nitrifying Archaeal and bacterial *amoA* genes (below detection to 10^4 ml^{-1}) and denitrifying bacterial *nirK*, *nirS*, and *nosZ* genes (below detection to 10^5 ml^{-1}). Follow-up laboratory incubations found the highest denitrification potential activities in FTW plant roots compared with water column samples, and there was no effect of neonicotinoid addition (100 ng L^{-1}) on potential denitrification activity. Based on these findings, (a) FTWs remove neonicotinoids from surface water through biomass incorporation, (b) neonicotinoids persist in biomass long-term (>1 yr after exposure), and (c) neonicotinoids do not adversely affect nitrate-N removal via microbial denitrification.

Abbreviations: DO, dissolved oxygen; DOC, dissolved organic carbon; FTW, floating treatment wetland; ORP, oxidation reduction potential; PCR, polymerase chain reaction.

1 | INTRODUCTION

Nutrient contamination in surface and groundwater is a growing concern in the United States and around the world (Biddau et al., 2017; Dwivedi & Mohanty, 2016; Exner et al., 2014; Katz et al., 2014; Mittelstet et al., 2019; Molenat et al., 2008). High concentrations of nitrogen (N) and phosphorus (P) have led to harmful algae blooms and fish kills (Yang, 2008). Floating treatment wetlands (FTWs) are proving to be a low-cost water treatment option to remove excess nutrients and total suspended solids in lakes, stormwater ponds, and lagoons (Di Luca et al., 2019; Keizer-Vlek et al., 2014; Tanner & Headley, 2011; Winston et al., 2013). In FTWs, buoyant hydroponic vegetated mats support the growth of wetland plant shoots above the surface water and roots within in the water column, utilizing excess nutrients for plant growth. However, other water quality concerns may affect the efficiency for FTWs to remove nutrients.

Pesticides can reduce the abundance and expression of genes responsible for N fixation, nitrification, and denitrification in soil (Singh et al., 2015), which may explain how pesticides negatively affect biogeochemical processes in soil (Wołejko et al., 2020; Yeomans & Bremner, 1985; Zhang et al., 2018). Studies focusing on aquatic microbial communities show similar responses; Sura et al. (2012) and Muturi et al. (2017) found that an herbicide (glyphosate) and insecticides (malathion, carbaryl, and permethrin) decreased bacterial production and decreased microbial community richness and diversity. Decreases in denitrification potential with the addition of certain fungicides to wetland water have also been reported (Milenkovski et al., 2010). Nevertheless, information about the specific effects of pesticides on denitrifying microbes in hydroponic rhizosphere communities is lacking and requires further investigation.

Among those emerging contaminants of concern are neonicotinoids, a class of insecticides used widely in agriculture for soybean [*Glycine max* (L.) Merr.] production and tree treatment as well as in urban settings in the form of pest repellents. Neonicotinoids are the fastest growing class of insecticides, accounting for over 25% of the global market in 2014 (Bass et al., 2015). Only 1.6–20% of active ingredients in neonicotinoid seed treatments are absorbed into the treated crop, creating the potential for water contamination via leeching and runoff (Goulson, 2013). Degradation of these insecticides occur primarily through photolysis and can be limited in the water column due to suspended solids inhibiting light. For many pesticides, metabolites can be as toxic or more toxic than the parent compound (Acero et al., 2019; Klarich et al., 2017). Negative impacts have been shown in nontarget organisms such as pollinators and aquatic invertebrates (de Jacob et al., 2019; DiBartolomeis et al., 2019; Finnegan et al., 2017). There is also concern for association with birth defects in exposed pregnant women and negative neurologic symptoms in humans (Han et al., 2018).

Core Ideas

- Neonicotinoid and byproducts were present in wetland biomass 21 d after exposure.
- Neonicotinoid and byproducts persisted in biomass 1 yr after exposure.
- Neonicotinoid exposure did not adversely affect nitrate-N removal via denitrification.

Imidacloprid and thiamethoxam are neonicotinoids widely used in the United States. Neonicotinoids have been found in midwestern groundwater, surface water, lacustrine, and tap water (Bradford et al., 2018; Hladik et al., 2018; Klarich et al., 2017; Satiroff et al., 2021). Therefore, new pesticide removal practices are needed in response to increasing concentrations and frequency of detection. Although FTWs are commonly used for nutrient removal, other treatment benefits should be explored. Wetland vegetation has been shown to reduce pesticide concentrations and detection frequency (Mahabali & Spanoghe, 2014; Main et al., 2017; Vymazal & Březinová, 2015). Floating treatment wetlands may offer a similar benefit. Therefore, we conducted mesocosm and microcosm studies of FTWs (a) to determine FTW neonicotinoid removal capacity, (b) to quantify neonicotinoid incorporation into biomass, and (c) to determine whether neonicotinoids affected nitrate-N removal rates by altering microbial denitrification potential.

2 | MATERIALS AND METHODS

2.1 | FTW mesocosm experiments

A mesocosm experiment was conducted during the summer of 2019 in the Messer Ecological Systems Observation Laboratory, a climate-controlled greenhouse at the University of Nebraska-Lincoln. Floating treatment wetland mesocosms consisted of 380-L black Rubbermaid feeding troughs filled with simulated greenhouse water (Figure 1; Supplemental Figure S1). Due to limited greenhouse space, control mesocosms (no FTWs) were 56-L buckets, as previously used in similar mesocosm experiments (Keilhauer et al., 2019; Messer Burchell, Birgand, et al., 2017; Messer et al., 2022). Floating treatment wetland mats (60 cm by 60 cm) were purchased from Beemats and stocked with 10 native Nebraska wetland plants that were planted in spring 2017 and established until the experiment began in 2019 with periodic fertilizer applications. Plant species consisted of longhair

