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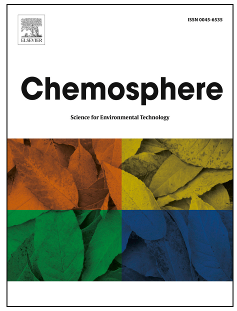

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Plants mediate precipitation-driven transport of a neonicotinoid pesticide

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Notes

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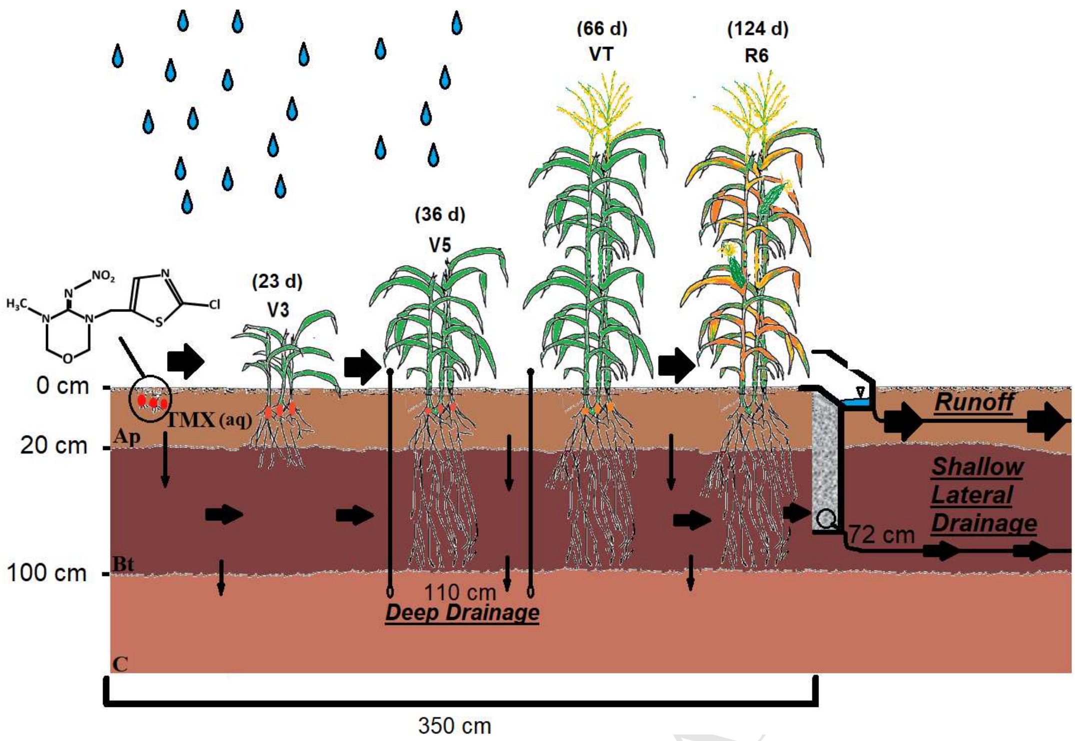
Abstract

Neonicotinoid insecticides provide crop protection via water solubility and systemicity, yet these chemical characteristics, combined with high toxicity to non-target invertebrates (e.g., honeybees), elicit concern of environmental transport. Neonicotinoids have been detected in soil and surface water throughout North America; however, no investigation has defined a direct connection to planted seed dressings. We quantified the physical transport of thiamethoxam (TMX), a neonicotinoid, under field conditions. We planted TMX-coated corn seeds and maintained plots with and without viable crops (n = 3 plots per treatment) to determine plant influence on pesticide transport. TMX concentrations were measured in soil and drainage throughout the growing season. Storm-generated runoff was the dominant transport mechanism (maximum TMX concentration 1.72 ± 0.605 μg L^{-1}; no viable plants), followed by shallow (<72 cm) lateral drainage (0.570 ± 0.170 μg L^{-1}; no viable plants), and deep (110 cm) drainage (0.170 ± 0.265 μg L^{-1}; viable plants). Soil samples confirmed vertical and lateral movement within 23 and 36 days of planting, respectively. Plants facilitated downward migration of TMX in soil but restricted TMX drainage. Altogether, these study results revealed that neonicotinoids can be transported from seed coatings both above and through the soil profile, which may enable migration into surrounding ecosystems.

Keywords

Insecticide, preferential flow, evapotranspiration, seed-coated neonicotinoid

Graphical Abstract



Note: The sizes of flux arrows reflect relative differences in drainage losses of TMX as detailed in the manuscript.

1. Introduction

Neonicotinoids have become the most extensively used class of insecticide worldwide as a result of a few novel chemical characteristics. These compounds exhibit low toxicity to mammals,[1] have a high selectivity for the invertebrate nicotinic acetylcholine receptor (*n*AChR),[2] and are water soluble (0.19-840 g L^{-1}),[2, 3] making them a top choice for systemic crop protection. Seed companies have resorted to coating the seeds of 140+ crops worldwide under the assumption that these compounds will be mostly taken up into the plant tissue and arm the crop throughout the growing season,[4] as opposed to conventional broadcast or foliar applications that present a higher risk of direct environmental exposure.[5] Nevertheless, high *n*AChR selectivity makes neonicotinoids lethal in sub-ppm levels to non-target organisms such as honeybees[6-8] and aquatic invertebrates.[9] Neonicotinoids have also been linked to increased mortality of insectivorous birds.[10] Additionally, their water solubility and low affinity for the soil matrix[3, 11] elicit concern of transport to aquatic environments. Seed coatings now account for more than 60% of global neonicotinoid application;[12] however, research has shown that a maximum of 20% of the applied dose is recovered in plant tissue[13] with the remainder left in the soil environment.

Previous laboratory and greenhouse studies have suggested that plant growth and development may influence neonicotinoid mobility in the soil. For example, as soil water content decreases during the growing season, corn (*Zea mays* L.) can apply suction forces of over 9.5 bars,[14] which may limit solute mobilization. Thus, plant-mediated evapotranspiration may retard downward migration of neonicotinoids, resulting in more evapo-concentration of the compounds near the root zone.[15] Researchers have also noted that neonicotinoids and other highly soluble pesticides develop a stronger affinity for the soil matrix with time, such that the sorption coefficient K_d increases. This process may reflect rate-limited migration into more protected sorption sites such as intra-aggregate micropores, thereby limiting transport and increasing retention time.[16-19] Still, non-equilibrium flow conditions resulting from high rainfall events may also drive downward advection or bulk flow of neonicotinoids and other solutes through newly formed root channels.[15, 20]

As a possible result of these advective pathways, the three most abundant neonicotinoids, imidicloprid (IMD), thiamethoxam (TMX), and clothianidin (CLO), are now detected in soil,[21] surface water bodies,[22-24] groundwater,[25] and drinking water.[26] Starner and Goh [27] detected IMD in 67 of 75 (89%) surface water samples collected in southern California, 14 of which exceeded the US Environmental Protection Agency's (EPA) chronic invertebrate aquatic life benchmark of

1.05 µg L^{-1}. Other studies in Ontario, Canada[28] and Iowa, USA,[29] detected neonicotinoid contamination in 100% of surface water bodies, likely related to the soybean and corn production of those regions. Both analyses showed that spring snowmelt[28] and large rainfall events[29] early in the growing season increased pesticide concentration in surface waters, with neonicotinoid-treated seeds identified as the most likely source. Neonicotinoid transport has been proven via dust drift associated with seed coatings and planting equipment,[30-32] although this contamination was exclusive to the time of planting[33] and did not account for the majority of pesticide introduced into soil with planted seeds.

