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ASSESSMENT OF NITRIFICATION AND UREASE INHIBITORS ON NITRATE LEACHING IN CORN (ZEA MAYS L.)

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ABSTRACT

Agricultural ecosystems are one of the largest global contributors to nitrate (NO₃⁻) contamination of surface- and ground-water through fertilizer application. Improved fertilizer practices are needed to manage crop nutrient supply in corn (Zea mays L.) while minimizing impacts to clean water reserves. The goal of this study was to compare current nitrogen (N) fertilizer practices (urea at planting) with ‘packages’ of improved management practices (a combination of right timing and product) that farmers potentially use. We conducted measurements in a continuous corn system from November 2015 to May 2017 at a large field scale (four 4 ha plots). Nitrate concentration was measured below the root zone and drainage estimated using a soil water budget approach where evapotranspiration was measured using the eddy covariance method. The objective was to compare NO₃⁻-N leaching from fields receiving urea vs. urea+NUI fertilizer applications at planting, urea-ammonium nitrate (UAN) vs. UAN+NUI applied at sidedress and a combination of these practices: urea+NUI at planting vs. UAN at sidedress. Drainage was only significant in the non-growing season. Neither fertilizer products applied with NUI at planting or sidedress proved to significantly reduce NO₃⁻-N leaching. The combination of delaying fertilization to sidedress and applying UAN significantly reduced the soil water NO₃⁻-N concentration compared to urea+NUI at planting (mean of 5.2 vs. 4.8 mg L⁻¹).
6.7 mg L\(^{-1}\)) but only in 2015-2016. Based on these results, applying UAN at sidedress is recommended, although additional study years are needed to confirm results.

Key words: Application timing, drainage, nitrogen, nitrate leaching, nitrification inhibitors and urease inhibitors.

INTRODUCTION

Corn (\textit{Zea mays} L.), one of the most prominent cereal crops cultivated in North America, requires large amounts of nitrogen (N) fertilizer to achieve high yields (Ma et al. 2003). In Canada, 66% of grain corn production occurs in Ontario (Statistics Canada 2011). The fertilizer most commonly applied to corn worldwide at this time is urea due to low cost and availability (USDA 2008; Witte 2011). Within 2 to 4 days of application, urea is hydrolyzed to ammonium (\(\text{NH}_4^+\)) and then nitrified within 2 to 4 weeks to nitrate (\(\text{NO}_3^-\)) (Firestone and Davidson 1989; Carmona et al. 1990; Grant et al. 1996). However, this can be problematic as N uptake is largest several weeks after planting (Aldrich and Hauck 1982; Fox et al. 1986). There is a time gap then, between pre-plant fertilizer application and significant plant N uptake. Another issue is that in humid regions such as Ontario, there is a surplus in the water budget as precipitation often exceeds evapotranspiration (ET) during spring (April and May) and also winter (December to March) (Fallow et al. 2003). The availability of excess water and the presence of \(\text{NO}_3^-\)-N in the soil water can result in downward movement of \(\text{NO}_3^-\)-N. This introduces a problem for possible \(\text{NO}_3^-\)-N contamination in drinking water and represents nutrient loss for the plant.

Good nutrient management has advocated proper use of fertilizer best management techniques (Singh and Sekkon 1978). Recently, the fertilizer industry has developed the 4R
nutrient stewardship as a guideline of best management practices (right source, right rate, right
time and right place) to improve crop N use efficiency (NUE) and mitigate N losses, without
compromising crop yields (Snyder et al. 2009). Synchronizing N fertilization with plant
requirement has been proposed as a best management practice to reduce NO$_3^-$-N leaching. One
way to accomplish this is by applying fertilizer several weeks after corn has emerged (sidedress
stage). However, application at planting is preferred by farmers due to the short window of time
that is often available for field operations before plant N requirements are high and as pre-plant
application is less costly to apply. Plant N requirement is strongest between V8 and silking,
while yield loss may occur when fertilization is delayed until V12 (Russelle et al. 1983; Scharf et
al. 2002).

Nitrification and urease inhibitors are compounds that can be added to urea-based
fertilizers to provide a possible alternative to sidedress application by potentially better
synchronizing nutrient release with plant requirement. Urease inhibitors (UI) slow the rate at
which urea is hydrolyzed to ammonium (NH$_4^+$) (Timilsena et al. 2015). The UI may reduce
gaseous losses of NH$_3$ by suppressing the rate at which NH$_4^+$ is produced (Abalos et al. 2012).
By doing this, UI delay the release of N in the soil until soil conditions are less likely to cause
loss, which may also reduce losses via NO$_3^-$-N leaching and/or N$_2$O emissions. Nitrification
inhibitors (NI) slow the microbial conversion of NH$_4^+$ to NO$_3^-$ (Timilsena et al. 2015). By
retarding the rate of nitrification with a NI, NO$_3^-$-N leaching and N$_2$O gas emissions may be
reduced by maintaining N in the less mobile NH$_4^+$ form (Motavalli et al. 2008; Liu et al. 2013).
Combining a UI and NI is a possible mitigation method to target all pathways for N losses.

