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Vadose Zone Processes and Chemical Transport

Soil organic matter can delay—but not eliminate—leaching of neonicotinoid insecticides

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Abstract

Soil organic matter (SOM) retains and attenuates many contaminants; however, its interactions with neonicotinoid insecticides under field conditions remain poorly understood. The goal of this study was to determine if SOM influences the persistence or leaching of two neonicotinoid insecticides: thiamethoxam (TMX) and its transformation-product clothianidin (CLO). Thiamethoxam-coated soybean [*Glycine max* (L.) Merr.] was planted into a clay soil containing different soil organic carbon (SOC) concentrations. Leachate and soil samples were collected for 10 wk after planting and were analyzed for insecticide concentrations using liquid chromatography–tandem mass spectrometry. Single and multiple linear regressions were performed between SOC, leaching volumes, and measured insecticide concentrations, focusing on rainfall events near the beginning, middle, and end of the study. Correlations were also tested between SOC and cumulative mass of leached insecticides. Neither SOC nor per-event leachate volumes explained variability in TMX leaching or residual CLO concentrations in soils; however, by the conclusion of the study residual thiamethoxam concentrations in soil were negatively correlated with cumulative volume of leached water. Initially, the concentration and total mass of leached CLO were significantly and negatively correlated with SOC content; however, this effect faded with time. Leachate dynamics also affected CLO transport, with positive correlations between leachate volume and CLO concentration during the latter events. This analysis demonstrates that SOM can reduce peak loading of neonicotinoids but may not alter cumulative leaching over the entire growing season.

1 | INTRODUCTION

Row crop seeds used in production agriculture typically come with an insecticidal coating and a fungicidal component. Fungicides are meant to control soil-borne fungal pathogens, while insecticides are designed to kill chewing and sucking pests in early stages of plant development (Nettles et al., 2016). Neonicotinoids are the most common class of insecticides used in seed coatings. Since 2011, over 80% of corn (*Zea mays* L.) hectares and approximately 30–40% of soybean [*Glycine max* (L.) Merr.] hectares planted in the United States have used neonicotinoid-treated seeds (Douglas & Tooker, 2015).

Neonicotinoids work by disrupting nicotinic acetylcholine receptors in insects and other macro-invertebrates. Insect

Abbreviations: CLO, clothianidin; HPLC, high-performance liquid chromatography; SOC, soil organic carbon; SOM, soil organic matter; TMX, thiamethoxam; UPLC/MS/MS, ultraperformance liquid chromatography with tandem mass spectroscopy.

exposure to neonicotinoids can cause both lethal (i.e., acute) and sublethal (i.e., chronic) effects, even at relatively low doses. By contrast, neonicotinoids have low binding efficiencies in vertebrates, conferring little to no mammalian toxicity (Simon-Delso et al., 2015). Transformations between neonicotinoid compounds can also alter toxicity to invertebrates. For example, the commonly used thiamethoxam (TMX) has a much lower binding affinity to the nicotinic receptors in insects as compared to other neonicotinoids (Nauen et al., 2003). By transforming into its main metabolite clothianidin (CLO), the insecticide becomes more effective at controlling pests, especially sucking insects such as aphids.

Neonicotinoids have high solubility in water, which allows for continued uptake into plant tissues and provides a consistent source of protection (Elbert et al., 2008). For instance, TMX has a solubility of 4.1 g L^{-1}, making it suitable for systemic applications (Maienfisch et al., 2001), whereas CLO has a lower solubility of ~0.3 g L^{-1} (Kah et al., 2018; Li et al., 2012). The soluble nature of these insecticides also allows them to migrate beyond the intended plants. Less than 20%, and as little as 1–2%, of the neonicotinoids applied as seed coatings is typically taken up by target crops (Alford & Krupke, 2017; Goulson, 2013; Sur & Stork, 2003). Although some of the residual chemicals are rapidly degraded (Baskaran et al., 1999), the remainder can move into the surrounding environment (Gupta et al., 2008). Insecticide-laden runoff can lead to high concentrations of neonicotinoids in waterways shortly after planting due to a "spring flush" (Hladik et al., 2014) caused by high rainfall and low plant uptake rates early in the season. This transport can be exacerbated by fields with artificial draining, like tile drains (Chrétien et al., 2017; Wettstein et al., 2016). As neonicotinoids leach into waterways, they can reduce aquatic insect populations, which can lead to the collapse of fisheries by removing this food source (Yamamuro et al., 2019).

Clay and organic particles can increase the persistence of pesticides in soil (Flury et al., 1995). In particular, organic matter increases retention of many contaminants, such as imidacloprid, even when present in small concentrations (Capri et al., 2001; Sheng et al., 2001). Sorption of neonicotinoids into the soil can increase their residence times (Nettles et al., 2016) and half-lives (Mahapatra et al., 2017). At the same time, sorption to soil organic matter (SOM) can decrease insecticide bioavailability and slow microbial degradation (Rouchaud et al., 1996). Greater SOM concentrations may therefore restrict leaching losses of pesticides but may also permit greater concentrations to persist in the near-surface environment, where they could possibly be mobilized in surface runoff (Ahuja et al., 1981; Radolinski et al., 2019). At the same time, the ability of SOM to retain different pesticides varies depending on the sorption characteristics of the chemicals. For example, TMX and CLO have relatively low affinity to soil organic carbon (SOC), with respective K_{oc} values of 70 ml g^{-1} (Vanderpont et al., 2022) and 84–345 ml g^{-1} (APVMA, 2007). Therefore, SOC may have limited influence on the persistence and transport of these two widely used insecticides, making it important to study these interactions under field conditions.