Laboratory and greenhouse studies have identified subsurface leaching as a potential mechanism for neonicotinoid movement from seed coatings. For instance, a recent laboratory experiment concluded that 24 h of inundation may drive up to 95% of seed-applied neonicotinoid mass into solution[34], while a different greenhouse study determined that seed-coated neonicotinoids could mobilize in soil under environmentally relevant conditions.[15] Still, transport in the field remains less certain. Three recently published field-scale experiments examined neonicotinoid transport from seed coats,[35-37] yet those studies did not quantify pesticide concentrations relative to background residues in soil, thus failing to isolate any direct effects from seed coatings. The studies also lacked sufficient hydrological data to identify and model soil and environmental controls on neonicotinoid transport. Thus, there exists a critical need to identify the mechanisms by which seed-coating pesticides mobilize through agricultural fields.

The purpose of this study was to quantify the transport of the common neonicotinoid thiamethoxam from commercially available TMX-coated corn seeds in a field setting and to identify the influence of viable plants throughout the growing season. We hypothesized that: i) TMX would become transported via multiple pathways, including surface runoff, shallow lateral drainage, and leaching below the corn root zone; ii) vertical and lateral transport of TMX would be detected in soil; and iii) higher concentrations of TMX would be detected in deep drainage for plots containing viable plants (compared to plots controlled for plant growth) due to preferential, vertical transport along newly formed root channels. By assessing neonicotinoid transport from planted seed coatings in the field, this study aims to provide the first direct connection between this widespread pesticide application method and potential environmental contamination.

2. Materials and Methods

2.1. Site Description and Soil Characterization

The field experiment was conducted at the Virginia Tech Urban Horticulture Center in Montgomery County, Virginia, on a 5% southeast facing slope. The soil was a Groseclose loam series (*Typic Hapludult*). The site had been in pasture for 10 years prior to the experiment, so the ground was tilled to a depth of 30 cm in spring and summer of 2015. The soil contained no background TMX, as determined using an LC-MS/MS analyzer (additional details on TMX analysis in soil, plant and water samples are presented in the Supporting Information).

Intact cores (5 x 5 cm) were taken from three depths representing distinct soil horizons: A_p (0-5 cm), B_t (25-30 cm), and C (105-110 cm). Cores were used to determine soil bulk density [M L^{-3}], porosity [L^3 L^{-3}], saturated hydraulic conductivity (K_s) [L T^{-1}], and water retention (n = 6 cores per horizon). K_s was measured using the falling head method with a UMS KSAT Benchtop Saturated Hydraulic Conductivity Instrument (UMS Inc., Munich, Germany). Unconsolidated soil samples were collected (n = 6 per horizon), air dried, sieved to 2 mm, and analyzed for cation exchange capacity (CEC), pH, total organic carbon (TOC), and texture. CEC was measured colorimetrically via ammonium acetate at pH 7 using a Lachat Quickchem 8500 autoanalyzer (Lachat, Loveland, USA), soil pH was measured in a 1:1 slurry (soil: $CaCl_2$), TOC was quantified by dry combustion using a Vario MAX CNS macro elemental analyzer (Elementar, Hanau, Germany), and textural analysis was conducted via the pipet method.[38] Soil physiochemical and hydraulic properties are shown in **Table 1**.

2.2. Field Plot Preparation and Experimental Design

Six runoff plots (300 cm x 350 cm) were constructed using sheet metal (30 cm tall, installed to approximately 15 cm depth) as borders to contain overland flow (see **Figure S1**). On the downslope side of each plot, a trench was excavated (10 cm wide x 300 cm long x 72 cm deep), lined with 10 cm ID perforated PVC pipe, and filled with coarse gravel to collect shallow lateral flow. A second PVC trough (13 cm wide x 300 cm long x 10 cm deep) was installed on the surface to collect surface runoff. Runoff and shallow lateral flow drainage were piped to separate, unlined 200 L steel barrels that were fitted with pressure transducers (HOBO U-20 L level logger; Onset, Bourne, USA) to determine drainage volume. Two suction lysimeters (1 bar, ceramic cups) were installed at random locations within each plot to a depth of 110 cm to collect TMX in gravity-driven deep drainage below the root zone. Tensiometers (Spectrum Technologies, Aurora, USA) and soil water content probes (Decagon Devices 5TM, Pullman, WA) were installed at random locations within each plot (30 cm and 110 cm depths; 1 of each probe per depth per plot) to measure soil matric potential [$M\ L^{-1}\ T^{-2}$] and soil water content [$L^3\ L^{-3}$]. A flow-through rain gauge (Spectrum Technologies, Aurora, USA) was used to record rainfall [L]. All loggers recorded at 15 min intervals. Daily potential evapotranspiration (ET_o; [L]) was determined using the FAO ET_o calculator.[39]

Prior to planting, agronomic rates of lime and fertilizer were applied. Cruiser Extreme® 1250 corn seeds (Syngenta; Greensboro, USA) were sowed to a depth of 4 cm within each plot as six 50 cm wide rows at a spacing of 33 cm, approximating a planting rate of 59,000 plants ha^{-1}, as recommended for the state of Virginia.[40, 41] Though 80 cm row spacing is sometimes recommended for increased corn yield, the 50 cm width chosen in this study has been shown to reduce yield by as little as 3%,[42] while better accommodating our 300 x 350 cm plots. Each seed carried 1.21 ± 0.04 mg of TMX in its seed coating (from n = 4 tested seeds) based on LC-MS/MS analysis. To understand the role of viable plants on the transport process, half of the plots were controlled for growth by snipping the plant upon emergence ("no viable plant" treatment; n = 3), while the remaining plots sustained viable corn plants ("viable plant" treatment; n = 3) throughout the 124-day growing season (June 6th – October 4th, 2016). The experiment followed a complete randomized design with one factor (plant presence) and two treatment levels (viable and no viable plants), whereby treatments were assigned randomly to each of the 6 plots.

2.3. Water Sampling

Drainage water samples from the three measured hydrologic compartments (runoff, shallow lateral flow, and deep drainage) were collected following nine rain events throughout the growing season (see **Table S1**). Runoff and shallow lateral drainage samples were retrieved from storage barrels and deep drainage was extracted from lysimeters by applying 60 kPa of suction for 10 minutes. It should be noted that the final rainfall event produced drainage volumes that exceeded the capacity of our storage barrels (>117 L); therefore, the final drainage volumes were estimated as the steady state water level at the time of sampling. Following retrieval from the field, all drainage samples were analyzed for TMX using method described in the Supplemental

Information with limit of quantitation (*LOQ*), limit of detection (*LOD*), and recoveries of 0.01 µg kg^{-1}, 0.005 µg kg^{-1}, and 104.2 ± 5.3%, respectively. Additionally, as a simple means of representing the effect of viable plants on TMX transport via drainage, we calculated a simple response ratio (R_r) as:

$$R_r = \frac{[TMX_{plant}]}{[TMX_{no\ plant}]} \quad (1)$$

where TMX_{plant} and $TMX_{no\ plant}$ represent concentrations of TMX in shallow lateral drainage for plots containing viable and no viable plants, respectively. We present these data as the natural logarithm of R_r ($\ln R_r$) such that more negative values of $\ln R_r$ correspond to more plant restriction of TMX transport.

2.4. Soil and Plant Sampling

TMX distribution in soil was measured at four corn growth stages (V3, V5, VT, and R6, corresponding to 23, 36, 66, and 124 days, respectively). Soil sampling periods were chosen to assess subsurface mobility of TMX during early (V3), middle (V5), and peak (VT) vegetative growth stages and one final point for physiological maturity (i.e., R6, which represents the end of the corn growing season).[43] At each of these four times, replicated 2 cm diameter by 5 cm tall soil samples were collected from the A_p (0-5 cm) and B_t (25-30 cm) horizons, with samples collected within the corn seed planting row (± 2 cm from row center) and in between corn rows (25 cm from planting rows; see **Figure S2** for a spatial description of soil sampling). Three samples were composited together into 1 sample per location (in-row versus between row) within each plot, which was then analyzed for TMX using the **2.5. Statistical Analysis**

One-way ANOVAs were used to compare per-event TMX concentrations [M L^{-3}] and final cumulative TMX mass [M], and drainage volumes [L^3] between "with viable plants" vs "no viable plants" treatments for each hydrologic compartment (surface runoff, , shallow lateral drainage ≤ 72 cm, deep drainage at 110 cm). For soil concentrations [M M^{-1}], data were subjected to two-way ANOVAs per combination of location (next to plant vs between rows) and horizon ($A_{p,}$, 0-5 cm vs B_t, 25-30 cm) using plant influence and corn stage (V3, V5, VT and R6) as factors. All data were rank transformed and analyzed for normality and homogeneity of variances using Fligner's test. Factorial ANOVA results were subjected to multiple comparisons via Tukey HSD. R version 3.2.2 was used to conduct all statistical analyses with $\alpha = 0.05$.

