FINAL REPORT

PROJECT TITLE: Balancing Production Gains against Environmental Impacts: A Study to Quantify Water and Air Quality Effects of Nitrogen Management on a Yield-Scaled Basis

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ABSTRACT

Modification of nitrogen (N) fertilizer application timing within the growing season has the potential to reduce soil nitrous oxide (N_2O) emissions and nitrate (NO_3^-) leaching but limited data are available to assess its effects. We compared cumulative growing season N_2O emissions (cN_2O) and soil NO_3^- intensity (SNI) following urea applied to corn (*Zea mays* L.) in a single application (SA) at planting or in three split applications (SpA) over the growing season. For both SA and SpA, granular urea was broadcast and incorporated at six fertilizer N rates in the corn phase of a corn-soybean [*Glycine max* (L.) Merr.] rotation and in a continuous corn system over two growing seasons. Daily N_2O flux was measured using chambers on 35 dates in 2012 and 40 dates in 2013 and soil NO_3^- -N concentration was measured weekly. Split application did not affect grain yield and did not reduce cN_2O. In contrast with expectations, split application actually increased cN_2O by 57% compared to single application at the highest N rate (210 kg N ha^{-1}). Conversely, split application did reduce SNI compared to single application, but only at the highest N rate (210 kg N ha^{-1}). Across N rates and rotations, cN_2O was 55% greater with SpA compared with SA in 2012. Increased cN_2O with SpA in 2012 likely resulted from a prolonged dry period prior to the second split application followed by large rainfall events following the third split application. Exponential relationships between cN_2O and fertilizer N rate explained 62 to 74% of the variance in area-based cN_2O and 54% of the variance in yield-based cN_2O. Applying urea to coincide with periods of high crop N demand does not necessarily reduce and may increase N_2O emissions. This project provided valuable information to corn farmers in demonstrating that the labor- and/or energy-intensive practice of supplying split N applications by itself may not have environmental benefits. Further study is needed to develop reliable mitigation strategies that can reduce N_2O and SNI while maintaining crop yields.

INTRODUCTION

Modification of nitrogen (N) management practices to reduce emissions of nitrous oxide (N_2O) has been identified as a strategy for reducing the greenhouse gas footprint of agricultural cropping systems (Ogle et al., 2014). Altering the timing of N fertilizer application is frequently mentioned as a potentially effective practice for reducing all forms of reactive N loss from fertilized soil including N_2O (Smith et al., 2007; Robertson and Vitousek, 2009; Ribaudo et al., 2011). For the majority of large-scale crop production systems, N fertilizer is often applied well before the crop has developed to a stage where the N can be efficiently assimilated. A survey of corn producers in Minnesota reported that only 9% of growers applied N fertilizer after planting (Bierman et al., 2012). The demand of the corn plant for N is low during early growth stages but increases and remains high for several weeks into the growing season (Abendroth et al., 2011). Therefore, applying N later in the growing season or in multiple split applications distributed across the growing season could improve the synchrony between soil N availability and crop N demand and reduce the amount of soil N available for conversion to N_2O. However, the benefits of altered N fertilizer timing on N_2O emissions are in question due to the limited number of studies and their inconsistent results.