Core Ideas

- Soil with more organic carbon leached less clothianidin during early-season storms.
- Residual thiamethoxam concentrations were reduced with greater total leaching volume.
- Building soil organic matter may reduce early-season neonicotinoid losses from fields.

The objective of this study was to evaluate if varying SOC concentrations (a) influence the concentrations of two neonicotinoids (TMX and CLO) moving as leachate through soil profiles and (b) alter the persistence of TMX and CLO in soil. We hypothesized that soils with greater C concentrations will have increased concentrations of TMX and CLO persisting in the upper soil profile, along with decreased amounts of these two chemicals lost in leachate. Also, because CLO is produced via degradation of TMX, we hypothesized that leached and residual TMX concentrations would decrease through time, whereas CLO concentrations would increase through time. Understanding these relationships is important for mitigating the amount of neonicotinoid insecticides that exit row-cropped fields.

2 | MATERIALS AND METHODS

2.1 | Experimental procedures

The field site was located at the Virginia Tech Turfgrass Research Center in Blacksburg, VA. The site was previously used for a study by Alvarez-Campos and Evanylo (2019), in which the authors examined differences in SOC after applying different biosolid amendments and planting a variety of summer and fall vegetables. In that study, a 22 m long by 11.5 m wide field was filled with Berks-Groseclose clay soil from a nearby location to a depth of 0.6 m. During construction, the site was graded to a horizontal plane (slope of 0%) to limit surface runoff and subsurface lateral flow. Twenty plots were laid out; each plot was 1.8 m long by 2.5 m wide, and plots were separated by 0.6-m alleys. The treatment plots received biosolid amendments in fall 2016, summer 2017, fall 2017, and summer 2018; at those same times the control plot received urea (46–0–0) applied at 112 kg ha^{-1}. The biosolid treatments used two products obtained from Blue Plains Advanced

Wastewater Treatment Plant (DC Water) in Washington, DC: biosolids (B) at an agronomic nitrogen rate (1×) and five times the agronomic nitrogen rate (5×), and biosolids + mulch at the agronomic nitrogen rate (1×) and five times the agronomic nitrogen rate (5×). Four physical replicates were installed per treatment.

We began the present study in May 2019 by spraying a 2% glyphosate solution to eliminate all vegetation growing in the plots. On 16 June 2019, we planted soybean (CL1561364, Syngenta Corp.) lengthwise across treatment blocks. The row spacing of the plants was ∼0.19 m, and within-row spacing was ∼0.13 m, resulting in a plant density of 407,000 plants ha^{-1} (∼180 plants per plot). Planting depth was 0.03 m. The plants were coated with Clariva Complete and Mertect 340-F seed treatments (Syngenta Corp.) at the labeled rate of 0.50 g TMX kg^{-1} seeds, resulting in ∼75 µg of TMX per seed (assuming 6,500 seeds per kg). Due to excessive weed and rodent pressures, after 6 wk we mechanically terminated the remaining soybean plants using a string trimmer. Weeds were terminated with glyphosate at the same time to eliminate the potential confounding effects presented by different amounts of plant growth between replications.

Rainfall was supplemented with irrigation whenever <10 mm of rainfall fell during a 1-wk period. For each irrigation event, 20 mm of water was applied using three pop-up sprinklers mounted inline on 50 mm (2-in) polyvinyl chloride pipes. The sprinklers were mounted 3 m apart, and all heads turned a full 360 degrees. Using distributed catch cans, we verified that the sprinklers provided an irrigation rate of 10 mm h^{-1} and that the irrigation was evenly applied across the plots. We checked irrigation uniformity bi-weekly throughout the experiment to ensure consistency.

2.2 | Leachate sampling and analysis

We sampled leachate in each plot using zero-tension lysimeters. Lysimeters were constructed from plastic snap end drainage pipe caps (Product no. 0867AA N-12, Advanced Drainage Systems) and were installed in the center of each plot ($n = 1$ per plot). These caps were 0.25 m tall and 0.25 m in diameter and were installed by boring holes (0.3 m diameter, 0.4 m deep) and then placing the cap open-end up on the bottom of the hole. To prevent clogging by fine particles, the lysimeters were filled to 0.13 m with acid-washed well gravel pack (Drillers Service, Inc.). The lysimeters were then back-filled using the original soil at a bulk density close to the initial state. This procedure resulted in the final depth of the soil–gravel interface being ∼0.27 m below the soil surface. The lysimeters had been installed 3 yr prior to this experiment, giving the repacked soil time to settle and develop more natural particle and pore arrangements.

We collected leachate samples following storm events with at least 10 mm of accumulated precipitation, with sampling specifically occurring on the following days after planting: 5, 15, 19, 24, 36, 38, 47, 53, 55, and 67, which corresponded to the period of 21 June to 22 Aug. 2019. During sampling each lysimeter was completely cleared, and the volume of collected water was recorded. We retained 15 ml of the sample and discarded the remainder away from the site. In the laboratory, leachate samples were vortexed (VWR VM-3000 Mini Vortexer) at ∼2,700 rpm for 15 s. Using a syringe, 3 ml of leachate was collected and then pressed through a series of 0.45- and 0.20-µm polytetrafluoroethylene filters (part numbers F2513-3 and F2513-4, Thermo Scientific) into a labeled 2-ml amber high-performance liquid chromatography (HPLC) vial (part numbers 5182-0716 and 5182-0717, Agilent Technologies), capped, and stored at 0 °C if not immediately analyzed on an ultraperformance liquid chromatography with tandem mass spectroscopy (UPLC/MS/MS).