3. Results and Discussion

Because our previous greenhouse investigations[15, 44] and the current study have shown that TMX uptake into corn plants represented a minor fraction of the applied seed dose (< 0.1%), the distribution of TMX in the drainage and soil is the focus of the current report. Thiamethoxam was detected in all three drainage compartments (surface runoff, shallow lateral drainage ≤ 72 cm, and deep drainage at 110 cm) as early as 10 days after planting and throughout the corn growing season (**Figure 1**). Though concentrations of TMX detected in drainage generally reflected each compartment's proximity to the seed source (runoff > shallow lateral drainage > deep drainage; **Figure 1**), TMX was transported in similar concentrations via shallow lateral drainage (no viable plants, 0.020 ± 0.0264 µg L^{-1}; **Figure 1b**) and deep drainage (no viable plants, 0.022 ± 0.017 µg L^{-1}; **Figure 1c**) following the first rain event. The total percent of seed-coated TMX quantified in drainage for viable plants (0.27 ± 0.02 %; **Table S1**) are comparable to estimates of total neonicotinoid losses to tile drains in sugar beet fields (same order of magnitude as IMD).[36] TMX concentrations in surface runoff and shallow lateral drainage from viable plant plots reached seasonal peaks by the V5 corn stage (~4 weeks), whereas plots with no viable plants showed more delayed and higher peak concentrations. Plants also affected late-summer concentrations in deep drainage, when TMX was only detected in the no-viable plant plots.

The cumulative mass transported via runoff and shallow lateral flow generally increased in response to storm-generated drainage (**Figure 2**), though drainage losses were more reduced following peak concentrations and less apparent for plots containing viable plants. Altogether, early detection of TMX in runoff, shallow lateral drainage, and analogous vertical migration to a depth of 110 cm indicate that the compound has a high potential for advective transport. Further, TMX detection through 113 days of plant growth suggest that transport is possible throughout the growing season, even as the mass of TMX available for transport decreases.

TMX mobility was also confirmed within the soil profile throughout the study period. For example, we detected vertical movement to the B_t soil horizon by the V3 corn stage (i.e., Day 23; **Figures 3b** and **3d**) and lateral migration between corn rows by the V5 stage (i.e., Day 36; **Figures 3c** and **3d**). The presence of TMX in soil was therefore dependent on elapsed time (two-way factorial ANOVA comparing concentrations at V3 and V5; $p < 0.05$), with a general trend of decreasing concentration through time for samples taken next to the plant (± 2 cm). In contrast, samples taken 25 cm from plants between rows showed TMX pulses at the V5 and VT corn stages.

The soil water content (θ) and matric potential (Ψ) data revealed that living plant roots reduced soil water content via uptake (Figure S3), particularly at the 110 cm depth (**Figure 4b** and **c**). Here, the 110 cm water content sensors in the viable plant plots showed diurnal fluctuations (i.e. higher θ values at night; lower values during the day with peak ET_o) beginning at the end of July, an indication that plant roots at that depth were actively transpiring water during that period. Plant response ratios (lnR_r) in shallow lateral drainage were lowest during the same time period (**Figure 4a**) suggesting plant alteration of the flow field could have limited the dose of TMX leaving the plots.

TMX transport was dominated by surface and subsurface runoff processes throughout the growing season (**Figure 1** and **Figure 2; Table S1**), which likely resulted from rapid mixing of seed coatings via newly formed macropores (from root growth and corn emergence) and the erosion of TMX-bound colloids. Maximum TMX concentrations in surface runoff were detected early in the growing season when cumulative mass losses and ET_o were low. The period of highest rainfall intensity (16 cm h^{-1} in early July 2016; **Figure 1a** and **Table S2)** resulted in lower TMX concentrations in surface runoff compared to less intense rainfall events before and after (both with rainfall intensities $\leq$ 1 cm h^{-1}). Low rainfall intensity may have produced higher doses of TMX in surface runoff due to longer water residence time and greater mixing near the seed coating, whereas high intensity rain likely diluted the signal. Thus, we detected the highest concentrations of TMX in 0-5 cm soil (up to 241 μg kg^{-1} at V3; **Figure 3a**) after low intensity rainfall preceding the V3 corn stage (**Figure 1a** and **b**). Similar early season losses to surface runoff may partially explain high concentrations of neonicotinoids detected in streams without comparable increases in discharge.[29, 45, 46] The potential risk to non-target organisms may be amplified under early season, low intensity rainfall, as runoff samples contained enough TMX in 1 mL to physically impair honey bees (> 1.4 ng)[6] exposed to ponded water.[47] Thus, fluxes into and out of this thin mixing layer[48, 49] near the soil surface may have broad implications for rapid environmental contamination of neonicotinoids.

The low intensity rainfall preceding the V3 corn stage transported TMX through B_t (**Figure 3b** and **1b)** soil into the deeper C horizon (**Figure 1c**), whereas larger, high intensity storms in early and late July 2016 produced lateral pulses of TMX throughout the profile (large spatial variation within **Figure 3c** and **d**). These latter data were characteristic of preferential flow events wherein high-intensity rainfall elevates pore water pressures and induces flow through larger macropores.[50] This bypass flow process often results in higher concentrations being detected in soil and water than predicted by conventional transport equations (e.g., advection-dispersion) through a homogenous medium.[51] Non-equilibrium flow conditions may have accented the differing subsurface architecture of viable plant vs no viable plant plots. For example, high and often variable saturated hydraulic conductivity measurements (**Table 1**) depict a heterogeneous soil pore structure with the potential for rapid mobilization of the highly soluble neonicotinoids. Further, higher concentrations of TMX detected in B_t soil of viable plant treatments (vs no viable plant) at V5 (Tukey, $p = 0.02$; **Figure 3b**) suggest that corn plants facilitated vertical transport of the pesticide. High intensity rain events in early July 2016 could have caused preferential transport of TMX, as newly formed and existing root channels may have provided conduits for infiltrating water and greater connectivity to an existing macropore network.[20, 52] This plant-mediated downward advection of neonicotinoids is consistent with observations collected during a previous greenhouse study,[15] which also detected maximum leachate concentrations and more vertical movement of TMX in soil containing viable plants around the V5 corn stage following heavy rainfall. However, after the V5 corn stage the subsurface network likely remained different between viable and no viable plant plots, yet we observed no significant differences between these treatments in soil TMX (**Figure 3**). Thus, drier soil conditions, less TMX mass available for transport (due to advective and degradation losses), and low intensity rainfall may have limited plant-driven mobility of the pesticide in the late growing season.

Though early July (V5 corn stage following intense rain) coincided with noticeable plant-mediated transport in the vertical soil profile (**Figure 3b**) and seasonal peak concentrations of TMX in shallow lateral flow (**Figure 1b**), this trend of higher plant-assisted pesticide mobility was not evident in drainage. Rather, plants appear to have restricted the mobility of TMX, seen as earlier peak concentrations for viable plant vs no viable plant plot drainage (**Figure 1b)** and significantly lower levels of the pesticide transported from plots containing viable corn plants in the late July event (one way ANOVA, $p < 0.05$; **Figure 1a** and **b**). As a result, the total mass of TMX in shallow lateral drainage became less sensitive to hydrological fluxes in plots containing viable plants, as indicated by asymptotic trend in **Figure 2**. The no-viable plant plots, in contrast, continued to leach TMX mass throughout the growing season. The cumulative mass of TMX transported from the shallow lateral compartment therefore differed by a factor of ~4 (no viable plants > with viable plants; one way ANOVA, $p < 0.05$; **Figure 2**), which may be partially attributed to reduced drainage volume in viable plant plots compared to those with no viable plants (one-way ANOVA, $p < 0.05$; **Figure 2**). Plants constrained the quantity of pesticide leaving the plots to a greater extent when cumulative $P – ET_o$ was at or below zero (**Figure 4a**). Similarly, plants began to limit TMX loss through surface runoff (with viable plants vs no viable plants, one way ANOVA, $p < 0.05$; **Figure 1b**) as evaporative demands increased in late July and early August, 2016 (**Figure 1b, c** and **Figure 4a**).