A few studies have compared single early-season N applications to split N applications distributed over the growing season. Zebarth et al. (2012) found no effect of single versus split application timing on N_2O emissions in a potato system. Burton et al. (2008) found reduced N_2O emissions with split application in one of two growing seasons. Split N application reduced N_2O emissions in sugar cane in Australia when 200 kg N ha^{-1} was applied, but did not affect N_2O when 100 kg N ha^{-1} was applied (Allen et al., 2010). One process-based N_2O emissions model that accounts for crop N uptake and soil N transformations predicted that N_2O emissions will decrease as the number of N fertilizer applications during the growing season increase (Hu et al., 2012) while another model was relatively insensitive to single versus split application (Del Grosso et al., 2009). While not examining split application per se, other studies have compared single N applications applied early versus later in the growing season. Phillips et al. (2009) found no significant difference in N_2O emissions following urea applied to corn 6 wk prior to planting compared to 3 d prior to planting. Similarly, Zebarth et al. (2008) found no difference in N_2O emissions following ammonium nitrate applied to corn at emergence compared to growth stage V6. Drury et al. (2012) found that urea application to corn at growth stage V6 decreased N_2O by 33% compared to pre-plant application in a conventional tillage system, but application timing had no effect on N_2O in no-tillage or zone-tillage systems. To date, no studies have evaluated effects of single versus split N fertilizer application on N_2O emissions in corn production systems in the U.S.

The total rate of N fertilizer applied to the field is usually the most reliable predictor of N_2O emissions (Shcherbak et al., 2014). Differences in the quantity and/or quality of crop residues from prior growing seasons or other residual effects of cropping history may also affect N_2O emissions (Mosier et al., 2006; Drury et al., 2008; Halvorson et al., 2008; Omonode et al., 2011). Thus, N fertilizer application timing effects may be influenced by the N rate as well the crop rotation. No studies to date have evaluated N application timing effects on N_2O emissions across a wide range of N rates or in different crop rotations.

OBJECTIVE AND GOAL STATEMENTS

The objective of this study was to examine the effects of single versus split fertilizer application on cumulative N_2O emissions and soil N availability in continuous corn (CC) and corn-soybean (CS) cropping systems across a range of N rates and over two consecutive growing seasons on a silt loam soil in Minnesota USA. Our aim was to investigate a practice that has the potential to reduce N_2O emissions, and not necessarily to simulate a practice that is currently common in the region. Our general hypothesis was that split application would reduce N_2O emissions with an expectation of interactions of timing with N rate, rotation, and/or year.

MATERIALS AND METHODS

Site Description and Experimental Design

The experiment was conducted in long-term research plots at the University of Minnesota Research Station in Rosemount, MN (44°45' N, 93°04' W). The soil is a naturally drained Waukegan silt loam containing 220 g kg^{-1} sand, 550 g kg^{-1} silt, and 230 g kg^{-1} clay with pH (in 0.01 M $CaCl_2$) of 6.0. The 30-yr (1984-2013) mean annual precipitation and temperature are 748 mm and 7.7°C, respectively (MCWG, 2014). The plots used in this study are part of a long-term experiment with tillage and rotation treatments in place since 1991 (Venterea et al., 2010). A 2-yr experiment (2012 and 2013) was conducted using a randomized complete block, split-split plot design with rotation as the main effect, fertilizer N application rate as the split-plot effect, and fertilizer N application timing as the split-split-plot effect. Each year, three main plots in a CC rotation and three main plots in the corn phase of a CS rotation were randomly subdivided into six split-plots; one split-plot (the control) received no N fertilizer, while the other five split-plots received one of five fertilizer N rates (50, 90, 130, 170, or 210 kg N ha^{-1}) which brackets the recommended rates for this region of Minnesota (Randall et al., 2008). Long-term management of these plots has used the same rate of N application (146 kg N ha^{-1}) to both rotations in order to avoid confounding rotation with N management. Each of the 5 split-plots receiving fertilizer N were further subdivided into two split-split-plots, which were randomly assigned to a single application (SA) at planting or to three split applications (SpA) distributed over the growing season.