2.3 | Soil sampling and analysis

We also collected soil samples from the experimental plots, selecting random locations along transects that were 0.05 m away from row centers. We used a 25-mm-diameter push probe to collect cores from the surface to the depth of the upper lysimeter surface (0.15 m). We collected three cores from each plot and then homogenized them to form one aggregated soil sample ($n = 1$). Soil samples were taken on the same schedule as water samples for the first six samples and then during every other leachate collection for the remainder of the experiment.

Gravimetric water content, volumetric water content, and bulk density were estimated for each sample by taking a subsample of known volume and drying it at 105 °C for 24 h. In addition, we measured total C on the first (21 June) and last (22 August) sets of soil samples, using a dry combustion unit (VarioMax CNS Analyzer, Elementar Americas Inc.). We quantified total organic C as total C minus 2.3 g kg^{-1}, based on the mean quantity of inorganic C measured in the soil plots by Alvarez-Campos and Evanylo (2019). We note that the assumption of a constant concentration of inorganic C is reasonable because the soils all had the same initial source during construction, and the biosolids did not supply additional carbonates (i.e., treatments with the greatest biosolid additions had similar amounts of inorganic C as the other treatments).

For neonicotinoid insecticide analysis, we placed ∼40 g of wet soil from each sample in a 50-ml centrifuge tube. The exact mass of added soil was recorded (wet mass), and the samples were then placed in a freeze drier for 48 h. At the end of that period, the samples were removed from the freeze drier and weighed immediately to determine dry

mass. We calculated gravimetric water content at time of sampling as difference between wet and dry mass divided by dry mass.

We then ground the soil samples one at a time. First, we poured them from the vial into a large plastic weigh boat and removed any hard material (rocks, sticks, etc.) before adding the remaining material to a coffee grinder (Model No. IDS57RB, Mr. Coffee). We ground the samples for 15 s and then poured the soil into the weigh boat, removing any remaining hard material at that time. The process was repeated until all small aggregates were broken apart. The soil was then poured back into the original tube, capped, and stored in the freezer at 0 ˚C.

For extraction, we weighed 1.0 ± 0.1 g of prepared soil into a 60-ml straight-wall centrifuge tube. The soil was spiked with deuterium-labeled TMX (TMX-d3) and CLO (CLO-d3) stock solution (539 ng g^{-1} TMX-d3 and 518.5 ng g^{-1} CLO-d3) as internal standards and left uncapped to allow the carrier solvent to evaporate. Internal standards were used to correct for variability due to TMX/CLO losses during extraction and cleanup as well as any matrix effect during instrumental analysis. We next added 15 ml of HPLC-grade acetonitrile to the solution with a pipette, and the tube was capped immediately. The tube containing the sample was vortexed for 2 min at 2,300 rpm using a VM-3000 Mini Vortexer (VWR, Inc.). After vortexing, we placed the solution in a centrifuge for 15 min at 5,000 rpm. We then removed 5.0 ml of the solution with a pipette and transferred it to a 12-ml screw-top glass tube without a cap and placed in a vacuum evaporator (Catalog No. 7900002, RapidVap, Labconco Corp.). The vacuum evaporator was run at 75 ˚C, 330 mbar, and 95% speed for 180 min or until a sample was completely dried. After the test tubes were cooled to room temperature, the dried residue in each glass tube was reconstituted using 1.0 ml of UPLC mobile phase (5 mM NH_4Ac in H_2O:5 mM NH_4Ac in MeOH, v/v = 1:9) and vortexed for 10 s. This solution was then extracted with a 1-ml syringe and filtered through a 0.2-µm polytetrafluoroethylene filter into a 2-ml amber HPLC vial with cap before analysis on an UPLC/MS/MS.

2.4 | UPLC/MS/MS analysis

We analyzed the processed leachate and soil extract samples for CLO and TMX and CLO-d3 and TMX-d2 using a UPLC (1290 Infinity, Agilent Technologies) coupled with a tandem mass spectrometer (6490 triple-quadrupole, Agilent Technologies). Additional details, including the mobile phase composition and gradients, the mass spectrometer settings and mass spectral conditions, the calibration and quantification procedures, and quality assurance/quality control, are detailed in Supplemental Tables S1–S3.

2.5 | Statistical analysis

We first performed a repeated measures ANOVA for TMX and CLO concentrations in leachate and soil samples, testing for significant differences between the previously installed biosolid amendment treatments ($n = 4$). No blocking effects were detected during these analyses.

To determine the effects of SOC and leachate volumes on TMX and CLO movement, we further analyzed three sampling dates: 1 July 2019 (Event 2), 24 July 2019 (Event 6), and 22 Aug. 2019 (Event 10). The 1 July sampling event was considered to represent the first part of the growing season and thereby to capture any spring flush-type behaviors. The 24 July sampling event was set near the midpoint of the growing season, and the 22 August sampling event was at the end of the experiment. For each sampling date we compiled TMX and CLO concentrations in leachate and soil samples from each plot. We also calculated the cumulative masses of TMX and CLO that had been leached from the beginning of the experiment by multiplying the measured leachate volume and concentration of either insecticide for each sampling events and then summing those products.

Our regression analyses considered two independent variables (mean SOC and per-event leaching volumes) and four dependent variables (TMX and CLO concentrations in leachate samples, $n = 20$ per sampling event; TMX and CLO concentrations in soil samples, $n = 20$ per sampling event). We performed single (univariate) linear regressions using each predictor variable independently (i.e., SOC and per-event leachate volume) and then performed multi-linear regression using both values. We checked for potential interactions between SOC concentration and leachate volume for each model by quantifying the p value associated with the interaction term, and we tested for covariance between the variables using the variance inflation factor (Thompson et al., 2017).