The effect of growing plants on pesticide transport is further illustrated through the observed depletion of deeper water pools. For example, reduced shallow lateral transport in viable plant plots (**Figure 1b**; **Figure 2; Figure 4a**) corresponded to lower drainage volumes

(**Figure 2**) and plant-mediated diurnal fluctuations in soil water content as deep as 110 cm (**Figure 4b**). These diurnal signals were also mirrored by a decrease in matric potential at depth, as plants exerted greater suction force on deeper water (110 cm soil, **Figure 4c**). Because the soil evaporation front in temperate North America rarely exceeds 30 cm in depth,[53] it can be deduced that diurnal signals in θ and steep decreases in Ψ at the 110 cm depth are the result of plant-induced drying of the soil profile. While $\ln R_r$ values were lowest in late July, the lack of detectable TMX in subsurface lateral drainage for viable plant plots in early September (denoted as "NA" in **Figure 4a**) suggest that plants exerted the strongest influence on transport when diurnal θ signals were at their highest amplitude (**Figure 4a** and **b**). Similarly, the two events in early August produced higher $\ln R_r$ values as $P - ET_o$ became larger (**Figure 4a**), as low intensity rain contributed to recharge (**Figure 1a** and **Figure S4),** and as diurnal θ signals became more damped (**Figure 4b**). Plants prompted a physical equilibrium between B_t and C horizons (Ψ in B_t $\approx \Psi$ in C; early-mid August, 2016; **Figure 4c**), while at the same time the matric potential decreased below -60 kPa, such that lysimeter water samples could no longer be obtained (**Figure 1c)**. Therefore, later in the growing season when evaporative demands were high, TMX likely diffused into the soil matrix (where pores held water at low Ψ). This process, which may have then physically isolated the compound from rapid hydrological fluxes, appears to have been amplified by the presence of viable plants (e.g., slightly higher fraction of TMX in soil with viable plants at R6; **Table S1**).

Rate-limited diffusion into more "protected" soil pores has been proposed as a mechanism to explain apparent increases in solute-matrix affinity with time for neonicotinoids[19] and other highly soluble agrochemicals.[52] In an extreme example, incubating the herbicide imazethapyr in undisturbed soil for 16 days resulted in a tenfold increase in observed K_d.[18]

Results of other sorption isotherms have also suggested that thiamethoxam displays a trend towards irreversible sorption in soil.[54] The results shown here suggest that rain events in the late growing season will mobilize less TMX than similar storms in the early growing season, likely due to diminished TMX concentrations in the soil (**Figure 3**). For example, the high rainfall storms that occurred in late September (**Figure 1**) mobilized only trace amounts of TMX via surface runoff and shallow lateral drainage (**Figure 2**). Although irreversible sorption of TMX to soil is not excluded,[54] degradation likely also played a role in the overall dissipation of TMX as this fertile soil (e.g. sufficient TOC, high porosity, and high CEC; **Table 1**) may have provided ideal conditions for microbial metabolism of the compound.[55]

4. Implications and Conclusions

Though levels of TMX transported from our experimental plots did not exceed any known lethal thresholds for non-target organisms, the apparent mobility of these compounds may still be concerning, as sub-part per billion exposure to neonicotinoids poses ecological risks to non-target terrestrial[6] and aquatic invertebrates.[56, 57] Moreover, this study can be taken as a conservative simulation of neonicotinoid transport potential due to: 1) the diminished dose of active ingredient (i.e. a reduced density of 59,000 plants ha^{-1}), 2) disturbed soil properties (reduced structural flow due to tillage prior to plot construction), and 3) rate-limited flow conditions (low K_s in B_t and C horizons; **Table 1**) in a highly reactive subsoil underlying the plow layer (e.g. high % clay and CEC in B_t and C horizons may retard movement due to partial positive charge on TMX; **Table 1**). Despite these potential limitations, TMX was transported via three drainage compartments and detected in the soil profile throughout the growing season. TMX concentrations were as high as 594 μg kg^{-1} (**Figure 3a**) for individual soil samples taken at day 23 (V3), representing one of the highest concentrations of a neonicotinoid yet detected in soil under environmentally relevant conditions. Further, TMX concentrations detected in surface soil between corn rows exceeded those of the samples taken 25 cm below the corn seed by over an order of magnitude (**Figure 3b,c**), suggesting that TMX can be preferentially transported even under small hydraulic gradients (**Figure S2**). This pattern of rapid advective transport could be exacerbated as farmers continue to adopt no-till practices which can promote development of soil structure and preferential flow pathways.[58] As of 2011, no-till accounted for 40% of the US acreage dedicated to corn, soybean, cotton, and wheat,[59] all of which currently employ neonicotinoid-coated seed treatments.[60]

Our results suggested that TMX transport potential decreases with time, with alteration of the water flow field caused by plant-induced drying out of the matrix representing a potentially important factor for environmental contamination of neonicotinoids. Thus, even though growing plants appear to facilitate bypass flow, they also act to mitigate plot-scale transport of TMX by amplifying flow retardation. As a consequence, early season rain events (e.g. pre-VT stage), whether intense (peak shallow lateral concentrations in early July; **Figure 1**) or mild and frequent (peak runoff concentrations in late June; **Figure 1**), may dictate total mass transport of these compounds. These early season pulses of TMX are consistent with rapid neonicotinoid transport to streams in the Midwest,[45, 46, 61] and our work provides a mechanistic link from plot to catchment scale contamination of these compounds. Taken altogether, these data serve as definitive proof that seed coated neonicotinoids can be transported throughout the growing season both above and through the soil profile in potentially harmful doses.

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Supporting Information

Details regarding our analytical approach, field plot design, rainfall events, soil sampling, and statistical analysis can be found in the Supporting Information.

References

1. Miranda, G. R.; Raetano, C. G.; Silva, E.; Daam, M. A.; Cerejeira, M. J., Environmental fate of neonicotinoids and classification of their potential risks to hypogean, epygean, and surface water ecosystems in Brazil. *Human and Ecological Risk Assessment: An International Journal* **2011,** *17*, (4), 981-995.
2. Jeschke, P.; Nauen, R.; Schindler, M.; Elbert, A., Overview of the status and global strategy for neonicotinoids. *J Agric Food Chem* **2011,** *59,* (7), 2897-908.
3. Kurwadkar, S.; Wheat, R.; McGahan, D. G.; Mitchell, F., Evaluation of leaching potential of three systemic neonicotinoid insecticides in vineyard soil. *Journal of contaminant hydrology* **2014,** *170,* 86-94.
4. Anderson, J.; Dubetz, C.; Palace, V., Neonicotinoids in the Canadian aquatic environment: a literature review on current use products with a focus on fate, exposure, and biological effects. *Science of the Total Environment* **2015,** *505*, 409-422.
5. Limay‐Rios, V.; Forero, L. G.; Xue, Y.; Smith, J.; Baute, T.; Schaafsma, A., Neonicotinoid insecticide residues in soil dust and associated parent soil in fields with a history of seed treatment use on crops in southwestern Ontario. *Environmental Toxicology and Chemistry* **2016,** *35*, (2), 303-310.