For the SpA treatments, one-third of the total N was applied in each of three applications. The first application occurred at planting coinciding with the SA treatment. The second and third applications occurred when the crop was at vegetative growth stage V6 and V14, respectively, which correspond with high rates of N accumulation in plant tissue (Abendroth et al., 2011). The V6 applications were made on 3 July 2012 and 9 July 2013 and the V14 applications were made on 24 July 2012 and 28 July 2013. Urea was hand-applied and immediately incorporated into the soil manually using metal garden rakes with 100-mm long tines. Corn was planted at 79,000 seeds ha^{-1} on 15 May 2012 (Cropland 3337VT2P) and on 16 May 2012 (Pioneer P0193AM) using a John Deere model 7100 MaxEmerge planter. After physiological maturity (on 24 Sep. 2012 and 18 Oct. 2013), corn ears were harvested from plants in the middle 4 rows of each split-plot. Ears were initially dried at 35°C, shelled, and further dried for 3 d at 65°C and weighed to obtain dry grain yield.

Each main plot was 27.4 m (36 rows with 0.76-m row spacing) wide by 61 m long, and each split-split plot was 4.57 m (6 rows) wide by 4.57 m long. Areas within the inner four rows of each split-split plot were used for collection of gas, soil and plant samples. The same set of three main plots was used both years in the CC rotation; in these plots, the plot areas were relocated each year to avoid re-using the same ground. In the CS rotation, a different set of main plots were used each year in accordance with the crop rotation. The main plots used in both rotations were selected from the same long-term tillage treatment (designated as 'conservation tillage'). According to the design of the long-term study, this tillage regime differed depending on the crop grown each year, with fall stalk chopping followed by fall disk-ripping occurring after corn and no fall tillage occurring after soybean. Thus, the rotation treatments of the long-term study and of this 2-yr study also have a tillage component embedded within them. Both rotations received secondary tillage (tandem disking) prior to planting each spring.

Nitrous Oxide Emissions

Soil-to-atmosphere N_2O fluxes were measured using static chambers (Venterea et al., 2010) on 35 dates between 12 Apr. and 5 Sep. in 2012 and on 40 dates between 1 May and 29 Sep. in 2013. Prior to planting, fluxes were measured weekly in two locations within each main plot. Following planting, fluxes were measured twice per week at one location in each split-split-plot. Approximately 4,500 individual N_2O flux measurements were collected over the 2-yr study period. In each split-split-plot, one stainless steel chamber anchor (0.50 m × 0.29 m) equipped with a 20-mm wide by 5-mm deep flange around its perimeter was installed to a depth of 0.07 m with the flange resting directly on the soil surface. On each sampling day between 1000 and 1300 local time, insulated and vented chamber tops (0.50 m ×

0.29 m × 0.10 m high) each also equipped with a 20-mm by 5-mm flange around their perimeter were sealed to the anchors by attaching the chamber flange to the anchor flange using binder clips. Gas samples were collected using 12-mL polypropylene syringes 0, 0.5, 1 and 1.5 h after sealing the chamber. Samples were immediately transferred to glass vials sealed with butyl rubber septa (Alltech, Deerfield, IL) and analyzed within 1 wk using a headspace autosampler (Teledyne Tekmar, Mason, OH) connected to a gas chromatograph (model 5890, Agilent /Hewlett-Packard, Santa Clara, CA) equipped with an electron capture detector. The equipment was calibrated with analytical grade standards (Scott Specialty Gases, MI) each day when samples were analyzed. Gas concentrations in molar mixing ratios were converted to mass per volume concentrations using ideal gas law and air temperatures at sampling. Fluxes of N_2O were calculated from the rate of change in chamber N_2O concentration using the restricted quadratic regression procedure (Parkin et al., 2012) and the chamber bias correction method to account for suppression of the surface-atmosphere concentration gradient (Venterea, 2010; Venterea and Parkin, 2012).