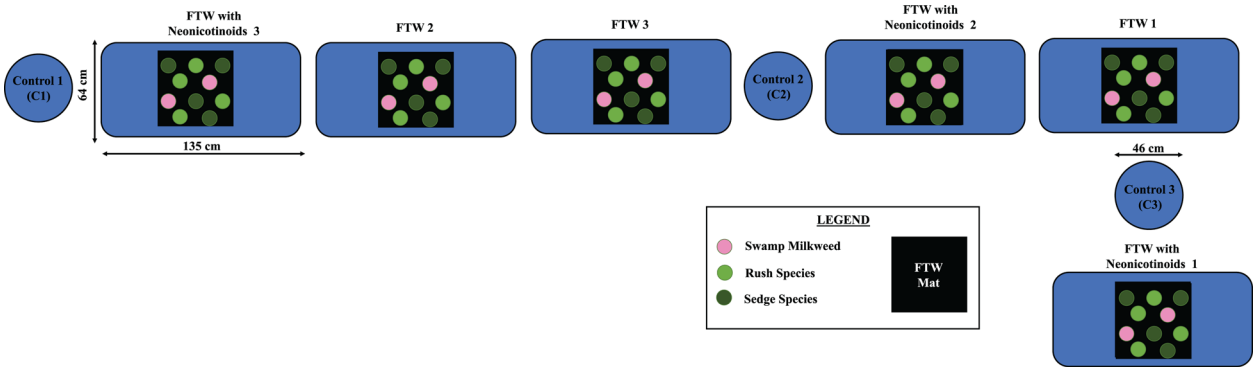

FIGURE 1 Mesocosm design setup in a climate-controlled greenhouse

sedge (*Carex comosa* Boott), fox sedge (*Carex vulpinoidea* Michx.), swamp milkweed (*Asclepias incarnata* L.), common rush (*Juncus effusus* L.), and Torrey's rush (*Juncus torreyi* Coville). An Onset HOBO light and temperature sensor were situated underneath each FTW mat to monitor temperature and light conditions in the mesocosms throughout the experiment.

Nine mesocosms were established, consisting of three FTW mesocosms enriched with neonicotinoids and nitrate-N (NO_3–N), three FTW mesocosms enriched with NO_3–N only, and three control mesocosms (no plants) with neonicotinoids and NO_3–N. Four days before the study, all mesocosms were cleaned and refilled with greenhouse tap water using a flow meter (P3 International Corporation) to ~285 L for those with FTWs and 50 L for lower-volume controls. On the first day of the experiment (Day 0), foil was placed over the mesocosms to limit ultraviolet light exposure to the water surface, which can result in substantial photodegradation of the insecticides (Todey et al., 2018). Biomass samples were collected from the six FTWs before potassium nitrate (KNO_3) or neonicotinoids were added.

All mesocosms were amended with KNO_3 (Fisher Scientific International, Inc.) to achieve initial NO_3–N concentrations of ~10 mg L^{-1}. Background NO_3–N concentrations in tap water yielded initial NO_3–N concentrations slightly above 10 mg L^{-1}. Imidacloprid and thiamethoxam, two commonly used neonicotinoids in the Midwest (Hladik et al., 2018) and found ubiquitously in Nebraska water systems (Satiroff et al., 2021), were selected. Stock insecticide solutions were spiked into three of the six FTW mesocosms and the three control mesocosms (no FTWs) to 100 ng L^{-1} for both imidacloprid and thiamethoxam, which is consistent with observed concentrations in rivers and lakes in eastern Nebraska (Satiroff et al., 2021).

Water samples were collected on Days 0, 1, 2, 3, 5, 7, 10, 15, and 21 of the experiment along with water depth and other physiochemical characteristics (temperature, pH, dissolved oxygen [DO], conductivity, oxidation reduction potential [ORP]), which were measured using a YSI EXO2 Sonde (Xylem). Prior to sample collection, water was recirculated in each of the mesocosms for 3 min using submersible pumps to ensure homogeneity. Grab samples were collected 15 cm below the air–water interface, placed in a cooler on ice, and transported immediately to the laboratory where they were passed through GF/F filters and stored in a refrigerator until analyzed for NO_3–N, ammonium-N (NH_4–N), phosphate-P (PO_4–P), and dissolved organic carbon (DOC). Unfiltered 20-ml samples were collected and frozen on Days 0, 7, 10, 15, and 21 for subsequent molecular microbial analysis. Insecticide samples (2 ml) were collected on Days 1 and 21, placed into amber glass bottles, and stored frozen until analysis for thiamethoxam, imidacloprid, and associated known degradation byproducts (imidacloprid desnitro, imidacloprid olefin, imidacloprid urea, clothianidin, 6-chloronicotinic acid, 6-cloronicotinic aldehyde, 6-chloro-N-methylnicotinamide, and 6-hydroxynicotinic acid).

Above- and below-surface biomass samples were collected on Day 21 of the experiment for neonicotinoid and neonicotinoid byproduct analysis (Supplemental Figure S2). Shoot samples from mesocosms with identical treatments were composites of three plant types: sedge, rush, and milkweed. Milkweed above-surface biomass samples consisted of leaves, whereas sedge/rush samples were cut from the top 25 cm of the plant. Root samples were cut from the bottom 15 cm of the rhizome but were not categorized by plant type due to entwining root mats between plant species. All plant samples were placed in Ziplock bags and stored frozen until freeze dried and analyzed. Destructive harvests were carried out at the end of the experiment to measure N and C content in the FTW biomass. One plant of each species was taken from the six FTWs; the roots were composited, and individual shoots were identified based on plant species. Fresh-frozen, freeze-dried samples were analyzed for insecticide residues. Subsamples of plant tissues were air dried and analyzed for N and C content and analyzed as described below.