6. Tosi, S.; Burgio, G.; Nieh, J. C., A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. *Scientific reports* **2017,** *7*, (1), 1201.
7. Whitehorn, P. R.; O'Connor, S.; Wackers, F. L.; Goulson, D., Neonicotinoid pesticide reduces bumble bee colony growth and queen production. *Science* **2012,** *336*, (6079), 351-352.
8. Sanchez-Bayo, F.; Goka, K., Pesticide residues and bees--a risk assessment. *PloS one* **2014,** *9*, (4), e94482.
9. Anderson, T. A.; Salice, C. J.; Erickson, R. A.; McMurry, S. T.; Cox, S. B.; Smith, L. M., Effects of landuse and precipitation on pesticides and water quality in playa lakes of the southern high plains. *Chemosphere* **2013,** *92*, (1), 84-90.
10. Hallmann, C. A.; Foppen, R. P.; van Turnhout, C. A.; de Kroon, H.; Jongejans, E., Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* **2014,** *511*, (7509), 341-343.
11. Zhang, P.; Ren, C.; Sun, H.; Min, L., Sorption, desorption and degradation of neonicotinoids in four agricultural soils and their effects on soil microorganisms. *Science of The Total Environment* **2018,** *615*, 59-69.
12. Goulson, D.; Kleijn, D., REVIEW: An overview of the environmental risks posed by neonicotinoid insecticides. *Journal of Applied Ecology* **2013,** *50*, (4), 977-987.
13. Sanchez-Bayo, F., Environmental science. The trouble with neonicotinoids. *Science* **2014,** *346*, (6211), 806-807.
14. Ionescu, A., Suction force variation in double-hybrid maize as a function of soil moisture. *Biologia Plantarum* **1969,** *11*, (5), 370-374.
15. Radolinski, J.; Wu, J.; Xia, K.; Stewart, R., Transport of a neonicotinoid pesticide, thiamethoxam, from artificial seed coatings. *Science of The Total Environment* **2018,** *618*, 561-568.
16. Katagi, T., Soil Column Leaching of Pesticides. In *Reviews of Environmental Contamination and Toxicology Volume 221*, Springer: 2013; pp 1-105.
17. Larsson, M.; Jarvis, N., Evaluation of a dual-porosity model to predict field-scale solute transport in a macroporous soil. *Journal of Hydrology* **1999,** *215*, (1-4), 153-171.
18. O'Dell, J. D.; Wolt, J. D.; Jardine, P. M., Transport of imazethapyr in undisturbed soil columns. *Soil Science Society of America Journal* **1992,** *56*, (6), 1711-1715.
19. Oi, M., Time-dependent sorption of imidacloprid in two different soils. *Journal of agricultural and food chemistry* **1999,** *47*, (1), 327-332.
20. Bundt, M.; Albrecht, A.; Froidevaux, P.; Blaser, P.; Flühler, H., Impact of preferential flow on radionuclide distribution in soil. *Environmental science & technology* **2000,** *34*, (18), 3895-3899.
21. Jones, A.; Harrington, P.; Turnbull, G., Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years. *Pest management science* **2014,** *70*, (12), 1780-4.
22. Starner, K.; Goh, K. S., Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010-2011. *Bulletin of environmental contamination and toxicology* **2012,** *88*, (3), 316-321.
23. Stone, W. W.; Gilliom, R. J.; Ryberg, K. R., Pesticides in U.S. streams and rivers: occurrence and trends during 1992-2011. *Environmental Science & Technology* **2014,** *48*, (19), 11025-11030.
24. Main, A. R.; Michel, N. L.; Cavallaro, M. C.; Headley, J. V.; Peru, K. M.; Morrissey, C. A., Snowmelt transport of neonicotinoid insecticides to Canadian Prairie wetlands. *Agriculture, Ecosystems & Environment* **2016,** *215*, 76-84.

25. Lamers, M.; Anyusheva, M.; La, N.; Nguyen, V. V.; Streck, T., Pesticide pollution in surface□and groundwater by paddy rice cultivation: a case study from northern Vietnam. *Clean–Soil, Air, Water* **2011,** *39*, (4), 356-361.

26. Klarich, K. L.; Pflug, N. C.; DeWald, E. M.; Hladik, M. L.; Kolpin, D. W.; Cwiertny, D. M.; LeFevre, G. H., Occurrence of neonicotinoid insecticides in finished drinking water and fate during drinking water treatment. *Environmental Science & Technology Letters* **2017,** *4*, 168-173.

27. Starner, K.; Goh, K. S., Detections of the neonicotinoid insecticide imidacloprid in surface waters of three agricultural regions of California, USA, 2010-2011. *Bull Environ Contam Toxicol* **2012,** *88*, (3), 316-21.

28. Schaafsma, A.; Limay-Rios, V.; Baute, T.; Smith, J.; Xue, Y., Neonicotinoid insecticide residues in surface water and soil associated with commercial maize (corn) fields in southwestern Ontario. *PloS one* **2015,** *10*, (2), e0118139.

29. Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M., Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environmental pollution* **2014,** *193*, 189-196.

30. Greatti, M.; Barbattini, R.; Stravisi, A.; Sabatini, A. G.; Rossi, S., Presence of the ai imidacloprid on vegetation near corn fields sown with Gaucho® dressed seeds. *Bulletin of Insectology* **2006,** *59*, (2), 99-103.

31. Girolami, V.; Marzaro, M.; Vivan, L.; Mazzon, L.; Giorio, C.; Marton, D.; Tapparo, A., Aerial powdering of bees inside mobile cages and the extent of neonicotinoid cloud surrounding corn drillers. *Journal of Applied Entomology* **2013,** *137*, (1-2), 35-44.

32. Tapparo, A.; Marton, D.; Giorio, C.; Zanella, A.; Solda, L.; Marzaro, M.; Vivan, L.; Girolami, V., Assessment of the environmental exposure of honeybees to particulate matter containing neonicotinoid insecticides coming from corn coated seeds. *Environmental science & technology* **2012,** *46*, (5), 2592-2599.

33. Nuyttens, D.; Devarrewaere, W.; Verboven, P.; Foqué, D., Pesticide□laden dust emission and drift from treated seeds during seed drilling: a review. *Pest management science* **2013,** *69*, (5), 564-575.

34. Smalling, K. L.; Hladik, M. L.; Sanders, C. J.; Kuivila, K. M. J. J. o. E. S.; Health, P. B., Leaching and sorption of neonicotinoid insecticides and fungicides from seed coatings. **2018,** *53*, (3), 176-183.

35. de Perre, C.; Murphy, T. M.; Lydy, M. J., Fate and effects of clothianidin in fields using conservation practices. *Environmental toxicology and chemistry* **2015,** *34*, (2), 258-265.

36. Wettstein, F. E.; Kasteel, R.; Garcia Delgado, M. F.; Hanke, I.; Huntscha, S.; Balmer, M. E.; Poiger, T.; Bucheli, T. D., Leaching of the neonicotinoids thiamethoxam and imidacloprid from sugar beet seed dressings to subsurface tile drains. *Journal of agricultural and food chemistry* **2016,** *64*, (33), 6407-6415.

37. Hartz, K. E. H.; Edwards, T. M.; Lydy, M. J. J. E., Fate and transport of furrow-applied granular tefluthrin and seed-coated clothianidin insecticides: Comparison of field-scale observations and model estimates. **2017,** *26*, (7), 876-888.

38. Day, P. R., Particle fractionation and particle-size analysis. *Methods of soil analysis. Part 1. Physical and mineralogical properties, including statistics of measurement and sampling* **1965,** *9*, (Agronomy), 545-567.

39. Raes, D.; Munoz, G., The ETo Calculator. *Reference Manual Version* **2009,** *3*.

40. DCR, V., Virginia Nutrient Management Standards and Criteria. Richmond, VA. In 2015.

41. Brann, D. E.; Abaye, A. O.; Peterson, P. R.; Chalmers, D. R.; Whitt, D. L.; Chappell, G. F.; Herbert, D. A.; McNeill, S.; Baker, J.; Donohue, S. J., Agronomy handbook. **2009**.