Soil and weather measurements

During each N_2O flux sampling period soil temperature (ST) and soil moisture content (SMC) were measured in two of the 11 split-split-plots within each main plot. Soil temperature was measured by inserting a temperature probe (Fisher, Hampton, NH) to the 0.05-m depth within 1 m of the chamber. Soil cores were collected from midway between the row and mid-row positions (referred to as the ¼-row position) within 1 hr of each flux measurement period using 0.05-m diameter brass rings to a depth of 0.05 m. Gravimetric water content was determined by drying at 105^{o}C. Additional soil samples were collected weekly for analysis of extractable soil N concentration. On each sampling date, two cores from each split-split-plot collected, one from the mid-row position and one from the ¼-row position, each to a depth of 0.15 m, and composited into a single bag. Samples were placed in a cooler and delivered to the lab within 2 h where approximately 10-g sub-samples were extracted in 40 mL of 2 M KCl, filtered (Whatman no. 42), and analyzed for total nitrite-N (NO_2^--N) plus nitrate-N (NO_3^--N) using the Greiss-Ilosvay method with cadmium reduction (Mulvaney, 1996) modified for use with a flow-injection analyzer (Lachat, Loveland, CO). Extractions and analyses were performed within 24 h of sample collection using procedures designed to minimize potential losses of NO_2^- during sample processing (Stevens and Laughlin, 1995). The sum of NO_2^- plus NO_3^- concentrations on a dry weight basis are reported here and referred to as 'soil nitrate-N' (SN). Gravimetric SMC over the 0-0.15 m depth was also determined in the soil samples collected weekly. A weather station located 1 km from the plots was used to collect air temperature and precipitation data which were recorded at 30-min intervals and converted to daily average and daily totals, respectively.

Data Analysis

Daily N_2O fluxes (dN_2O) measured on each sampling date were used to determine cumulative growing season N_2O emissions (cN_2O) by trapezoidal integration versus time (Parkin and Venterea, 2010). The fertilizer-induced N_2O emission factor (EF) was calculated for each treatment by subtracting cN_2O in the control treatment of each main plot from cN_2O in each treatment of that plot, dividing by the amount of fertilizer N applied, and expressing the result as a percentage (Venterea et al., 2012). Soil nitrate-N concentrations for each sampling date were used to determine soil nitrate-N intensity (SNI) by trapezoidal integration versus time (Burton et al., 2008). Yield-based N_2O emissions (cN_2O-y) and yield-based SNI (SNI-y) were calculated by dividing cN_2O and SNI, respectively, by grain yield (Mosier et al., 2006). Data were analyzed at $P \leq 0.05$ using the MIXED procedure of SAS (SAS Institute, 2006). In order to maintain a balanced experimental design, data from the non-fertilized control treatment, which lacked a fertilizer N application timing, were excluded from tests of fixed effects and mean comparisons but were included in regression and correlation analyses. Year, crop rotation, fertilizer N rate, and fertilizer N application timing were considered fixed effects and block and interactions with block were considered random effects. Residuals were assessed for normality and common variance using the UNIVARIATE procedure of SAS and scatterplots of residuals versus predicted values (Kutner et al., 2004). Data for cN_2O emissions, cN_2O-y, SNI, SNI-y, and EF did not meet the assumptions of normality and common variance, so data for these dependent variables were logarithm base 10 transformed prior to statistical analysis.

Mean comparisons were made using independent pairwise *t* tests at $P \leq 0.05$ using the PDIFF option in the MIXED procedures of SAS (SAS Institute, 2006). When the main effect of fertilizer N rate or interactions among fertilizer N rate and other fixed effects were significant at $P \leq 0.05$, linear and nonlinear regression equations were developed to describe the response of the dependent variables to fertilizer N rate using the MIXED and NLIN procedures of SAS (SAS Institute, 2006), respectively. Several regression models were evaluated based on scatterplots of residuals versus predicted values (Kutner, 2004), and selected regression models were significant at $P \leq 0.05$. The agronomic optimum fertilizer N rate for corn grain yield were predicted by setting the first derivative of the fit quadratic regression equation to zero