The nitrification inhibitor dicyandiamide (DCD) is one of the most popular compounds
used with urea as it is inexpensive, water insoluble and efficient in cold climates (Di and
Cameron 2004; Hatch et al. 2005; Chien et al. 2009; Sanz-Cobena et al. 2012). Urea treated with DCD has been found to reduce nitrous oxide (N$_2$O) emissions and NO$_3$-N leaching in grazed pastures, vegetable systems (capsicum, amaranth, radish), wheat, barley and corn (Ball-Coelho and Roy 1999; Majumdar et al. 2002; Cui et al. 2011; Di and Cameron 2012; Roche et al. 2016).

The most widely used and most effective UI is $N$-(n-butyl) thiophosphoric triamide (NBPT or NBTPT) (Chai and Bremner 1987; Trenkel 2010). In these studies, urea+NBPT was confirmed to reduce ammonia volatilization in grazed pastures and corn (Schlegel et al. 1986; Bronson et al. 1989; Sanz-Cobena et al. 2008; Zaman et al. 2009). Field and laboratory studies showed that urea+NBPT is effective in reducing NO$_3$-N losses from well-drained loam-textured soils (Bronson et al. 1989; Carmona et al. 1990; Clay et al. 1990; Rawluk et al. 2001).

Few studies have examined the effectiveness of the combination of NI and UI (NUI) in humid climates or in corn systems (Halvorson et al. 2014; Connell et al. 2011; Soares et al. 2012; Parkin Hatfield 2014; Venterea et al. 2016). Additional studies in these conditions are needed as the effectiveness of NUI is highly dependent on weather variables, particularly soil moisture, with effectiveness being reduced under dry conditions (Sanz-Cobena et al. 2012). Of existing research, it has been found that NO$_3$-N leaching was reduced with urea+NUI in intensive pasture production and corn (Zaman and Blennerhassett 2010). In contrast to these results, other studies have found that NO$_3$-N losses were greater with urea+NUI compared to urea or urea+DCD (Gioacchini et al. 2002; Sanz-Cobena et al. 2012). More research is needed to assess if urea+NUI at planting may provide a beneficial reduction in NO$_3$-N similar to applying nitrogen at sidedress. To our knowledge there has been no prior field-scale studies in southern Ontario investigating this comparison. In addition, it is not clear whether a further reduction may be obtained by applying NUI at sidedress.
We hypothesized that using fertilizer products with NUI would reduce NO$_3^-$-N leaching compared with a conventional fertilizer product. In addition, we hypothesized that applying fertilizer products with NUI at planting would provide similar benefits as applying a conventional fertilizer as a sidedress. We tested these hypotheses at a large field scale (four 4 ha plots) where NO$_3^-$-N concentration was measured below the root zone and drainage estimated using a soil water budget approach where evapotranspiration was measured using the eddy covariance method. This approach required large field plots and prevented the use of a typical agronomic factorial experiment. Instead, our intention was to compare current practices (urea at planting) with ‘packages’ of improved management practices (a combination of right timing and product) that farmers potentially use. The objective was to compare NO$_3^-$-N leaching from fields receiving urea vs. urea+NUI fertilizer applications at planting, urea-ammonium nitrate (UAN) vs. UAN+NUI applied at sidedress and a combination of these practices: urea+NUI at planting vs. UAN at sidedress.

**MATERIALS AND METHODS**

*Site Description and Experimental Design*

This study was commenced in May 2015 and measurements started in November 2015 until May 2017. Drainage was not significant during the growing season of 2016; therefore, presentation of results and discussion is concentrated in two non-growing season periods: November 2015 to May 2016 and November 2016 to May 2017. The experimental site was at the University of Guelph Elora Research Station near Elora, Ontario (43°39’ N 80°25’ W, 376 m elevation). The soils at the research site are classified as a Grey-Brown Luvisol or Albic Luvisol and the soil at our site is an imperfectly drained Guelph silt loam with 29% sand, 52% silt and
19% clay (Morwick and Richards 1946; Jayasundara et al. 2007). The soil chemical characteristics have been previously assessed as follows: pH: 7.6 (water) in the 0-15-cm layer, organic carbon: 26.9 g Kg\(^{-1}\), total N: 2.4 g Kg\(^{-1}\), available phosphorus: 24 mg Kg\(^{-1}\), available potassium: 146 mg kg\(^{-1}\) (Jayasundara et al. 2007). The field was tile drained in the 1960s with distance between tiles of 15-m and approximate depth of 80-cm.