We also tested for significant correlations between cumulative masses of leached TMX and CLO and SOC using univariate regression models. Because the cumulative mass of leached insecticides included all previous leaching events, we did not test correlations with leachate volumes collected during individual events. We instead examined correlations between cumulative volume of leached water and cumulative mass of leached TMX and CLO but did not perform statistical analyses on these relationships because leachate volumes and masses were related to one another (i.e., mass leached was calculated based on leachate concentration multiplied by leachate volume).

Because CLO is a metabolite of TMX, we also analyzed the relationships between the two insecticides as well as the combined transport of both chemicals. We first tested correlations between TMX and CLO in leachate concentrations,

soil-bound concentrations, and cumulative leached mass for each of the three studied events. We next calculated the combined concentrations of TMX and CLO on a molar basis using their molecular weights (TMX = 291.7 g mol^{-1}; CLO = 249.7 g mol^{-1}). We assessed the cumulative number of moles of both compounds that had leached by each sampling event using the same molecular weights to convert between mass and moles. Finally, we performed the same repeated-measures ANOVA and univariate and multiple linear regression analyses on the combined TMX + CLO data.

Statistical analyses were performed using R (version 4.1.2) via RStudio (version 2022.02.0-443). We used $\alpha = .1$ to determine significance for all tests and reported exact p values and standard errors of predictor variables.

3 | RESULTS

3.1 | Weather

Air temperature exceeded 32 ˚C on several days, with an overall mean of 22.7 ˚C (Supplemental Figure S1). A total of 318 mm of precipitation fell at the soil surface, of which 199 mm came from irrigation (Supplemental Figure S2).

3.2 | Soil C concentrations

Soil organic C values did not differ between the two sampling dates (t test; $p = .46$), with June samples typically having slightly greater numerical values compared with those collected in August (Supplemental Figures S3 and S4). When the concentrations from the two sampling dates were averaged for each plot, the control and B-1× treatments had significantly lower concentrations than the B-5× and B+M-5× soils (Supplemental Figure S5). In addition, the B+M-1× treatment had a significantly greater mean C concentration than the control ($p = .068$).

3.3 | Leachate samples

The cumulative volume of leachate collected from the lysimeters through all sample dates was 6,407 ± 3,213 ml (mean ± SD). There were no significant differences in the cumulative volume leached between any treatments ($p \geq .1$) (Supplemental Table S4).

3.4 | Repeated-measures ANOVA

The repeated-measures ANOVA indicated that soil-bound TMX concentrations were not different between treatments ($p = .12$) (Supplemental Figure S6), whereas the CLO concentrations measured in soil had a treatment effect ($p = .042$) (Supplemental Figure S7). The combined soil-bound concentrations of TMX+CLO had no treatment effects ($p = .40$) (Supplemental Figure S8). Leachate concentrations had similar results, with no significant effect for TMX concentrations ($p = .19$) (Supplemental Figure S9) but with a potential treatment effect for CLO concentrations ($p = .093$) (Supplemental Figure S10). The two insecticides had opposite trends through time, with TMX showing a decrease in median concentrations through time and CLO having an increase in median concentrations. The combined concentration of TMX+CLO in leachate had a similar trend as TMX (Supplemental Figure S11), with no significant differences between treatments through time ($p = .19$).

3.5 | Regression analyses

All significant correlations ($p < .1$) from the single- and multi-linear regression models are presented in Supplemental Table S5. The multi-linear regression models gave similar results as the univariate regression models, and the interaction term between SOC and leachate volume was never significant. Therefore, all correlations presented in the results come from the single linear regression models.

3.6 | Neonicotinoid retention and transport

Residual TMX concentrations in soil ranged from 0 to 16 μg kg^{-1}. The TMX concentrations had similar distributions for Events 2 and 10 and a smaller range and median concentration in Event 6 (Figure 1a). Soil TMX concentrations were negatively correlated with cumulative leachate volume for Events 6 and 10 (Figure 1b); the correlation was significant for Event 10 ($r = -.44$; $p = .054$). Neither SOC content (Supplemental Figure S12) nor leaching volume (Supplemental Figure S13) had significant correlations with soil TMX concentration.

Soil CLO concentrations tended to be small, with only three profiles having non-zero values in Event 2 and only four profiles having non-zero values in Event 6 (Supplemental Figure S14). Approximately half of the soil profiles had measurable CLO by Event 10, but the concentrations were not associated with any of the measured predictor variables (e.g., SOC). The combined TMX+CLO measured in the soil samples showed similar trends as the TMX (Supplemental Figures S15 and S16), with the strongest correlation detected with SOC during Event 6 ($r = -.34$) (Supplemental Figure S15b).

Leachate TMX concentrations were negatively but not significantly correlated with SOC concentrations for the early-season sampling event (Event 2) (Figure 2a). The two variables had a negligible correlation for the mid-season sample (Event 6) (Figure 2b) and a significant positive correlation

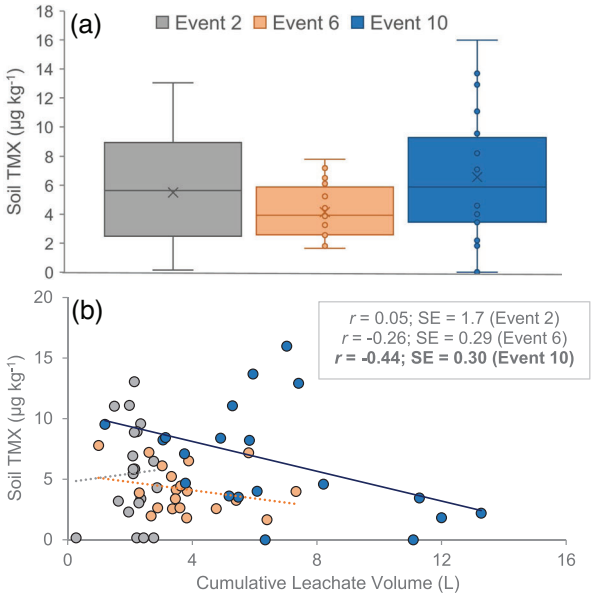

FIGURE 1 Concentration of thiamethoxam (TMX) measured in soil samples collected during Event 2 (1 July 2019), Event 6 (24 July 2019), and Event 10 (22 Aug. 2019). (a) Distributions. (b) Correlations with cumulative leachate. Bold text and solid regression lines indicate significant correlations ($p < .1$)