42. Brann, D. E., Optimum row width for corn -- revisited

1998.

43. McWilliams, D. A.; Berglund, D. R.; Endres, G., Corn growth and management quick guide. **2010**.

44. Cushman, J. A. Environmental Fate of Animal Manure-associated Antibiotics and Seed-coated Pesticide in Soils. Virginia Tech, 2017.

45. Schnoebelen, D. J.; Kalkhoff, S. J.; Becher, K. D.; Thurman, E., Water-quality assessment of the eastern Iowa basins: Selected pesticides and pesticide degradates in streams, 1996–98. *US Geological Survey Water-Resources Investigations Report* **2003,** *3*, (4075), 62.

46. Thurman, E. M.; Goolsby, D. A.; Meyer, M. T.; Mills, M. S.; Pomes, M. L.; Kolpin, D. W., A reconnaissance study of herbicides and their metabolites in surface water of the midwestern United States using immunoassay and gas chromatography/mass spectrometry. *Environmental science & technology* **1992,** *26*, (12), 2440-2447.

47. Samson-Robert, O.; Labrie, G.; Chagnon, M.; Fournier, V., Neonicotinoid-contaminated puddles of water represent a risk of intoxication for honey bees. *PloS one* **2014,** *9*, (12), e108443.

48. McGrath, G. S.; Hinz, C.; Sivapalan, M., A preferential flow leaching index. *Water resources research* **2009,** *45*, (11).

49. Steenhuis, T. S.; Boll, J.; Shalit, G.; Selker, J. S.; Merwin, I. A., A simple equation for predicting preferential flow solute concentrations. *Journal of environmental quality* **1994,** *23*, (5), 1058-1064.

50. Jarvis, N. J., A review of non-equilibrium water flow and solute transport in soil macropores: principles, controlling factors and consequences for water quality. *European Journal of Soil Science* **2007,** *58*, (3), 523-546.

51. Allaire, S. E.; Roulier, S.; Cessna, A. J., Quantifying preferential flow in soils: A review of different techniques. *Journal of Hydrology* **2009,** *378*, (1), 179-204.

52. Jørgensen, P. R.; Hoffmann, M.; Kistrup, J. P.; Bryde, C.; Bossi, R.; Villholth, K. G., Preferential flow and pesticide transport in a clay-rich till: Field, laboratory, and modeling analysis. *Water Resources Research* **2002,** *38*, (11), 28-1-28-15.

53. Sprenger, M.; Leistert, H.; Gimbel, K.; Weiler, M., Illuminating hydrological processes at the soil□vegetation□atmosphere interface with water stable isotopes. *Reviews of Geophysics* **2016,** *54*, (3), 674-704.

54. Li, Y.; Su, P.; Li, Y.; Wen, K.; Bi, G.; Cox, M., Adsorption-desorption and degradation of insecticides clothianidin and thiamethoxam in agricultural soils. *Chemosphere* **2018,** *207*, 708-714.

55. Myresiotis, C. K.; Vryzas, Z.; Papadopoulou-Mourkidou, E., Biodegradation of soil-applied pesticides by selected strains of plant growth-promoting rhizobacteria (PGPR) and their effects on bacterial growth. *Biodegradation* **2012,** *23*, (2), 297-310.

56. Beketov, M. A.; Liess, M., Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates. *Archives of environmental contamination and toxicology* **2008,** *55*, (2), 247-253.

57. Sánchez-Bayo, F.; Goka, K.; Hayasaka, D., Contamination of the aquatic environment with neonicotinoids and its implication for ecosystems. *Frontiers in Environmental Science* **2016,** *4*, 71.

58. Alletto, L.; Coquet, Y.; Benoit, P.; Heddadj, D.; Barriuso, E., Tillage management effects on pesticide fate in soils. A review. *Agronomy for sustainable development* **2010,** *30*, (2), 367-400.

59. Wade, T.; Claassen, R. L.; Wallander, S., *Conservation-Practice Adoption Rates Vary Widely by Crop and Region*. United States Department of Agriculture, Economic Research Service: 2015.

60. Douglas, M. R.; Tooker, J. F., Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in US field crops. *Environmental science & technology* **2015,** *49*, (8), 5088-5097.

61. Hladik, M. L.; Kolpin, D. W.; Kuivila, K. M., Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Environmental Pollution* **2014,** *193*, 189-196.

Table 1.

Soil physiochemical properties. CEC = Cation Exchange Capacity; ρ_b = Bulk Density; K_s = Saturated Hydraulic Conductivity; TOC = Total Organic Carbon.

Horizon	Depth (cm)	Texture	Sand (%)	Silt (%)	Clay (%)	CEC (meq 100 g^{-1})	Porosity (%)	ρ_b (g cm^{-3})	K_s (cm d^{-1})	pH (-)	TOC (%)
A_p	0-20	silt loam	24.0	62	13.9	8.5 ± 0.9	58 ± 0.03	1.26 ±0.09	4460 ± 1670	5.7 ± 0.3	2.00 ± 0.45
B_t	20-100	silty clay loam	14.7	46.4	38.9	8.4 ± 2.6	53 ± 0.04	1.49 ±0.12	50.4 ± 99.3	4.4 ± 0.2	0.25 ± 0.06
C	100+	clay	4.1	20.9	75.0	12 ± 2.7	60 ± 0.03	1.34 ±0.05	81.2 ± 81.4	6.4 ± 0.1	0.40 ± 0.03

CEC, porosity, bulk density, K_s, pH and TOC are expressed as mean ± standard deviation.