The experiment was conducted on a large homogeneous 30 ha micrometeorological study area, necessary to directly measure evapotranspiration and derive drainage (see below). The experimental approach consisted of applying the following fertilizer treatments to four 240 m by 170 m (4 ha) side-by-side fields: 1) urea broadcast and incorporated at planting, 2) urea+NUI broadcast and incorporated at planting (NBPT and DCD; SuperU©, Koch Agronomic Services), 3) UAN injected at sidedress and 4) UAN+NUI (NBPT and DCD; AgrotainPlus©, Koch Agronomic Services) injected at sidedress. The site was a continuous corn (Zea mays L.) system with a conventional tillage management consisting of fall moldboard plowing and pre-seeding cultivation. Study fields received the OMAFRA (2016) recommended fertilizer treatment rate of 150 kg N ha\(^{-1}\) differing by timing of N application (planting or sidedress) and product (conventional or conventional+NUI). At planting, all fields received monoammonium phosphate (MAP) side-banded at 250 kg ha\(^{-1}\) containing 30 kg N ha\(^{-1}\). Urea and urea+NUI were broadcast at rates of 120 kg N ha\(^{-1}\) one day before planting and incorporated to 10-cm (May 12, 2015 and May 11, 2016). UAN and UAN+NUI were injected between 70-cm rows at rates of 120 kg N ha\(^{-1}\) at sidedress (June 25, 2015 and June 17, 2016, V6 and V4 respectively). Differences in vegetative stage at time of fertilizer side-dress application were due to weather conditions and the available window for fertilizer application in both years.
Nitrate-N Leaching

The amount of NO$_3$-N leached below the root zone ($[\text{NO}_3^-\text{-N}]_{\text{leached}}$) was calculated by multiplying the NO$_3$-N concentration sampled from the soil water solution at 80-cm depth by drainage volume estimated from the water budget (see below) according to the following equation:

$$[\text{NO}_3^-\text{--N}]_{\text{Leached}} = [\text{NO}_3^-\text{--N}]_{80\,\text{cm}} \times D \times 0.01$$

where D is drainage in mm (L m$^{-2}$), explained further in a following section, and $[\text{NO}_3^-\text{-N}]_{80\,\text{cm}}$ is the NO$_3$-N concentration (mg N L$^{-1}$) sampled from soil water at 80-cm depth with porous ceramic cup water samplers and 0.01 (kg ha$^{-1}$) is a unit conversion factor to give units of kg N ha$^{-1}$ to $[\text{NO}_3^-\text{-N}]_{\text{leached}}$.

Soil water samplers consisted of a porous ceramic cup mounted to a 2.54-cm diameter PVC pipe and sealed with rubber stopper. Two sets of plastic tubing were inserted into each sampler with a shorter tube for applying internal pressure and water extraction. Porous ceramic cups have low cost, provide minimal soil disturbance, are easy to install and collected soil solution has been found to accurately represent NO$_3$-N in drainage water compared with monolith lysimeters and soil cores (Grossmann and Udluft 1991; Webster et al. 1993).

Samplers were installed in November 2015 to a depth of 80-cm using a 45° angle to minimize above-cup soil disturbance and the influence of preferential flow (Lord and Shepherd 1993). Nine samplers were installed in a grid pattern in each field to provide spatial coverage. Installation was accomplished by augering with a metal rod and using a 45°-angled ramp as a guide. Flexible metal tubing was seated at the top of the PVC pipe to protect against rodent damage and to allow burial beneath the soil for planting and harvest operations. Plastic containers were secured to the metal tubing to house sample extraction connections.
Soil water was sampled from soil water samplers every 1 to 2 weeks during the drainage period from November to May each year. Sampling intervals were longer during dry periods or when freezing temperatures prevented water extraction. When the soil was dry a suction of 50 kPa was applied to each water sampler using a hand vacuum pump. Samples were extracted from the water sampler using pre-evacuated vials and stability of the NO$_3^-$ ion was maintained post-sampling by transporting samples from the field on ice. Samples were transferred to plastic containers and frozen until analyzed using an autoanalyzer (Seal Analytical AA3). Arithmetic mean NO$_3^-$-N concentration was calculated from the nine sub-samples for each field and reported for each sampling time.

**Drainage**

Direct measurements of drainage are costly and difficult to accomplish with minimal soil disturbance (Ramos and Kücke 2001). A soil water budget approach provides a non-invasive method to estimate drainage flow which combined with a measurement of NO$_3^-$-N concentration at depth can provide an estimation of NO$_3^-$-N leaching (McCoy et al. 2006). Drainage at 80-cm depth was calculated by a soil water budget approach (McCoy et al. 2006):

$$D = P - ET - \Delta S$$

where D is drainage, P is precipitation (rainfall + snowmelt), ET is evapotranspiration, and $\Delta S$ is the change in soil water storage for the soil profile, all terms expressed in mm, and runoff assumed negligible due to the level field conditions (McCoy et al. 2006). Factors D and $\Delta S$ can be positive or negative values. When D is positive, drainage occurs and when D is negative there is capillary rise in the soil profile. Data for each factor in Equation 2 was measured in 30-minute periods before being aggregated to daily, then weekly values.
Rainfall and snowfall data were obtained from the Environment Canada Elora Weather Station adjacent to the study site. To provide an accurate estimate of precipitation and of the winter snow water balance, snowmelt was added to rainfall. Rainfall was measured using a tipping-bucket rain gauge from the Environment Canada Elora Weather Station. Water added to the soil in the form of snowmelt was estimated by determining the snow water equivalent (SWE, kg m\(^{-2}\)) by multiplying the snow density (kg m\(^{-3}\)) by snow depth (m) (Dingman 1994). Snow depth and density measurements were collected at the experimental site when there were gains or losses in snow on ground. Snow depth was measured using a meter stick and a known volume of snow was collected using a snow tube, melted and then weighed to determine snow density (Dingman 1994). Since manual snow depth measurements were not taken daily, snowfall was also obtained from the Environment Canada Elora weather station from November to May each year to gap fill between measured data. From these measurements, snowmelt was then calculated using snow depth at the end of a week subtracted from the snow depth at the beginning of that week. For periods when there was less snow remaining at the end of the week compared with the beginning, snowmelt was added to precipitation to determine total precipitation (McCoy et al. 2006).