2.2 | Denitrification potential incubations

A laboratory incubation study was conducted to assess the primary site for denitrification (water column versus root-associated) and the effect of neonicotinoid pesticide exposure (Supplemental Figure S3) on denitrification rates after the greenhouse mesocosm study was completed. Triplicate incubations using mesocosm water or FTW roots were made in 150-ml serum bottles buffered to pH 7.4 with potassium phosphate with and without imidacloprid and thiamethoxam. Root and water column samples from three mature mesocosms were collected, and once in the laboratory root biomass was cut into 2-cm pieces and mixed. For mesocosm water incubations with or without neonicotinoids (treatments WN and W, respectively), 90 ml of mesocosm water was combined with 10 ml of 10 mM potassium phosphate buffer (pH 7.4, 1 mM final concentration). For root incubations with or without neonicotinoids (treatments RN and R, respectively), 10 g of root matter was added to 90 ml of 1 mM potassium phosphate buffer (pH 7.4). A control treatment with no added FTW water or roots and no neonicotinoids (Treatment C) also contained 90 ml of 1 mM potassium phosphate buffer (pH 7.4). Stock solutions were measured into specific treatments (WN and RN) to a final concentration of 100 ng L^{-1} of both thiamethoxam and imidacloprid (i.e., the same level as in the mesocosm experiment). Glucose and chloramphenicol were added to all treatments (5 mM and 0.5 g L^{-1} final concentration, respectively), and then the serum bottles were stoppered and made anaerobic in repeated cycles of evacuation and N flushing. Potassium nitrate was added aseptically by syringe to all incubation vials to a 10 mg N L^{-1} final concentration. Samples were collected by syringe on Days 0, 1, 2, 3, 4, 5, 7, and 10; immediately frozen; and subsequently analyzed for NO_3–N and NH_4–N as described in Section 2.3. After 10 d, root mass was recovered from the RN and R treatments and analyzed for volume, dry mass, and nutrient concentration.

2.3 | Nutrient and C analysis

Concentrations of NH_4–N and NO_3–N in FTW and laboratory serum bottle water samples were determined by automated spectrophotometry using a Seal Analytical AQ300 autoanalyzer according to USEPA methods 351.2 and 353.2, respectively. Dissolved P was measured spectrophotometrically according to USEPA method 365.3 using a Beckman DU-800 spectrophotometer. Dissolved organic C was determined using a 1010 TOC Analyzer (Oceanography International Corporation) with the Standard Method 5301D. Above- and below-surface biomass samples were analyzed for total N and total C content at Ward Laboratories, Inc. using the Dumas Combustion Method (Plank, 1991; Sweeney, 1989).

2.4 | Neonicotinoid residue analysis

All samples were analyzed for insecticide residues using liquid chromatography–tandem mass spectrometry. Standards and solvents were purchased from Sigma-Aldrich, PlusCHEM, or ChemService and included clothianidin, thiamethoxam, imidacloprid, acetamiprid, thiacloprid, dinotefuran, metalaxyl, dimethoate, pyraclostrobin, trifloxystrobin, azoxystrobin, picoxystrobin, imidacloprid urea, imidacloprid olefin, imidacloprid desnitro HCl, thiamethoxam urea, 6-hydroxynicotinic acid, 6-chloronicotinic acid, 6-chloronicotinic aldehyde, 6-chloro-N-methylnicotinamide, sulfoxaflor, and indoxacarb. Stable isotope labeled internal standards were purchased from Sigma-Aldrich or Cambridge Isotope Laboratories. Stock solutions (1.0 µg $µl^{-1}$) of each analyte and standard were prepared in methanol (Optima, Fisher Scientific) and stored at −20 °C.

Frozen plant tissue samples were freeze-dried using a Labconco 4.5L Freezone system prior to grinding using a mortar and pestle. Extraction and subsequent analysis of freeze-dried plant tissue for pesticide residues generally followed procedures outlined in Botías et al. (2015). Briefly, 0.2 g of freeze-dried biomass tissue in a 50-ml polypropylene centrifuge tube was mixed with 2 ml of reagent water to rehydrate, followed by 2.5 ml acetonitrile and 0.75 ml hexane. The mixture was spiked with 10 µl of a surrogate mix (0.10 ng $µl^{-1}$ nitenpyram and terbuthylazine) to measure recovery, capped, and shaken for 10 min on a wrist action shaker. A salting out reagent (1.25 g 4:1 magnesium sulfate/sodium acetate) was then added, and the solution was hand shaken and centrifuged at 2,500 rpm for 5 min. Liquid supernatant was pipetted into a clean centrifuge tube containing 625 mg SupelQue cleanup sorbent (PSA/C18/ENVI-Carb, Sigma-Aldrich) and vortexed. The tissue sample was extracted a second time using an additional 1.75 ml of acetonitrile, and the supernatant was combined with the first portion. The purified extract was evaporated to ∼1 ml and filtered using 25-mm, 0.45-µm pore size glass microfiber into a glass culture tube. The solvent was evaporated to near dryness, spiked with 50 µl deuterium labeled internal standards (0.2 ng $µl^{-1}$ d3-clothianidin, d3-thiamethoxam, d4-imidacloprid, d6-metalaxyl, pyraclostrobin-(N-methoxy-d3), and mixed with 200 µl of purified reagent water to a solvent ratio 20:80 methanol/water.

Compounds were separated and analyzed on a Aquity UPLC interfaced with a Xevo TQS triple quadrupole mass spectrometer using a UniSpray source (Waters Corp.). Chromatographic separation was performed with an Aquity BEG C_{18} 50 mm by 2.1 mm by 1.7 µm reverse phase column. Mobile phase solvents A (0.1% v/v formic acid in water) and B) (0.1% v/v formic acid in methanol) at a flow rate of 0.6 ml min^{-1} began with 95:5 A/B, increasing to 5:95 A/B until

3 min, and held for 0.5 min before switching back to original conditions 95:5 A/B at 3.60 min for a total run time of 5 min per injection.

Multiple reaction monitoring was used for each compound, and five deuterium-labeled internal standards were used for quantitation (Supplemental Table S1). Quality controls analyzed at a frequency of 5% or better included laboratory method blanks, laboratory fortified blanks, laboratory fortified matrix, and laboratory duplicates. Instrument detection limits were determined as three times the standard deviation of a 2.0 pg μl^{-1} standard average 0.22 ± 0.19 pg on-column, equivalent to 0.11 µg L^{-1} in filtered water samples. Method detection limits for plant tissue samples, determined 8–10 replicates of a low-level fortified blank matrix, averaged 0.030 ± 0.030 ng g^{-1}.