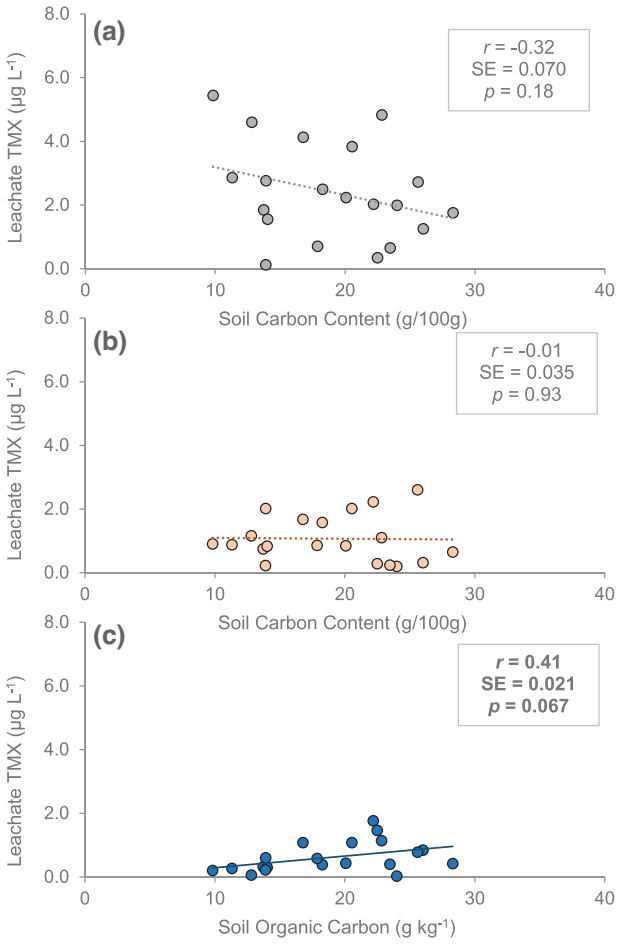

FIGURE 2 Leachate concentrations of thiamethoxam (TMX) versus soil organic carbon (SOC) concentrations for leachate samples collected during (a) Event 2 (1 July 2019), (b) Event 6 (24 July 2019), and (c) Event 10 (22 Aug. 2019). Bold text and solid regression lines indicate significant correlations ($p < .1$)

for the final set of samples (Event 10) (Figure 2c). Maximum TMX concentrations decreased through time from 5.4 µg L^{-1} in Event 2 to 1.8 µg L^{-1} in Event 10. No significant correlations existed between TMX leachate concentration and leachate volume (Supplemental Figure S17).

Leachate CLO concentrations were significantly ($p < .1$) and negatively correlated with SOC concentrations for Event 2 (Figure 3a) and Event 6 (Figure 3b) but not the final sampling event (Event 10) (Figure 3c). The Pearson coefficients indicated that correlations between the two variables decreased through time, with $r = -.50$ in Event 2, $r = -.42$ in Event 6, and $r = -.17$ in Event 10. Unlike TMX, the CLO concentrations increased by a factor of five between Event 2 (maximum of 0.12 µg L^{-1}) and Event 10 (maximum of 0.60 µg L^{-1}). This increase was likely caused by the metabolism of TMX to CLO occurring throughout the growing season.

Leachate CLO concentrations were also correlated with leachate volumes collected during each sampling event. For Event 2, the correlation was negative but not significant ($p = .13$) (Figure 4a), indicating that a dilution effect may have occurred. That event was also associated with lower leaching volumes and relatively small CLO concentrations, which may have limited the relationship between the two variables. The correlations were positive and significant for the other selected events: $r = .48$ and $p = .033$ for Event 6 (Figure 4b) and $r = .61$ and $p = .0046$ for Event 10 (Figure 4c).

The relative increase of CLO through time also influenced the results for the regression performed with the combined TMX and CLO concentrations (Supplemental Figures S18 and S19). For Events 2 and 6, the combined regression had very similar correlations as those of only TMX (Figure 2; Supplemental Figure S17). By Event 10, the combined leached concentrations were significantly correlated with leachate volume ($p = .028$) (Supplemental Figure S19c), likely due to the strong correlation that was detected between leachate CLO and leachate volume (Figure 4c).