The ET was measured using two eddy covariance systems (EC), one installed in the East side and one in the West side of the 30-ha field. Measurement of ET decreases the uncertainty in drainage estimates using Eqn. (2). The main EC system was comprised of a sonic anemometer (CSAT3, Campbell Scientific) and a closed-path EC155 (Campbell Scientific) analyzer, when this was not available data from the open path LI-7500 (LI-COR) was used. The ET was assumed to be representative of the entire 30-ha area and equal across all fields. The EC measured the water vapour flux as an average latent heat flux (W m\(^{-2}\)) in 30-min averages.
latent heat flux data was filtered for friction velocity (u*) less than 0.11 m s$^{-1}$ to eliminate data when there was weak turbulence, commonly during night time. Additionally, the data was filtered for wind direction to eliminate periods where tower shadowing occurs when the angle between horizontal wind and sonic axis was between 60° and 120°. Once filtered for u* and wind direction, latent heat (LE) was converted to ET (kg m$^{-2}$ or mm) by using the latent heat of vaporization (2.5 x 10$^{6}$ J kg$^{-1}$).

Missing half-hourly ET values were gap-filled using a fit through origin linear regression equation derived from the measured EC data and the equilibrium evaporation (\(\lambda ET\)) (Priestley and Taylor 1972):

\[
\lambda ET = \frac{\Delta}{\Delta + \gamma}(Rn - G) \tag{3}
\]

where \(\lambda ET\) is equilibrium evaporation, \(\Delta\) is the slope of saturated vapour pressure at a given temperature, \(\gamma\) the psychrometric constant, \(Rn\) is net radiation and \(G\) soil heat flux. Equations from Allen et al. (1998) were used to calculate \(\Delta\) and \(\gamma\). Net radiation was measured at the site using a net radiometer. Gaps in \(Rn\) data were filled by running a linear regression between solar radiation and \(Rn\). The soil heat flux was assumed to be 10% of net radiation according to approximation suggested by Allen et al. (1998). A fit through origin equation was calculated for each non-growing season: 2015 to 2016 and 2016 to 2017. If there were missing data in the measured EC data, then the slope from the fit through origin equation was multiplied by the \(\lambda ET\) value to gap fill. In 2015-2016, the linear equation was \(EC=1.09\lambda ET\) with a \(R^2=0.71\), compared with 2016-2017 of \(EC=1.43\lambda ET\) with a \(R^2=0.86\). The agreement coefficient (\(R^2\)) is high providing confidence that this technique provided an adequate gap-filling method.
Change in soil water storage, $\Delta S$, was calculated as an average for the whole field based on volumetric soil water content (SWC) measurements derived from sensors in four locations. In June 2015 water content reflectometers (WCR, Campbell Scientific Model CS616) were installed horizontally in the soil. Two sensors were installed in each field at depths of 5-cm and 25-cm for a total of 8 sensors per depth and one sensor in each field at 55-cm and 85-cm for a total of 4 sensors per depth. During field operations, the 5-cm and 25-cm WCR sensors were removed to prevent possible damage.

The WCR allows for an indirect measurement of soil water content. The output from the WCR gives a period of 15-40 µs or (0.7-1.6 ms). This output was converted to volumetric water content ($\theta$) according to the calibration equation by Kulasekera et al. (2011):

$$\theta = 0.0008\tau^2 - 0.0168\tau + 0.0609. \quad [4]$$

where $\tau$ is the WCR period ($\mu$s).

Soil temperature was also measured at the same locations as SWC at 5-cm depth using soil copper-constantan thermocouples. The WCR and thermocouples were wired to a CR23X (Campbell Scientific) datalogger and a solar panel for a power source. The CR23X datalogger recorded data in 10-minute intervals from the sensors. The average SWC was calculated for the two sensors at every depth per field and for soil temperatures at 5-cm. Missing values were gap filled using linear interpolation. These data were then aggregated to 30-min, 60-min and one-day values. For SWC, a weighted average function was then used to determine the average SWC for each soil increment across the four fields (1-cm through 85-cm). Storage was calculated using the average weighted function by multiplying average soil water content for one-day across fields by the soil increment. The total storage in each layer was obtained from the product of the volumetric water contents for each soil increment of 1 through 85-cm. $\Delta S$ was calculated as the
difference in storage between one-day and the previous day. The values for $\Delta S$ were then aggregated to weekly values.