2.5 | DNA extraction and microbial community analysis

Frozen 20-ml FTW water samples were thawed and centrifuged for 5 min at 2,500 × *g* to pellet bacterial biomass. The supernatant was carefully decanted, and the pellet was resuspended in sterile distilled water and transferred to 2-ml vials. Following the protocol of Ausubel et al. (1989), vials were boiled to lyse microbial cells and then centrifuged at 10,000 × *g* for 10 min at 2 ˚C before transferring the clarified supernatant containing DNA to a clean tube. Six genes, including 16S ribosomal RNA (Muyzer et al., 1993), archaeal and bacterial ammonia monooxygenase (*amoA*) from nitrifying microorganisms (Tourna et al., 2008; Rotthauwe et al., 1997), and two nitrate reductases (*nirS* and *nirK*) and a nitrous oxide reductase (*nosZ*) from denitrifying bacteria (Braker et al., 1998), were quantified in DNA extracts using quantitative polymerase chain reaction (PCR). Primer descriptions and PCR procedures can be found in Supplemental Table S2. Quantitative PCR reactions were carried out with QuantiTect Syber Green master mix (QIAGEN) using a StepOnePlus real-time PCR system (Applied Biosystems Inc.). Each sample was measured in triplicate, averaged, converted to copies per milliliter of original sample, and log transformed. Archaeal *amoA* and bacterial *amoA*, *nirS*, *nirK*, and *nosZ* gene abundances were analyzed relative to 16S concentration.

2.6 | NO_3–N removal

First-order NO_3–N removal rates were calculated for all mesocosms following both experiments (Benjamin, 2010; Brezonik & Arnold, 2011; Keilhauer et al., 2019; Messer, Burchell, & Birgand, 2017):

$$C_T = C_0 * e^{-kt} \quad (1)$$

where C_T is the final NO_3–N concentration (mg L^{-1}), C_0 is the initial NO_3–N concentration (mg L^{-1}), t is time from the beginning experiment to when NO_3–N concentrations were below detectable limits (days), and k is the removal rate (g m^{-2} d^{-1}).

2.7 | Neonicotinoid, NO_3–N percent removal, and NO_3–N removal rates

For each mesocosm, NO_3–N, thiamethoxam, and imidacloprid percent removals were calculated using concentrations from Day 1 to the last day of the experiment where concentrations were above the minimum detection limit of 0.05 mg L^{-1} for NO_3–N and 2.00 µg L^{-1} for imidacloprid and thiamethoxam (Benjamin, 2010; Brezonik & Arnold, 2011; Keilhauer et al., 2019).

$$\%\text{Removal} = \frac{C_0 - C_T}{C_0} \times 100\% \quad (2)$$

Daily NO_3–N removal rates and overall neonicotinoid removal rates were calculated for all mesocosms after the experiment using Equation 3:

$$J_{XX} = \frac{(x_{i-1} - x_i)}{A \times t} \quad (3)$$

where J_{xx} was the analyte removal rate (mg m^{-2} d^{-1}), X_{i-1} was the analyte loading from the previous sampling day (mg), X_i was the analyte loading from given sampling day (mg), A was the area of the FTW mat, and t was the time since nutrient enrichment (days).

2.8 | Statistics

Statistical analyses were performed on NO_3–N concentrations, DO, conductivity, ORP, pH, and temperature to determine differences between the three treatments through time during the experiment. Because FTW and control mesocosms lost 1.5–10 cm of water due to evapotranspiration during the experiment, statistical comparisons were carried out after adjusting NO_3–N and neonicotinoid concentrations with daily water depth measurements. All gene abundance data were normalized by log transformation, and ANOVA regression analysis was performed on each treatment using Minitab 17. Significant differences between treatments were assessed

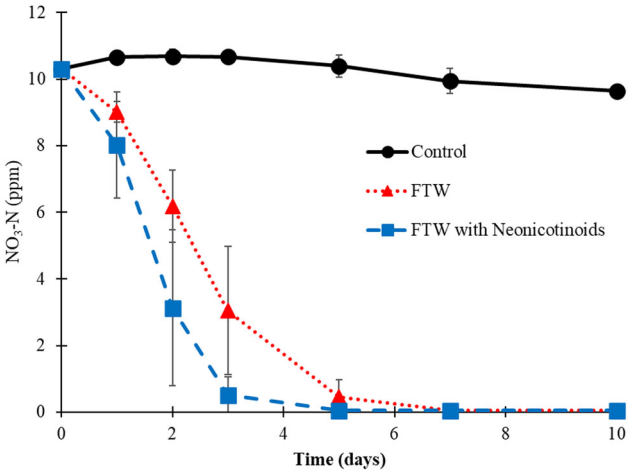

FIGURE 2 Concentrations of NO_3–N, adjusted for evapotranspiration, in control (no floating vegetation), floating treatment wetland (FTW) without neonicotinoids, and FTW with neonicotinoids during the mesocosm experiment. A summary of nutrient concentrations from mesocosms can be found in Supplemental Table S3

using Tukey pairwise comparison test at a significance test of $\alpha < .05$.

3 | RESULTS AND DISCUSSION

3.1 | Mesocosm NO_3–N removal

By Day 10, all six FTW mesocosms had NO_3–N concentrations below the analytical detection limit (0.05 mg L^{-1}), whereas control mesocosms were unchanged from the initial 10 mg NO_3–N mg L^{-1} concentrations (Figure 2). An ANOVA performed on the first 10 d of the mesocosm experiment identified significant differences ($p < .001$) in NO_3–N concentrations between control mesocosms and FTW mesocosms, but no difference was observed between the two FTW treatments (with and without neonicotinoids). Concentrations of NH_4–N ranged from 0.20 to 0.84 mg L^{-1} on Day 0 of the experiment and remained stable in controls and fell below 0.05 mg L^{-1} within 24 h in the FTW mesocosms. Although trends in NO_3–N concentrations were very similar between neonicotinoid and non-neonicotinoid treatments in the FTWs, a 1-to-2-d lag in NO_3–N concentration reduction in the FTW without neonicotinoids was observed likely due to lesser available DOC compared with the FTWs with neonicotinoids in the water column (Table 1).