We also calculated the cumulative mass of leached TMX and CLO through time, based on the product of leachate insecticide concentrations and leachate volumes. Both insecticides showed an inverse correlation with SOC content (Figure 5), with r coefficients ranging from −.17 (TMX in Event 2) to −.50 (CLO in Event 10). A relatively high mass of TMX was mobilized in the first two events (i.e., the Event 2 data), and in many profiles >50% of the total mass loss occurred by Event 2. This result was likely due to the greater leachate

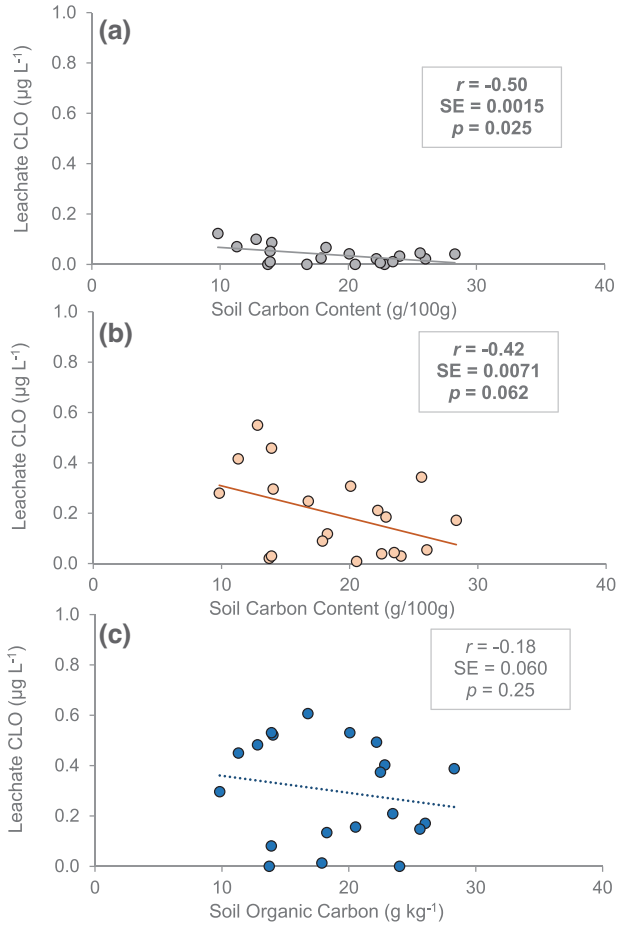

FIGURE 3 Clothianidin (CLO) concentrations in leachate versus soil organic carbon (SOC) concentrations for samples collected during (a) Event 2 (1 July 2019), (b) Event 6 (24 July 2019), and (c) Event 10 (22 Aug. 2019). Bold text and solid regression lines indicate significant correlations ($p < .1$)

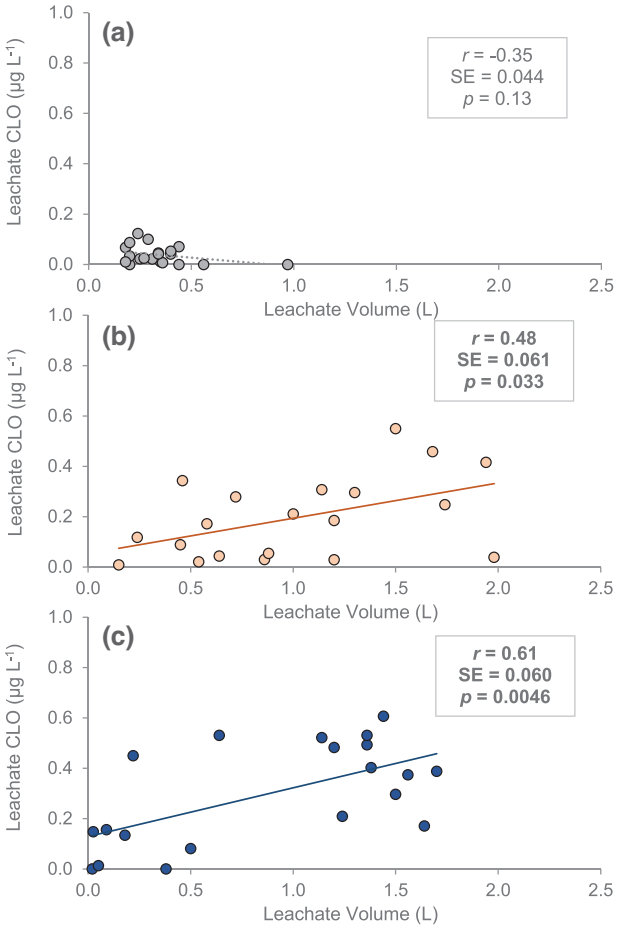

FIGURE 4 Clothianidin (CLO) concentrations versus water volume for leachate samples collected during (a) Event 2 (1 July 2019), (b) Event 6 (24 July 2019), and (c) Event 10 (22 Aug 2019). Bold text and solid regression lines indicate significant correlations ($p < .1$)

concentrations measured early in the experiment (Figure 2a). Total mass of leached TMX was similar between Events 2 and 6 and between Events 6 and 10, as evidenced by the similar spacing in Figure 5a between the Event 2 and Event 6 versus the Event 6 and Event 10 regression lines. The CLO data, by comparison, showed very little mass leached by Event 2, with a substantial increase in leached mass by Event 6 and then an even greater increase by Event 10 (Figure 5b).

The cumulative mass leached of each insecticide was also strongly coupled to the cumulative amount of leaching (Supplemental Figures S20 and S21), with $r = .71$ for TMX and $r = .84$ for CLO in the final sampling event. The same trend was detected for the combined cumulative molar losses of TMX+CLO (Supplemental Figure S22). These relationships were expected because leachate volumes were used to calculate mass losses, but they also emphasize that leaching volume is an important factor determining insecticide loads. The comparisons between TMX and CLO concentrations and total leached masses likewise indicated the relative increase in CLO versus TMX through time (Supplemental Figures S23–S25). By the final sampling event, the correlations between the two insecticides had become stronger both in terms of soil-bound concentrations ($r = .58$) (Supplemental Figure S24) and mass losses ($r = .79$) (Supplemental Figure S25).