*Statistical Analysis*

Comparisons of NO$_3$-N leaching from the four fields were performed based on the soil water NO$_3$-N concentrations since drainage values used were common to all four plots. The Wilcoxon signed rank-test (Rstudio 1.1.423; R Core Team, 2017) was selected for comparison of paired treatment means as data were non-normally distributed (Shapiro-Wilks test $P < 0.05$). This test is applicable to repeated measurements and has been used to compare N$_2$O emissions from a similar four plot setup (Wagner-Riddle et al., 2007; Tenuta et al., 2016). Three comparisons were made to examine the objectives: 1) urea vs. urea+NUI at planting, 2) UAN vs. UAN+NUI applied at sidedress and 3) a combination of these practices: urea+NUI at planting vs. UAN at sidedress. We acknowledge that method of application (broadcasting for urea and injection for UAN) confound comparison 3 but our intent was to compare a ‘package’ of practices as they would likely be applied at the farm scale by producers, who typically inject UAN at sidedress and do not broadcast urea at that time. Comparisons were made using measured values of NO$_3$-N concentrations (before data was interpolated) from nine pseudo-replicate observations per treatment in two separate analyses for years 2015-2016 and 2016-2017. A Bonferroni correction was applied as a more conservative approach to reduce the chance of receiving a significant result due to chance and account for pseudo-replication. From this correction, values were considered significantly different for all analyses when $\alpha < 0.0167$ (0.05/3; significance level divided by the number of paired tests).
RESULTS AND DISCUSSION

Precipitation and Evapotranspiration

The 2015 growing season (May-October) started wet in May and June followed by a drier period, but overall was wet with precipitation of 532 mm compared to climate normals of 502 mm (Table 1). In contrast, the 2016 growing season started off very dry in May and June followed by wet months in July and August, but overall drier than 2015 and climate normals with precipitation of 401 mm. The 2015 non-growing season (November-April) was drier than climate normals except March. In contrast, the 2016-2017 non-growing season precipitation exceeded climate normals every month except November which was drier. As a result, overall precipitation in the non-growing season of 2015-2016 was lower compared to 2016-2017. Over the whole year (May-April), precipitation for 2015-2016 was only slightly higher with 977 mm vs. 930 mm in 2016-2017, compared to climate normal of 916 mm. However, the seasonal distribution was different in the two experimental years with precipitation in 2015-2016 being concentrated in the growing season and in 2016-2017 in the non-growing season.

Similar to the precipitation trend, cumulative ET during the non-growing season (November-April) was greater in 2016-2017 than 2015-2016 with 298 mm compared to 193 mm, demonstrating that precipitation influenced ET (Fig 1). The observed differences in ET between experimental years could also have been related to higher air temperature in 2016-2017 compared with 2015-2016 (Table 1). These ET measurements are higher than normal compared to average ET estimated by Fallow et al. (2003) over a 47-year period where ET in November-April was 87 mm. In both non-growing seasons, ET was relatively low until February when thawing events and increasing temperatures increased ET, as expected (Fig. 1).
Soil water content and storage

Despite a drier growing season in 2016 compared to 2015, average soil water content was similar with 0.16 m$^3$ m$^{-3}$ at the start (November) of both non-growing seasons (Fig. 2). Over the two-year experimental period, soil water content at 85-cm averaged 0.26 m$^3$ m$^{-3}$ and at 55-cm 0.31 m$^3$ m$^{-3}$. At 25-cm depth, soil water content was similar in both experimental years with 2015-2016 ranging from 0.27 m$^3$ m$^{-3}$ to 0.34 m$^3$ m$^{-3}$ and 2016-2017 from 0.20 m$^3$ m$^{-3}$ to 0.34 m$^3$ m$^{-3}$. The 5-cm depth showed the largest variations, and these were associated with liquid water moving into and out of the solid phase during freeze-thaw events in the non-growing season (Fig. 2). Liquid soil water content at 5-cm decreased when soil was frozen at 5-cm depth and increased when there was thawing (Fig. 2). The 5-cm range was narrower in 2015-2016 with 0.03 m$^3$ m$^{-3}$ to 0.24 m$^3$ m$^{-3}$ and wider range in 2016-2017 with 0.02 m$^3$ m$^{-3}$ to 0.33 m$^3$ m$^{-3}$. In January through March of both experimental years, thawing periods can be observed with increases in air temperature with higher peak values in soil water content observed in 2016-2017 at 5-cm depth up to 0.33 m$^3$ m$^{-3}$ compared to 0.18 m$^3$ m$^{-3}$ in 2015-2016 (Fig. 2). Thus, when thawing periods occurred, there was higher liquid soil water content at the 5-cm depth for 2016-2017.

There were large variations in weekly changes in soil water storage between both non-growing seasons, with 2015-2016 varying less ranging from -16 mm to 29 mm, and 2016-2017 varying more from -43 mm to 51 mm (Fig. 3). In 2016-2017, the soil profile gained water ($\Delta S>0$) over a longer period (DOY 321-335, 356-364, and 81-95) than 2015-2016, although times with water loss ($\Delta S<0$) were less frequent in 2015-2016 (Fig. 3). It can be observed that some storage gains were from thawing and storage losses were due to freezing as liquid water moved in and out of the solid phase (Fig. 2). In 2015-2016, weekly gains in liquid water were
apparent from November-March, with losses in water most prominent in January, February and March (Fig. 3a). In 2016-2017, there were weekly gains and losses from November-May, with large gain and loss events in February and March which were associated with freeze-thaw cycles as liquid soil water and air temperature also increased during these periods (Fig. 2b and 3b).