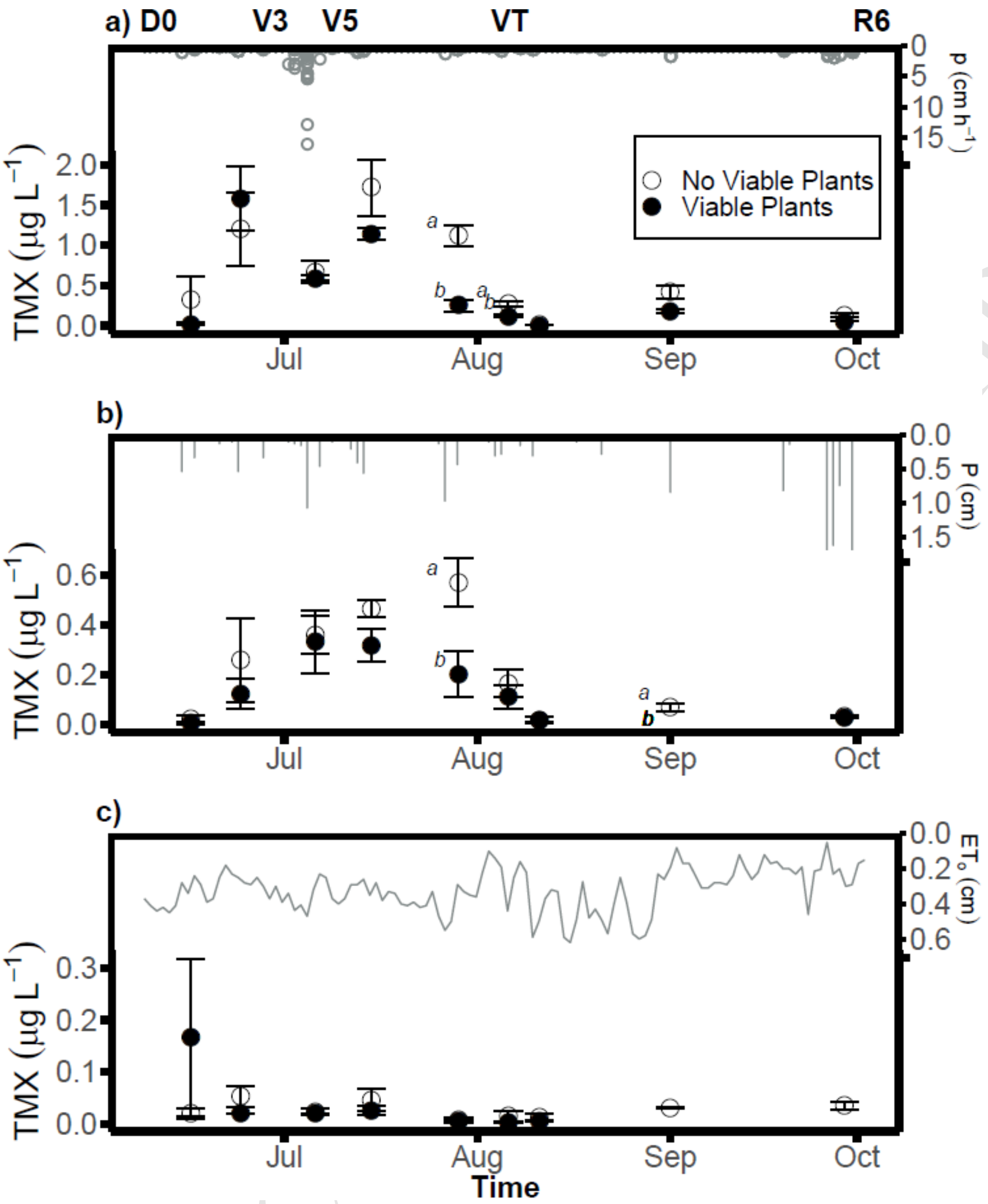

Figure 1. TMX concentrations detected throughout the 2016 corn growing season in **a)** surface runoff, **b**) shallow lateral drainage at 72 cm in the B_t horizon, and **c)** deep drainage collected via suction lysimeters at 110 cm in C horizon. The rainfall intensity (p), rainfall accumulation (P) and daily potential evapotranspiration (ET_o) are plotted in the top panel of **a)**, **b)**, and **c)**, respectively. Time of planting (D0) and corn growth stages (V3, V5, VT, and R6) are shown above **a)** as a reference for time of soil sampling. Error bars represent standard error (SE; n = 3). *Different letters* denote significant differences between plant and no viable plants treatments at each sampling event ($p \leq 0.05$). The "**b**" for the 9/01/16 event in panel **b)** denotes differences in concentration between detectable (no viable plant) and non-detectable (viable plant) samples. Viable plant plots yielded no water for lysimeter sampling for the final 2 rain events in **c)**.

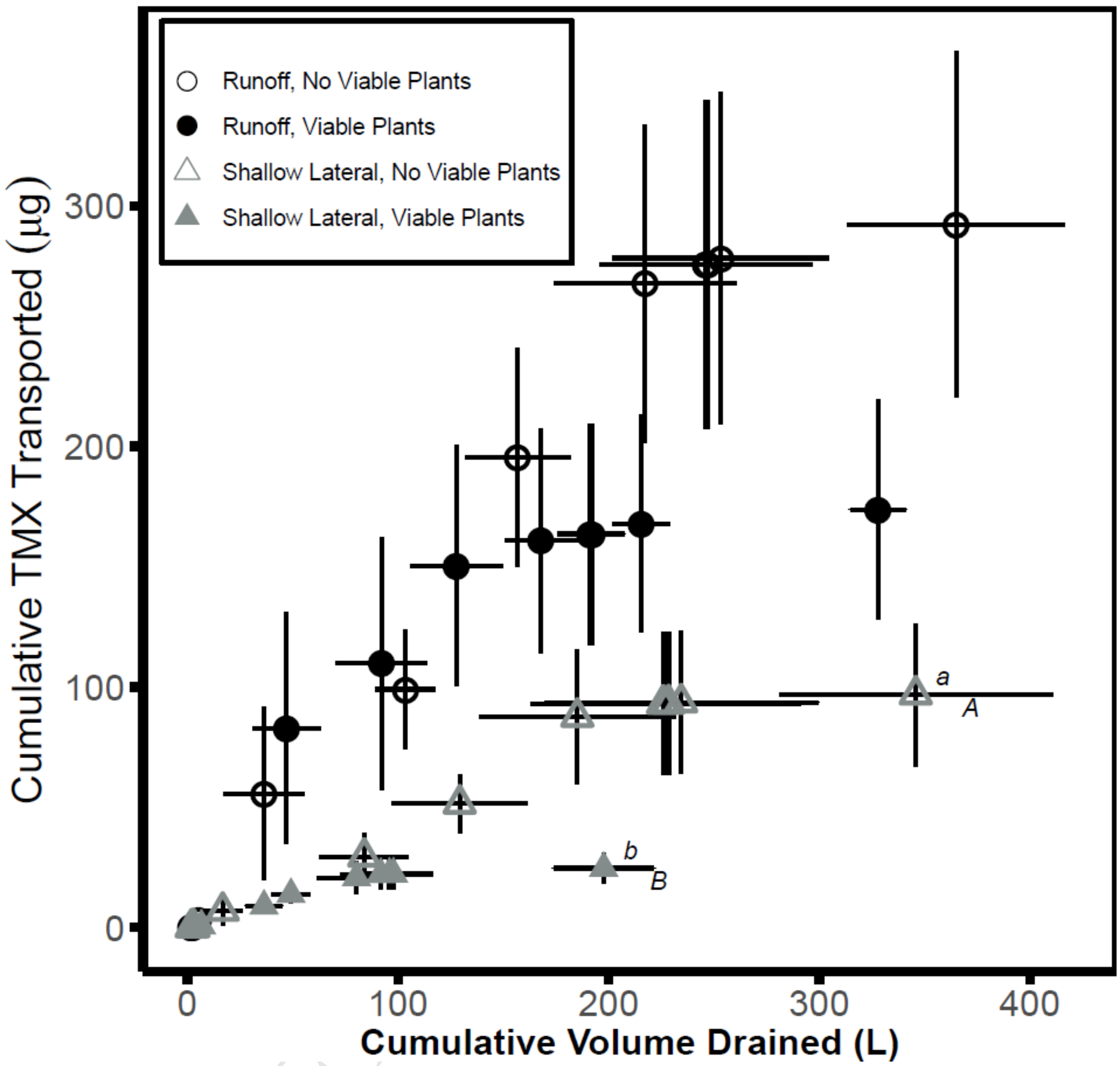

Figure 2. Cumulative mass of TMX transported in runoff and shallow lateral drainage vs cumulative volume drained. Note, deep drainage results are not considered here due to lack of flow records below the shallow lateral drainage outlet. *Lower case letters* denote significant differences between viable plant and no viable plant treatments in cumulative TMX while *capital letters* designates differences in cumulative volume ($p < 0.05$) and errors bars represent SE (n = 3). All statistical tests compared the final cumulative observation points. ***Intended for color reproduction***.