4 | DISCUSSION

The main objectives of this study were to evaluate if SOC concentration (a) influences the ability of TMX and CLO to leach through soil profiles or (b) alters the persistence TMX and CLO in the soil. Our study was conducted under realistic field conditions and thereby avoided limitations associated with commonly used laboratory or column studies. The post-planting concentrations of TMX detected in our soil were similar to those measured in production corn fields in Canada (Schaafsma et al., 2015), confirming realistic TMX loading in our experiment. We also focused our analysis on

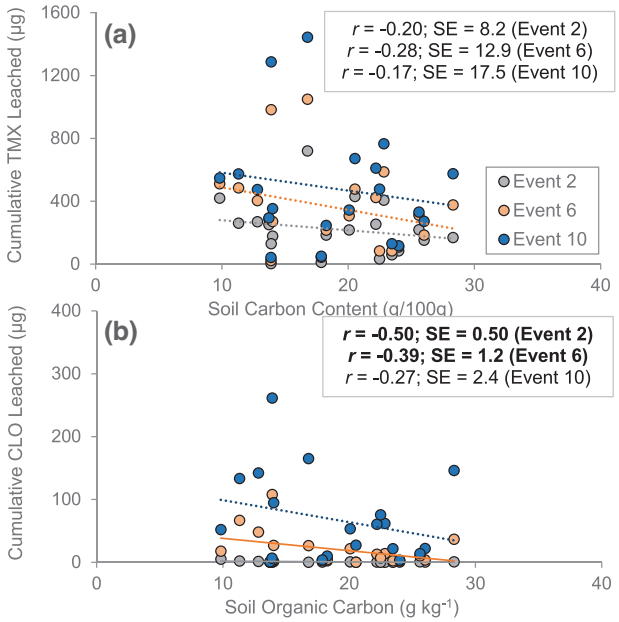

FIGURE 5 Cumulative masses of (a) leached thiamethoxam (TMX) and (b) leached clothianidin (CLO) versus soil organic carbon (SOC) concentrations based on leachate samples collected through Event 2 (1 July 2019), Event 6 (24 July 2019), and Event 10 (22 Aug. 2019). Bold text and solid regression lines indicate significant correlations ($p < .1$). Note that the y-axes have different scales between panels

vertical leaching, which is an important mechanism by which contaminants such as insecticides and antibiotics become transported from crop fields (Flury, 1996; Radolinski et al., 2022; Willkommen et al., 2021). Leaching is particularly important in low-relief topographies and landscapes with drain tiles (Blann et al., 2009; Dolliver & Gupta, 2008) and can also drive lateral subsurface flow in sloping fields (e.g., Radolinski et al., 2019). Therefore, these results should be relevant to many common agricultural settings.

We hypothesized that soils with greater C contents would leach less insecticides (i.e., the study plots with the highest SOC contents would have lower concentrations and less cumulative mass of transported TMX and CLO). The experimental results provided partial support for this hypothesis because SOC concentration was negatively correlated with CLO concentration in leachate (Figure 3) and with the cumulative amount of CLO leached through the soil profile (Figure 5b). Nonetheless, the effect of SOC appeared to diminish throughout the growing season. By the final sampling event, the amount of leached water became the most important factor affecting CLO concentrations in leachate. At the same time, the TMX leaching dynamics were relatively complex. Although the hypothesized relationship existed for Event 2 (i.e., lower TMX concentrations were measured in leachate from soils with greater C content), the correlation was not significant. Moreover, the correlation had changed to become positive (and significant) by the final event. This contrast may be indicative of more consistent leaching that occurred in the lower-C soils, as evidenced by the negative (though not significant) correlations that were observed between cumulative mass loss of TMX and SOC concentration (Figure 5a). In other words, TMX may have leached more rapidly in plots with lower SOC, leaving less available for transport during subsequent precipitation events.

We also hypothesized that soils with greater C concentrations would have increased concentrations of TMX and CLO persisting in the upper soil profile. However, the experimental results did not support this hypothesis. Rather, the first two selected sampling events showed a negative relationship between soil TMX concentrations and SOC (Supplemental Figure S12a,b), and the final sampling event had similar concentrations of retained TMX across the study plots, despite a nearly 3 times difference in SOC concentrations (Supplemental Figure S12c). These findings contrast with results of previous studies showing that soils with relatively high C content had greater persistence of TMX and CLO (Mörtl et al., 2016) as well as other insecticides (Pérez-Lucas et al., 2021; Worrall et al., 2001). Other work has found positive correlations between SOC and the sorption coefficients (K_d) for both TMX and CLO (Dankyi et al., 2018). Larger K_d values should favor retention in the soil solid phase, a trend we did not observe. One possibilitiy for this discrepancy lies in the chemical structure of the insecticides, which both have a nitro functional group ($-NO_2$) with strong electron withdrawing capacity (Supplemental Figure S26). This group allows the chemicals to become electrostatically attracted to electron-donating functional groups on SOM but also limits interactions with nonpolar surface functional groups that may have dominated the organic matter in the studied soils. The comparison between the different biosolid amendment treatments showed that the type of organic matter had limited effects on TMX concentrations in soil ($p = .12$) (Supplemental Figure S6) and leachate ($p = .19$) (Supplemental Figure S9) and versus more substantial effects on CLO concentrations in soil ($p = .042$) (Supplemental Figure S7) and leachate ($p = .093$) (Supplemental Figure S10). These differences may reflect the greater solubility of TMX (~4.1 g L^{-1}) (Maienfisch et al., 2001) versus CLO (~0.3 g L^{-1}) (Kah et al., 2018; Li et al., 2012).