**Drainage**

Modelling of drainage began in November 2015 (DOY 319) as this was when soil water samplers were installed, and NO$_3$-N leaching could be calculated. With the exception of two weeks in 2016 where there was a total of 70 mm drainage (DOY 297-310), the remainder of the weeks between DOY 104 to DOY 293 consisted of small negative values. After DOY 136, 2017, drainage concluded and similar to that of 2016, remained negative throughout the growing season.

Drainage during the non-growing season was slightly higher in 2016-2017 with cumulative totals from November-April equal to 202 mm, compared to 2015-2016 with 186 mm (data not shown). Drainage was higher than 30-year averages by Fallow et al. (2003) for the same region where 151 mm of drainage was predicted from November-April. The seasonal distribution of weekly drainage showed different trends with drainage being higher in Nov-Jan in 2016-2017 and higher in Feb-April in 2015-2016. Weekly values varied in 2015-2016 from -29 mm to 35 mm, with a greater range observed in 2016-2017 from -49 mm to 61 mm. Drainage events coincided with peaks in precipitation, as expected (Fig. 3). Variations in drainage (positive and negative) were much less in April continuing into May in both years most likely from increased ET starting in June when plants are actively growing.
Nitrate-N Concentration

The average soil water NO$_3^-$-N concentrations in 2015-2016 for urea, urea+NUI, UAN and UAN+NUI were 6.1, 6.7, 5.2 and 4.6 mg L$^{-1}$ respectively. For the 2016-2017 non-growing season, average soil water NO$_3^-$-N concentrations were very similar for all treatments with 2.0, 2.1, 2.1 and 1.9 mg L$^{-1}$, respectively, for urea, urea+NUI, UAN and UAN+NUI (Fig. 4). Among all fields, NO$_3^-$-N concentrations were below the Health Canada (2013) MAC guideline of 10 mg L$^{-1}$ throughout both years (Fig. 4). Overall, 2015-2016 had a trend for greater NO$_3^-$-N concentration than 2016-2017. Beginning in November 2015, NO$_3^-$-N was already higher than in 2016, with a steady increase continuing into winter then decreasing in April-May. In contrast, in 2016-2017 NO$_3^-$-N concentrations were low throughout November-March, except for urea+NUI showing a slight increase, with a small increasing trend towards the end of the measurement period starting at the end of April.

The highest NO$_3^-$-N concentrations in 2015-2016 were likely due to low corn yields and the high amount of residual NO$_3^-$-N remaining in the soil after harvest. In 2015, the corn crop performed poorer compared to 2016, resulting in significant differences in corn yield with UAN resulting in significantly less yield (8.89 Mg ha$^{-1}$) compared with urea, urea+NUI and UAN+NUI (10.91, 10.85 and 10.21 Mg ha$^{-1}$) (Ferrari Machado 2017). Compared to 2015, in 2016 there were no significant differences in corn yield with an average of 11.9 Mg ha$^{-1}$ (Ferrari Machado 2017). With increasing yield, there is more N removed from the field therefore a decrease in mineral N in soil. This is indicated in higher soil NO$_3^-$-N concentrations from 0-15-cm depth in 2015, with November, 2015 concentrations for urea, urea+NUI, UAN and UAN+NUI at 3.68, 2.90, 5.24, 3.45 mg L$^{-1}$ as determined by a separate study at the same site, respectively (Ferrari Machado 2017). In contrast, soil NO$_3^-$-N at 0-15-cm depth in 2016 was
lower for urea by a factor of 2.97, urea+NUI by 2.10, UAN by 3.77, and for UAN+NUI by 1.33 (with NO$_3^-$-N concentrations of 1.24, 1.39, 1.39, and 2.59 mg L$^{-1}$, respectively) (Ferrari Machado 2017). Thus, the lower corn yield in 2015 left more residual NO$_3^-$-N in the soil explaining the higher NO$_3^-$-N concentrations sampled from soil water compared to 2016. The higher growing season precipitation in 2015 compared to 2016 may have contributed to wet conditions restricting crop growth leaving residual soil NO$_3^-$-N in the soil post-harvest. Other studies have reported poor crop yield to be associated with high residual NO$_3^-$-N post-harvest (Randall and Mulla 2001; Kladivko et al. 2004; Jayasundara et al. 2007).

In 2015-2016, the combination of delaying fertilization to sidedress and applying UAN significantly reduced the soil water NO$_3^-$-N concentration compared to urea+NUI at planting (mean of 5.2 vs. 6.7 mg L$^{-1}$; Fig. 4a), although we are not able to determine which was the controlling factor (timing or product) and caution that method of application also differed between treatments. Regardless, we are able to state that in this year applying NUI with urea did not have a similar effect on soil water NO$_3^-$-N concentration as delaying fertilizer application to a time when crop uptake was larger. There were no significant differences between urea at planting and urea+NUI at sidedress (6.1 vs. 6.7, respectively; Fig. 4a), or between UAN+NUI at sidedress and UAN at sidedress (5.2 and 4.6 mg L$^{-1}$; Fig. 4a). In 2016-2017 there were no significant differences observed between treatments (Fig 4b).