Daily NO_3–N removal rates were calculated between Day 1 and Day 10 or on the last day NO_3–N concentrations were above the analytical detection limit (0.05 mg L^{-1}). Average NO_3–N losses from the water column in the FTWs with neonicotinoids and without neonicotinoids were 3.33 ± 1.17 and 1.47 ± 0.17 g m^{-2} d^{-1}, respectively, but did not significantly differ ($p = .206$). These rates are consistent with those observed by Yang et al. (2008), which ranged from 0.24 to 1.90 g m^{-2} d^{-1} but are larger than the rates (0.12 g m^{-2} d^{-1}) observed by Saeed et al. (2016) for a horizontal flow pilot FTW. First-order removal rates were 1.16 ± 0.43 and 0.61 ± 0.14 g m^{-2} d^{-1} for mesocosm FTWs with and without neonicotinoids, respectively. Although no differences were observed between the two FTW treatments, the experiment did demonstrate that FTW NO_3–N removal was not affected by neonicotinoids, contrary to the original hypothesis.

Low ORP and DO concentrations indicated conditions were conducive for denitrification (Table 1). Denitrification may occur when ORP is low (Li & Irvin, 2007) and minimal DO is available. All FTW mesocosms had a DO <1 mg L^{-1} by Day 2, although FTWs with neonicotinoids exhibited anaerobic conditions slightly sooner (Table 1). Additionally, ORP dropped below 250 mV and was negative in all FTW mesocosms by Day 7. Control mesocosms exhibited more aerobic conditions throughout the experiment (1.54–7.25 mg DO L^{-1}), which did not have FTWs. The FTW treatments had FTWs established in the mesocosms for several months prior to the beginning of this experiment. Further, observations of lower DO concentrations in the FTW treatments were consistent with previous observations in environments with prolonged floating treatment wetland establishments (Strosnider et al., 2017). Although ORP dropped below 250 after Day 5 in control mesocosms, the presence of DO (4.0–5.1 mg L^{-1}) and low C source (1.1–1.8 mg mg L^{-1}) availability may account for limited NO_3–N removal. The pre-establishment of the FTWs likely contributed to the higher DOC levels in those treatments compared with the controls and has been observed in other mesocosm treatment wetland studies (Keilhauer et al., 2019; Messer, Burchell, Birgand, et al., 2017). Plant uptake of N incorporated into the biomass during the 2019 growing season was 90.0 ± 6.82 g m^{-2} in above-surface biomass and 54.9 ± 3.09 g m^{-2} in below-surface biomass (Messer et al., 2022).

3.2 | Neonicotinoid removal/transformation

Imidacloprid concentrations in the water were significantly reduced ($p < .001$) in FTW mesocosms when compared to control mesocosms without FTWs (Figure 3). Thiamethoxam concentrations decreased to a similar extent in the water of FTWs; control mesocosms exhibited a similar decrease. Neonicotinoid transformation product concentrations in the water (including imidacloprid desnitro, imidacloprid urea, and clothianidin) were negligible compared with the parent insecticides on Day 21 (Supplemental Table S4).

Neonicotinoids and measured degradation byproducts accumulated in above- and below-surface biomass (Figure 4;

TABLE 1 Mean (± SD) for dissolved oxygen (DO), conductivity, oxidation-reduction potential (ORP), pH, water temperature, and dissolved organic carbon (DOC) in floating treatment wetland (FTW) mesocosms with neonicotinoids (FTW + N), FTW mesocosms (FTW), and control mesocosms

Day of experiment	Treatment	DO	Conductivity	ORP	pH	Temperature	DOC
		mg L^{-1}	µS cm^{-1}	mV		°C	mg L^{-1}
Day 0	Control	7.25 ± 0.60	687.00 ± 1.73	332.23 ± 24.20	6.57 ± 0.12	25.07 ± 0.42	8.58 ± 0.31
	FTW	3.28 ± 1.17	705.67 ± 26.10	136.27 ± 7.92	6.43 ± 0.10	23.47 ± 0.21	5.59 ± 0.72
	FTW + N	3.38 ± 1.21	692.33 ± 20.26	201.37 ± 16.74	6.41 ± 0.08	23.70 ± 0.44	14.15 ± 1.80
Day 1	Control	4.86 ± 0.51	688.00 ± 1.00	378.03 ± 19.23	6.96 ± 0.07	25.20 ± 0.26	N/A
	FTW	1.06 ± 0.64	721.33 ± 12.50	189.10 ± 54.14	6.80 ± 0.30	24.20 ± 0.53	N/A
	FTW + N	0.32 ± 0.17	714.33 ± 10.97	221.90 ± 71.95	6.52 ± 0.11	24.36 ± 0.35	N/A
Day 2	Control	5.42 ± 0.51	688.67 ± 2.08	352.90 ± 23.10	7.05 ± 0.08	25.93 ± 0.45	N/A
	FTW	0.54 ± 0.22	731.33 ± 9.45	165.97 ± 22.75	6.50 ± 0.05	25.27 ± 0.46	N/A
	FTW + N	0.15 ± 0.06	716.33 ± 9.29	155.10 ± 29.58	6.48 ± 0.04	25.50 ± 0.44	N/A
Day 3	Control	4.39 ± 0.49	689.33 ± 1.53	272.30 ± 32.60	7.20 ± 0.09	26.03 ± 0.45	N/A
	FTW	0.43 ± 0.24	742.00 ± 17.58	151.00 ± 27.40	6.58 ± 0.12	25.40 ± 0.53	N/A
	FTW + N	0.20 ± 0.09	718.00 ± 14.18	−88.03 ± 180.76	6.45 ± 0.04	25.67 ± 0.42	N/A
Day 5	Control	4.51 ± 0.56	703.33 ± 14.57	218.40 ± 25.90	7.40 ± 0.04	26.70 ± 0.30	N/A
	FTW	0.29 ± 0.10	769.67 ± 24.01	−45.57 ± 180.82	6.45 ± 0.09	26.53 ± 0.32	N/A
	FTW + N	0.14 ± 0.04	749.33 ± 32.01	−273.03 ± 62.52	6.38 ± 0.08	26.60 ± 0.26	N/A
Day 7	Control	2.80 ± 2.34	690.00 ± 5.57	70.50 ± 40.92	7.20 ± 0.03	26.90 ± 0.62	N/A
	FTW	0.20 ± 0.09	771.33 ± 20.53	−206.53 ± 102.16	6.49 ± 0.14	26.33 ± 0.51	N/A
	FTW + N	0.11 ± 0.04	752.33 ± 29.87	−318.13 ± 30.06	6.38 ± 0.06	26.60 ± 0.44	N/A
Day 10	Control	1.54 ± 1.34	698.33 ± 1.53	6.30 ± 62.78	7.09 ± 0.14	24.20 ± 0.36	N/A
	FTW	0.27 ± 0.23	791.33 ± 26.58	−261.63 ± 70.13	6.72 ± 0.24	23.60 ± 0.56	N/A
	FTW + N	0.13 ± 0.03	770.67 ± 28.29	−288.93 ± 25.03	6.51 ± 0.02	23.80 ± 0.17	N/A
Day 21	Control	5.39 ± 0.06	706.67 ± 2.08	24.00 ± 57.65	6.45 ± 0.06	25.40 ± 0.78	3.61 ± 0.04
	FTW	0.35 ± 0.22	873.33 ± 23.59	−285.57 ± 36.00	6.58 ± 0.15	24.27 ± 0.50	12.69 ± 3.50
	FTW + N	0.15 ± 0.07	845.33 ± 29.57	−346.70 ± 23.68	7.24 ± 0.13	24.40 ± 0.56	13.69 ± 4.90