Even though TMX and CLO did not appear to form strong bonds with SOM, temporary sorption may have helped facilitate microbial degradation of the neonicotinoid insecticides. We detected a significant and negative correlation between soil TMX concentrations and the cumulative volume of water leached through the profile at the final soil sampling event (Figure 1b). Further, SOM may affect the rate at which TMX becomes transformed to CLO, which is an important factor affecting the concentrations of both chemicals (Karmakar et al., 2009). Although the kinetics of this reaction

and its underlying mechanics are not well understood, it is possible that the previous additions of organic amendments may have increased soil microbial biomass, as noted in other research (Casida, 2011; Stark et al., 2007). The additional presence or diversity of microorganisms could have caused TMX to degrade into other daughter compounds (e.g., urea, nitrosoguanidine, and desnitro compound) rather than CLO (Rana et al., 2015). Likewise, the absence of CLO in much of the soil samples, particularly those collected early on, may reflect the rapid conversion of CLO to other compounds that were not identified in our analysis. A mass balance performed on each soil plot showed that we recovered an average of 25–50% of the applied TMX (Supplemental Figure S27), indicating that the majority of the neonicotinoids applied in the seed coatings may have been transformed into other compounds.

In terms of timing, SOC appeared to have the most pronounced influence on insecticide transport the early-season sampling events, which may be most analogous to the "spring flush" often observed in the literature (Hladik et al., 2014; Thurman et al., 1992). This period is particularly critical because seed-coating chemicals are typically present in relatively high concentrations (Frame et al., 2021) and soil water content is often elevated (Findell & Eltahir, 1997). We note that the "spring flush" phenomenon is not exclusive to the season in which coated seeds are planted because TMX and CLO have both been detected in runoff and tile drain water in the second year after planting TMX-coated seeds (Chrétien et al., 2017). Because CLO is more toxic to invertebrates than TMX (Nauen et al., 2003), the ability of SOM to reduce CLO leaching early in the cash crop growing season is likely an important benefit to surrounding ecosystems. At the same time, our results showed that >50% of the total TMX mass loss occurred during the first two precipitation events. Even though the relationships were not significant, we observed negative correlations between TMX concentrations in the early-season leachate and SOC as well as consistent negative correlations between TMX mass loss and SOC. Building up SOM may therefore be a useful tool for decrease TMX loading to the environment during the early growing season. This process could be considered a co-benefit from ongoing efforts to increase SOM to sequester atmospheric C (Srivastava et al., 2012) and build better soil health (Jian, Du, et al., 2020), for example by amending soils with biosolids and other composts (Alvarez-Campos & Evanylo, 2019; Singh & Agrawal, 2008), implementing conservation tillage (Haddaway et al., 2017), and cover cropping (Stewart et al., 2018). These practices can also enhance farm profitability (LaCanne & Lundgren, 2018), giving another potential benefit for building SOM in traditional corn and soybean production systems.

The data collected later in the growing season can also inform management practices. Even though the total amount of TMX and CLO leached by the growing season end was not significantly different between study plots, neonicotinoid insecticide leaching became strongly correlated with the total amount of water that leached. Farming practices that limit water leaching out of the root zone, such as use of over-winter cover crops (Jian, Lester, et al., 2020), may be particularly effective at preventing neonicotinoid insecticide losses via this pathway. Our late-season samples also confirmed that the CLO concentrations increased during the growing season and reached their highest values during the final sampling event, supporting our hypothesis. This behavior was likely due to conversion of TMX to CLO and further implies that CLO leaching may have continued for some time beyond the study period. Other investigations have found that TMX and CLO can persist in detectable concentrations long after the cash crop has been harvested. In an example from eastern England, springtime soil sampling revealed CLO concentrations of up to 10.3 μg kg^{-1} more than 1 yr after planting CLO- or TMX-coated cereal seeds (Jones et al., 2014). Similarly, wetland water samples collected between snowmelt and spring planting in the Canadian prairie had detectable TMX in 43% of samples and CLO in 96% of samples (Main et al., 2016). The insecticides were found in several wetlands adjacent to fields where neonicotinoids had not been applied in the year prior, indicating both the long-term persistence of these chemicals and their ability to become mobilized through time.

Even though our study focused on a single growing season, the results provide evidence that interactions with SOC may be most effective for reducing the rate of TMX and CLO transport, rather than total loads. This behavior fits the conceptual framework that solute-solid phase interactions delay, but do not fully prevent, chemical transport (Cameron & Klute, 1977; Radolinski et al., 2022). Here we note that our lysimeters were installed at relatively shallow depths (with the collection surfaces ~0.27 m below the soil surface), so the effective transport distance was much less than the length of a typical rooting zone. Additional study may be needed to determine if the delays in leaching associated with greater soil C content would translate to longer-lasting reductions in mass transport through the entire soil profile.

5 | CONCLUSIONS

The goal of this study was to examine the relationship between SOC concentrations and leaching versus retention of TMX and CLO under field conditions. Using TMX-coated soybean, we found that greater concentrations of SOC were associated with reduced CLO concentrations in leachate collected early in the season, corresponding to a spring flush. A similar, but not significant, trend was observed for TMX. Early-season precipitation events are often the most problematic in terms of environmental contamination, and in our study >50% of the total TMX mass losses via leaching occurred in the first two

rainfall events. By the end of the experiment, the volume of water leached through the profile became a primary control on the amount and concentrations of leached insecticides. Therefore, strategies that both build soil C and reduce subsurface drainage may provide outsized environmental benefits.

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AUTHOR CONTRIBUTIONS

Benjamin A. Morrison: Formal analysis; Investigation; Methodology; Writing – original draft. Kang Xia: Conceptualization; Funding acquisition; Methodology; Project administration; Resources; Supervision; Validation; Writing – review & editing. Ryan D. Stewart: Conceptualization; Data curation; Funding acquisition; Methodology.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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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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