*Nitrate-N leaching*

Nitrate leaching was calculated as the product of drainage and NO$_3^-$-N concentration (Fig. 5). Although monitoring of NO$_3^-$-N concentration occurred throughout the growing season in 2016, drainage was negligible and NO$_3^-$-N leaching was not significant. Discussion presented here focuses on non-growing season NO$_3^-$-N leaching. In 2015-2016 there were high NO$_3^-$-N
concentrations and low drainage compared with 2016-2017 where there were low NO$_3^-$-N concentrations and high drainage. In 2015-2016 there were several short NO$_3^-$-N leaching events in November and December, with a prolonged leaching event in January-March, followed by one more event (DOY 83; Fig. 5a.) before drainage ceased in April (DOY 97; Fig. 5a). In contrast, in 2016-2017 there was one very small event in December, followed by frequent events in January and February of small magnitude, before a larger event in March then a decrease until increasing again in April (Fig. 5b).

Differences in NO$_3^-$-N leaching between non-growing seasons were associated with amount of drainage and NO$_3^-$-N concentration. For example, in January-February 2016 when NO$_3^-$-N concentrations were high, NO$_3^-$-N leaching was also high when drainage events occurred (Fig. 4a and 5a). There was also a period of NO$_3^-$-N leaching in January-February 2017; however, NO$_3^-$-N concentrations were low and drainage values were low, thus leaching was minimal (Fig. 4b and 5b).

Significant differences in NO$_3^-$-N leaching between treatments were evaluated based on NO$_3^-$-N concentration comparisons given that the same drainage value was used for all treatments. In 2015-2016, the combination of delaying fertilization to sidedress and applying UAN significantly reduced NO$_3^-$-N leaching compared to urea+NUI at planting. There were no differences observed between other fertilizer treatments (Fig. 6a). Similarly, in 2016-2017 there were no differences observed between any treatments (Fig. 6b).

There was a trend of higher NO$_3^-$-N leaching in 2015-2016 than 2016-2017 likely due to higher NO$_3^-$-N concentrations, higher drainage, residual NO$_3^-$-N and non-growing season precipitation. Other studies have indicated these to be the main factors controlling NO$_3^-$-N leaching (Drury et al. 1996; Tan et al. 2002; Kladivko et al. 2004; Arregui and Quemada 2006;
Nitrate leaching was highest during January-March in both non-growing seasons, while other research has found the highest NO$_3^-$-N leaching losses in the fall from high precipitation and residual NO$_3^-$-N, although these studies did not evaluate winter NO$_3^-$-N leaching (Bergström and Brink 1986; Kladivko et al. 2004). Nitrate leaching is more prominent during the non-growing season as low ET with adequate P create ideal conditions for D and with adequate NO$_3^-$-N concentrations, leaching can occur. This is supported by research from Tan et al. (2002) and Drury et al. (1996) that found NO$_3^-$-N leaching occurred in this region only during the non-growing season of November-April. Similarly, Jayasundara et al. (2007) found that 80% of NO$_3^-$-N leaching occurred from November through April.

Fertilizer with UAN at sidedress resulted in significantly less NO$_3^-$-N leaching than urea+NUI at planting in 2016-2017 but it is difficult to ascertain what caused the observed difference since UAN was injected and also was a different form of N fertilizer than urea (Fig. 6a). Plant N use efficiency is maximized when fertilizer is applied several weeks after plant emergence by supplying N when plant N requirement is high (Aldrich and Hauck 1982). Thus, by increasing the synchronicity between fertilization and plant N demand, the UAN treatment could have maximized N use efficiency and resulted in significantly lower NO$_3^-$-N leaching compared to urea+NUI. However, this result is inconsistent with post-harvest 2015 and also 2016 soil NO$_3^-$-N levels at 0-15-cm depth since UAN had higher NO$_3^-$-N in the soil compared to the other treatments. Injection would have minimized NH$_3$ volatilization likely also keeping more N in the soil (Drury et al., 2017).

The lack of significant reduction in NO$_3^-$-N leaching between urea and urea+NUI in both experimental years (Fig. 6) could potentially be explained by NBPT and DCD favouring immobilization and maintaining N as NH$_4^+$ in the soil. Gioacchini et al. (2002) suggest that the
availability of NH$_4^+$ in the soil may stimulate organic matter mineralization and the release of mineral N promoting NO$_3^-$-N leaching losses. There have been several studies that have shown an increase in NO$_3^-$-N leaching from the use of urea+NUI (DCD+NBPT, specifically) (Gioacchini et al. 2002; Sanz-Cobena et al. 2012). Based on the findings of this study and previous research, the abatement effect of NUI may be countered by soil N retention, which may be why no difference was observed between urea and urea+NUI.

Although reduction in losses was expected with UAN+NUI compared to UAN, they did not occur. A study on N$_2$O emissions by Parkin and Hatfield (2014) also found no significant differences between UAN and UAN+NUI, although they did not evaluate NO$_3^-$-N leaching. However, an opposing result was found by Ferrari Machado (2017) with UAN+NUI significantly reducing N$_2$O emission compared with UAN, which is in agreement with other studies (Halvorson et al. 2010). Ferrari Machado (2017) suggests that the beneficial properties of N can be negated by high water filled pore space after application. Thus, no difference may have been found between UAN+NUI in this study as there was high water filled pore space after application.