Note. $n = 3$ per treatment on each sampling day. N/A, not available.

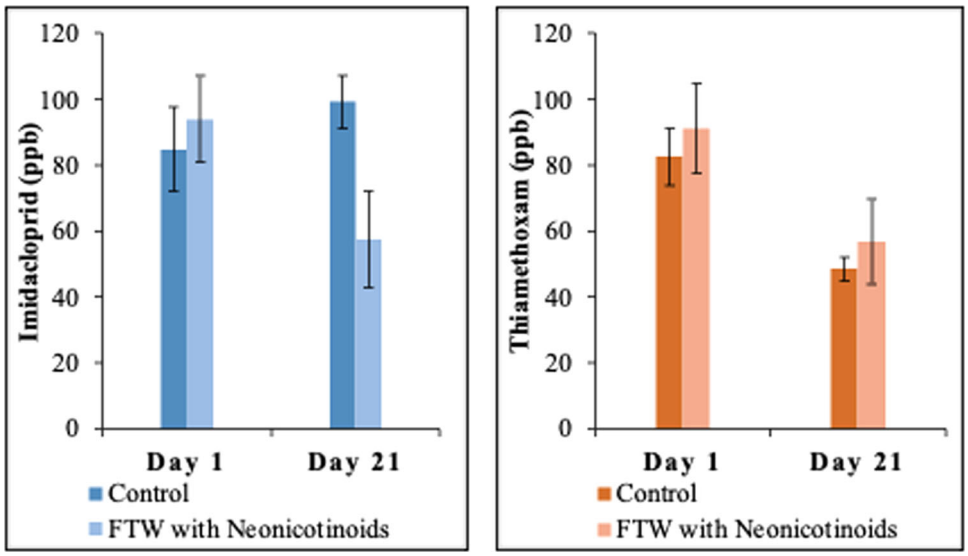

FIGURE 3 Average imidacloprid and thiamethoxam water concentrations at the beginning and end of the mesocosm experiment in water from control (no floating treatment wetlands [FTWs]) and FTW. Concentrations were adjusted for evapotranspiration. Error bars represent 1 SD ($n = 3$)

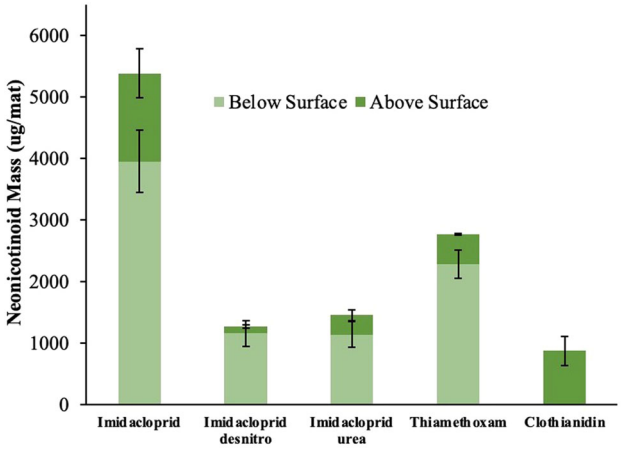

FIGURE 4 Mass distribution of neonicotinoids and neonicotinoid byproducts in floating treatment wetland FTW roots (below surface) or leaves/stems (above surface). Error bars represent 1 SD ($n = 3$)

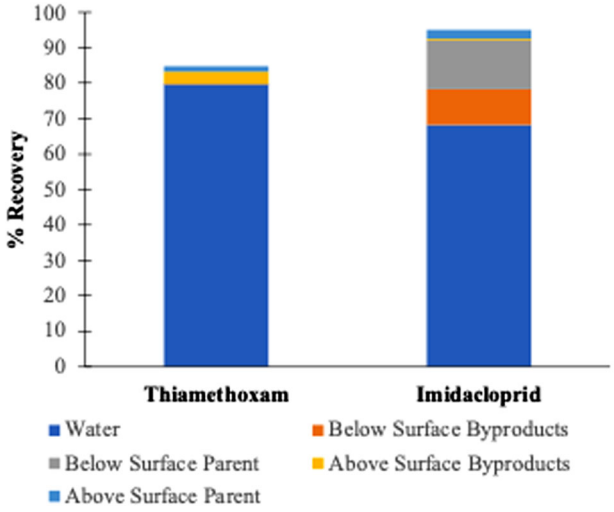

FIGURE 5 Mass balance of recovered imidacloprid and thiamethoxam and byproducts in floating treatment wetland (FTW) mesocosms on experiment Day 21. All mass values are averages of the three FTW plants in pesticide enriched mesocosms

Supplemental Table S5). Most compounds accumulated in the roots compared with the above-surface biomass except for clothianidin, a byproduct of thiamethoxam, which was found exclusively in the above-surface biomass. Above-surface concentrations for clothianidin, imidacloprid, imidacloprid desnitro, imidacloprid urea, and thiamethoxam were 353–557, 617–832, 41–97, 123–289, and 170–2,275 ng g^{-1}, respectively (Supplemental Table S5). Imidacloprid metabolites, particularly desnitro imidacloprid, have been found to be more toxic in mammals than their parent compound (Brunet et al., 2004; Lee Chao & Casida, 1997; Thompson et al., 2020; Tomizawa & Casida, 2003, 2005). Further, imidacloprid has been shown to highly absorb in a human intestinal cell model (Brunet et al., 2004).