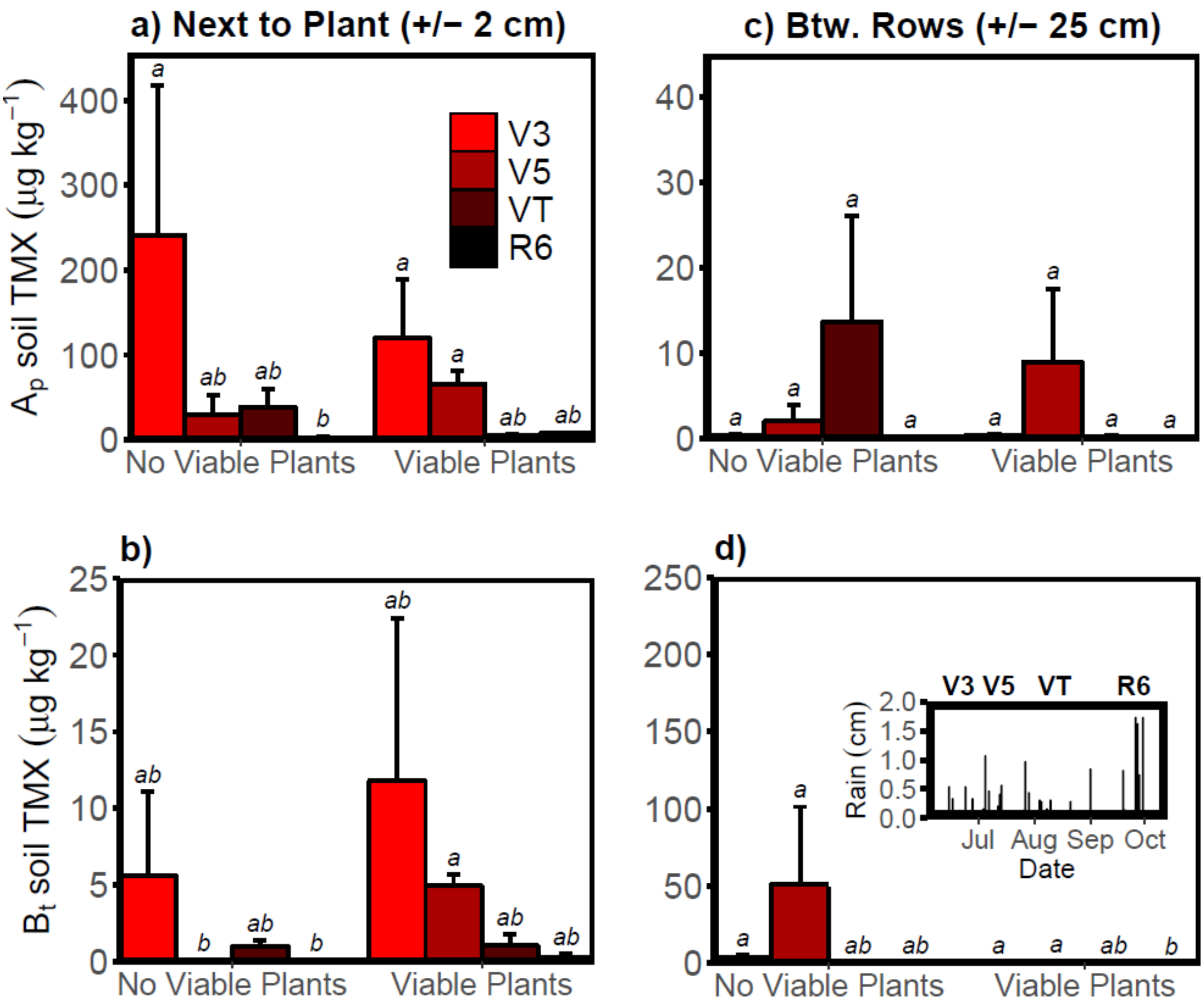

Figure 3. TMX concentrations in soil collected at 0-5 cm (A_p soil horizon) and 25-30 cm (B_t soil horizon) at 2 cm (**a** and **b**) and 25 cm (between two rows) from corn plants or sown seeds (**c** and **d**). The inset of **d)** illustrates corn growth stage and rainfall during the growing season. *Different letters* denote significant differences between corn stage and with viable plant versus no viable plants treatments ($p < 0.05$) within each figure panel (i.e. sample location and depth) and errors bars represent SE ($n = 3$). **Note** that the scale in **c)** is 1/10 of that in **a)** and the scale in **d)** is 10 times that of **b)**. ***Intended for color reproduction***.

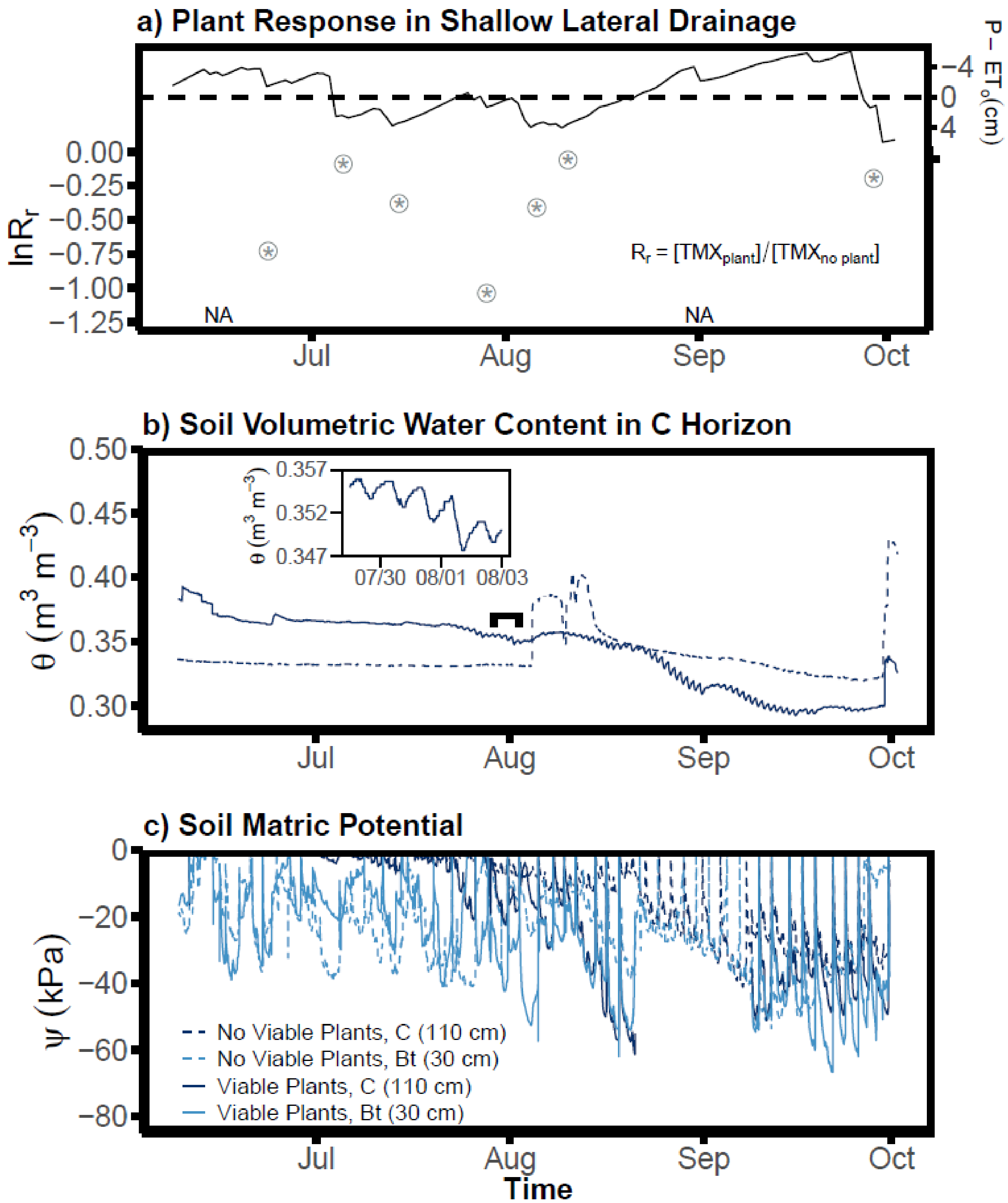

Figure 4. a) Temporal change of shallow $\ln R_r$ and P – ET_o (cumulative precipitation minus evapotranspiration). **b)** Volumetric soil water content (θ; *n* = 3 plots per treatment) and **c)** matric potential (Ψ; *n* = 2 plots per treatment) in B_t and C horizon soil. The inset of **b**) Shows a 5-day period with plant-driven diurnal fluctuations in θ. Sharp increases in Ψ represent tensiometer refill events and missing time periods for plant treatments denote logger and probe

malfunctioning periods following high suction (low Ψ). Sampling events labeled as “NA” were removed from analysis in **a)** due to comparison issues with TMX at or below detection limit. ***Intended for color reproduction***.

Highlights

- Thiamethoxam was transported from corn seed coatings throughout the growing season
- Low intensity storms produced the highest levels of TMX in early season runoff
- Plants enhanced vertical mobility of TMX but constrained losses to drainage outflow