When expressing the NO$_3^-$-N as a fraction of N fertilizer applied we observe slightly higher values in 2015/2016 for urea+NUI and urea at planting (13.6% and 12.5%) than for UAN and UAN+NUI at sidedress (9.9% and 8.8%). This is comparable to results from Jayasundara et al. (2007) that found a NO$_3^-$-N leaching loss of 16% from corn in a conventional system; however, use of the 15N tracer indicated only 2% of this was derived from fertilizer. Thus, a low percentage of NO$_3^-$-N leaching losses are likely directly from fertilizer and some of the N lost could be derived from mineralization of organic matter. Thus, there is a large percentage of N
that is unaccounted for that could be lost by other pathways such as denitrification (N₂O), or NH₃ volatilization or immobilized in the soil as organic N.

CONCLUSIONS

Drainage was only significant in the non-growing season, therefore only this period was evaluated for NO₃⁻-N leaching. There were differences in precipitation between the growing seasons preceding the non-growing seasons with more precipitation received in 2015-2016 than 2016-2017. The environmental conditions during the non-growing seasons were also very different as 2016-2017 received more precipitation, had higher ET and higher D, experienced greater variation in 5-cm soil water content and change in soil water storage due to thawing events. Also, 2015-2016 had higher NO₃⁻-N concentration in sampled soil water due to higher soil residual NO₃⁻-N from a poor corn yield, compared to 2016-2017. As a result, NO₃⁻-N leaching showed a trend of higher values in 2015-2016 than 2016-2017.

Combining the 4R fertilizer strategies of right product and right timing was shown to influence NO₃⁻-N leaching. Neither fertilizer products applied with NUI at planting or sidedress proved to significantly reduce NO₃⁻-N leaching. Applying UAN at sidedress presented the best management practice to reduce NO₃⁻-N leaching presumably due to synchronizing nutrient supply with plant requirement. However, these results were inconsistent, and a significant difference was only observed in 2015-2016. Based on these results, applying UAN at sidedress is recommended, although additional study years are needed to confirm results.

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Figure Captions

Figure 1. Cumulative gap-filled ET for Nov to May in 2015-2016 (black) and 2016-2017 (grey). DOY = day of year.

Figure 2. Daily soil volumetric water content (θ) at 5-cm (light black), 25 (light grey), 55-cm (black) and 85-cm (dark grey) depths, soil temperature at 5-cm (soilT; medium grey) and air temperature (airT; dotted line) from November 2015 to May 2016 (a) and November 2016 to May 2017 (b). Gaps in data are from equipment failure or removal for planting/harvesting operations.

Figure 3. Weekly components of the water budget: P (rainfall + snowmelt; bar graph with horizontal line), ET (evapotranspiration; black line), D (drainage; dashed line) and ΔS (change in storage; dashed line) from November 2015 to May 2016 (a) and November 2016 to May 2017 (b).

Figure 4. The NO₃-N concentrations at 80-cm depth during the non-growing seasons for urea (light grey), urea+NUI (light black), UAN+NUI (black) and UAN (dark grey) from November 2015 to May 2016 (a) and November 2016 to May 2017 (b). Error bars represent standard error of mean. Note that NO₃-N was monitored throughout the growing season; however, D was negligible and as such only the non-growing season was included in this study.

Figure 5. Weekly amounts of NO₃-N leached during the non-growing seasons for urea (light grey), urea+NUI (light black), UAN+NUI (black) and UAN (dark grey) from November 2015 to May 2016 (a) and November 2016 to May 2017 (b). Note that NO₃-N was monitored throughout the growing season; however, D was negligible and thus, NO₃-N leaching did not occur.
Figure 6. Paired NO$_3$-N leaching comparisons between treatments of urea (white) vs. urea+NUI (black), UAN (diagonal cross) vs, UAN+NUI (dotted), and urea (black) + NUI vs. UAN (diagonal cross) from November 2015 to May 2016 (a) and November 2016 to May 2017 (b). Asterisk denotes significant differences of paired means according to the Wilcoxon sign rank test applied at a significance level of 0.0167 (=0.05/3 or significance level divided by the number of paired tests; Bonferroni correction) to reduce the chance of receiving a significant result due to chance and account for pseudo-replication. Error bars represent mean uncertainty +/- 10\%.
Table 1. Average monthly temperature and monthly precipitation (rainfall + snowfall) at Elora Research Station compared with climate normals (1981-2010) (Waterloo Wellington: http://climate.weather.gc.ca/climate_normals/index_e.html).

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<th>Month</th>
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*aDaily average temperature (°C) in May 2017 of 10.8.*
Figure 1

143x102mm (240 x 240 DPI)
Figure 2

99x121mm (240 x 240 DPI)
Figure 3

93x120mm (240 x 240 DPI)
Figure 4

88x119mm (240 x 240 DPI)
Figure 5

90x118mm (240 x 240 DPI)
Figure 6

82x120mm (240 x 240 DPI)