Similar biomass accumulation results have been documented in other studies. For example, milkweed leaves have been observed to retain 10.8–2,193 ng g^{-1} of clothianidin after the soil was dosed with 0.6–1.5 g of clothianidin per pot (Bargar et al., 2020). Relatedly, glyphosate and its metabolite aminomethyl phosphonic acid were shown to accumulate in the roots of tea plants and eventually translocate to the leaves (Tong et al., 2017). Similar metabolism occurred in thiamethoxam-treated rice (*Oryza sativa* L.) plants, where concentrations of clothianidin were found in plants 6–10 d before any clothianidin was detected in the surrounding soil (Ge et al., 2017).

In this study, the mass balance of the neonicotinoids in the FTW systems accounted for ~85–95% of added imidacloprid and thiamethoxam either in the water or plant biomass (Figure 5). The 5–15% of unaccounted insecticide could have been lost through adsorption onto mesocosm walls and FTW mat; conversion into an unknown/undetected byproduct; or decomposition via photolysis, hydrolysis, and/or mineralization (Muerdter & Lefevre, 2019; Todey et al., 2018; Yari et al., 2019). Hydrolysis of thiamethoxam has been observed to be a base catalyzed process with minimal hydrolysis in neutral and acidic waters, like the water used in this experiment (Karmakar et al., 2009; Klarich Wong et al., 2019; Liqing et al., 2006; Thompson et al., 2020). Imidacloprid and thiamethoxam have a low vapor pressure; therefore, loss via volatilization was not expected (Bonmatin et al., 2015). However, further research is needed to identify specific biological and/or abiotic transformation mechanisms for these insecticides.

These observations are consistent with previously reported persistence of neonicotinoids in *Lemna* duckweed (Muerdter & Lefevre, 2019) and broadleaf arrowhead (*Sagittaria latifolia* Willd.) (McKnight et al., 2021). Muerdter and Lefevre (2019) observed that imidacloprid in the water column was reduced by *Lemna* duckweed after 6 d of exposure; however, desnitro imidacloprid was minimal in the plant tissue, which differed from this experiment. Similarly, *S. latifolio* was observed to reduce imidacloprid from the water column by 79.3% after 56 d, whereas the nonplanted containers had 0% reduction (McKnight et al., 2021). Further, the study reported that degradation of imidacloprid only occurred in the presence of both plants and microbes. However, degradation was not observed when solely plants or microbes were present.

3.3 | Pesticide persistence in wetland plants

Floating treatment wetlands resided in the temperature-controlled greenhouse for 1 yr after the initial mesocosm experiment without addition of neonicotinoids to the water.

During this period, potassium nitrate (KNO_3) was added on a weekly basis to mesocosms to maintain NO_3–N concentrations at ~5 mg L^{-1} and PO_4–P concentrations of 1 mg L^{-1}, and plants died back and re-emerged following their seasonal life cycle. Water was drained periodically from the mesocosms over the year, but plants were left undisturbed. One year after the mesocosm experiment, additional above- and below-surface biomass samples were analyzed for neonicotinoids and degradation byproducts (summarized in Supplemental Table S6). Imidacloprid and imidacloprid byproducts (desnitro, urea, 6-chloronicotinic acid) decreased from the sampling time the previous year; however, a substantial amount continued to reside primarily in the roots in the form of imidacloprid desnitro and imidacloprid urea. In contrast, thiamethoxam and thiamethoxam byproducts (clothianidin, urea) decreased substantially with concentrations primarily observed in the above-surface biomass in the forms of thiamethoxam and thiamethoxam urea. Specifically, thiamethoxam urea has been reported to form via hydrolysis (Todey et al., 2018); however, more controlled experiments would be required to definitively determine this was the sole transformation process producing thiamethoxam urea in this study.

3.4 | FTW functional gene quantification

The presence and abundance of key nitrifying and denitrifying genes involved in N transformations can provide insight into wetland nutrient transformation processes (Bowen et al., 2020; Peralta et al., 2010). The absolute abundance of *amoA*, *nirS*, *nirK*, and *nosZ* in control and FTW water column samples during the FTW greenhouse mesocosm study is shown in Supplemental Figure S4. Every sample contained microbial biomass (16S rRNA), bacterial ammonium oxidizers (*amoA*), and denitrifying microorganisms (*nirS*), and the other genes indicative for Archaeal ammonia oxidizers (*amoA*). Denitrifying microorganisms (*nirK* and *nosZ*) were also routinely detected. The low abundances of all these functional genes ($<10^5$ ml^{-1}) reflects a low overall microbial community abundance in water column samples (10^5 and 10^6 16S ribosomal RNA gene in control and FTW mesocosms, respectively). These observations were unsurprising because it is well known that increased abundance of microbial communities is typically detected in sediments (Duhamel et al., 2017; Zhang et al., 2022). Examining temporal trends, both bacterial and archaeal *amoA* and *nirK* were initially lower in the control mesocosms but increased by 1 log during the study to similar abundances in FTW mesocosm water samples. However, absolute 16S rRNA remained ~1 log lower throughout the course of the study in the control mesocosms. When functional genes were normalized to 16S rRNA gene abundance (Supplemental Table S4), these patterns held true: overall microbial community abundance was larger ($p < .05$) in the six mesocosms with FTWs when compared to control mesocosms. No differences were observed in 16S rRNA gene abundance between the two FTW treatments (with and without neonicotinoids). Additionally, no differences were observed between mesocosm treatments for archaeal *amoA*, *nirS*, and *nosZ* after normalizing to 16S abundances.

The presence of these functional genes is indicative of classical denitrification processes in FTW systems, where organic matter decomposition is linked to NO_3–N reduction under low oxygen conditions. Aerobic microbial nitrification is also indicated where NH_4–N is oxidized to nitrite and then NO_3–N, providing the energy for new microbial biomass. With these insights, the importance of the FTW rhizosphere as a potential source for root exudates (i.e., the organic matter source fueling denitrification) and for organic N and NH_4–N (to fuel nitrification) becomes a crucial component of the system. Our new hypothesis was that denitrifying microbial communities within the rhizosphere were likely the primary sites for denitrification in the system. Thus, a follow-up study of rhizosphere and water column samples collected from the FTW mesocosms after the initial greenhouse mesocosm study was conducted at the microcosm scale to determine the relative contributions of water column and rhizosphere microbial communities in denitrification.

3.5 | Root versus water column potential denitrification activity

A laboratory study of potential denitrifying activity in root-associated versus water column denitrifying communities was conducted after the greenhouse mesocosm study to further define neonicotinoid effects on denitrification. The low-abundance denitrifying community in the water column samples showed no potential to remove NO_3–N in the incubations (Figure 6). However, NO_3–N was quickly consumed (below the detection limit of 0.05 mg L^{-1}) in the vials containing fresh FTW roots by Day 3 of the incubation. These results suggest that a more abundant microbial community attached to FTW roots, as noted in earlier FTW experiments (Urakawa et al., 2017), was responsible for the bulk of denitrification observed in the mesocosm study and that very little NO_3–N was likely removed by microbes in the water column. Floating treatment wetland roots are known to provide attachment sites for microbes, allowing for microbial denitrification (Samal et al., 2019). Additionally, the presence of organic matter released from FTW roots would not only provide denitrifying microorganisms with C sources but would also stimulate oxygen-utilizing microbes to establish a low-oxygen microenvironment within the rhizosphere. Concentrations of NO_3–N between the two root treatments (with and without neonicotinoids) did not differ throughout the 5-d

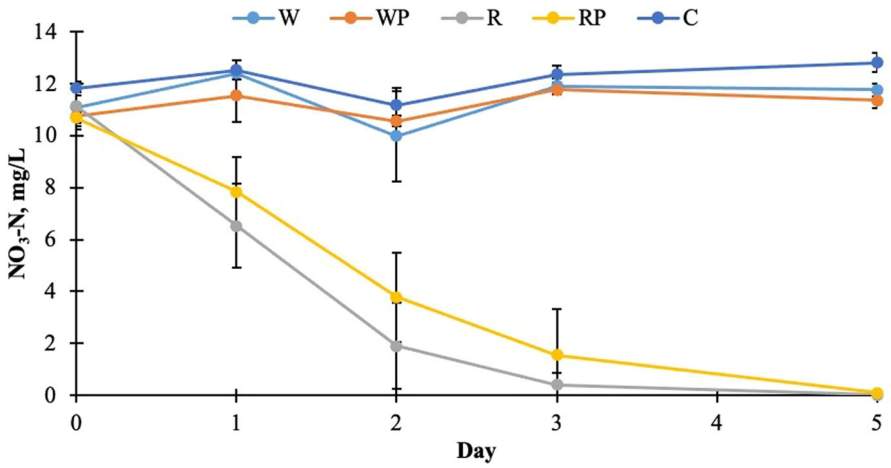

FIGURE 6 Average NO_3–N concentrations ($n = 3$) in vials with mesocosm water (W), mesocosm water with neonicotinoids (WP), roots (R), roots with neonicotinoids (RP), and sterilized buffer (C). Error bars indicate concentration SD for each treatment

experiment ($p = .307$). Furthermore, the NO_3–N first-order removal rates in root vials with and without neonicotinoids were similar (1.10 ± 0.27 and 0.85 ± 0.1 g m^{-2} d^{-1}, respectively) and did not differ. Under these incubation conditions, there was little evidence that the presence of neonicotinoids at a concentration of 100 ng L^{-1} affected denitrification rates in FTW root systems.

Taken together, the mass loss of NO_3–N observed in FTW, detection of key N transforming genes in water column samples, and high denitrifying potential in the roots are consistent with a root-associated denitrifying microbial community responsible for NO_3–N losses in FTWs. Although it is possible that NO_3–N uptake by the FTW roots in the serum bottles was responsible for N loss, root nutrient analysis after the incubation showed a net N decrease (38.1 ± 4.4 and 40.9 ± 3.8% in roots from no neonicotinoid and neonicotinoid vials, respectively) from Day 0 to Day 5. Presumably, microbes utilized the extra N released from damaged/decomposing roots.

4 | CONCLUSIONS AND FUTURE IMPLICATIONS

In this study, neonicotinoids at approximately 100 μg L^{-1} concentration were not observed to significantly alter FTW NO_3–N removal potential in both a greenhouse mesocosm experiment and the potential denitrification microcosm serum bottle experiment. Complete NO_3–N removal was achieved using FTW systems through either plant uptake and/or denitrifying communities associated with the roots of the floating wetland plants. Denitrification potential was not inhibited after the exposure of imidacloprid and thiamethoxam in surface water. Removal of NO_3–N from the water column was unaffected by imidacloprid and thiamethoxam. Further, FTWs were observed to reduce neonicotinoid concentrations in surface waters; however, the formation of desnitro imidacloprid in plant tissue indicates the need for further assessment regarding mammalian ecotoxicity risks. Future studies should also use mRNA to investigate the mechanistic effects of neonicotinoid exposure and other emerging contaminants at the genetic level.

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AUTHOR CONTRIBUTIONS

Julia K. Lindgren: Data curation; Formal analysis; Visualization; Writing – original draft. Tiffany L. Messer: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Validation; Writing – review & editing. Daniel N. Miller: Data curation; Methodology; Resources; Supervision; Validation; Writing – review & editing. Daniel D. Snow: Data

curation; Resources; Validation; Writing – review & editing. Thomas G. Franti: Writing – review & editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Tiffany L. Messer https://orcid.org/0000-0001-7772-9842

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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