Linear associations between dN_2O, SMC, ST and SN, and between cN_2O and SNI were evaluated for each combination of year, crop rotation, and fertilizer N application timing with Pearson's correlation coefficient (*r*) at $P \leq 0.05$ using the CORR procedure of SAS (SAS Institute, 2006). Because SN was measured weekly and dN_2O was measured twice weekly and not necessarily on the same day as SN, linear interpolation was used to estimate SN for association with dN_2O data. Linear multiple regression models with dN2O as dependent variable and SMC, ST and SN as potential independent predictors were evaluated for each combination of year, crop rotation, and fertilizer N application timing using the REG procedure of SAS (SAS Institute, 2006). Models for all combinations of one, two, or three of these predictors were evaluated and final models were selected based on the adjusted R^2, Mallow's Cp, Akaike's, and Schwarz' Bayesian criteria (Kutner et al., 2004). Selected regression models and all parameter estimates were significant at $P < 0.001$.

RESULTS AND DISCUSSION

Weather

Total precipitation amounts during April through September were similar in 2012 (646 mm) and 2013 (591 mm) compared to the 30-yr mean of 542 mm (Table 1, Fig. 1a). In 2012, more of the precipitation occurred in larger rainfall events. There were 9 d in 2012 during which > 31 mm of precipitation was recorded compared to only one day in 2013. Periods with lower than normal rainfall and dry soil conditions occurred during the latter half of both growing seasons. In 2012, a dry period persisted from two weeks prior to until one week following the V6 urea application (i.e., 21 June through 12 July). During this period, the seasonal maximum ST values (> 28^oC) and minimum SMC values were observed, with SMC at 0-0.05 m remaining below 0.12 g H_2O g^{-1} on four consecutive sampling dates (Figs. 1b-c). A second dry period in 2012 started on 16 Aug, 3 wk following the V14 application, after which only 18 mm of rain was recorded compared to the 30-yr mean of 72 mm for the month of September. Soil moisture content steadily declined over the last four sampling dates of the 2012 season. In 2013, SMC steadily declined starting 1 wk after the V14 application on 28 July; only 98 mm of precipitation was recorded for the months of August and September combined, compared to the 30-yr mean of 180 mm.

Table 1. Rainfall patterns by month and growing season.

Period	2012	2013	30-yr mean†
April	80	146	71
May	188	148	99
June	153	117	103
July	117	82	89
Aug	92	62	108
Sept	14	36	72
Total	646	591	542

† Calculated for 1984-2013 using data available at http://climate.umn.edu/doc/historical.htm.

Grain yield

Fertilizer N rate was the only factor that had a significant effect on corn grain yield (Table 2). Across years, crop rotations, and N application timings, there was a quadratic response of grain yield to fertilizer N rate (R^2 = 0.89, Fig. 2), with maximum yield occurring at 155 kg N ha^{-1}. Widely varying effects of split N application on corn yield have been reported, including no effects (Randall et al., 1997) increased yield in some but not all growing seasons (Jaynes, 2013); and decreased yield (Jaynes and Colvin, 2006). The lack of a yield response to timing across a range of fertilizer N rates in the current study suggests that application timing had no overall effect on crop utilization of fertilizer N across the whole growing season. It is possible there was more efficient crop N utilization associated with the first SpA application compared to the larger single SA application at planting which could have promoted greater N loss via leaching of soluble N below the undeveloped root zone. However, lower N losses and more efficient crop N utilization associated with the first SpA application could have been offset by greater N losses and/or less efficient crop N utilization of the mid-season (V6 and V14) applications. Lack of timely rainfall and relatively low SMC during specific periods before and after the V6 and V14 applications each year may have resulted in limited mobilization of fertilizer N throughout the soil profile which may have restricted root N uptake. Volatilization of ammonia (NH_3) may have also contributed to lower crop N utilization following the mid-season applications due to warmer conditions which can promote NH_3 loss (Jones et al., 2013). We incorporated the urea following each application consistent with recommended practices and with the majority of Minnesota farmers according to a recent survey (Bierman et al., 2012). Even though mechanical incorporation following mid-season applications may be impractical or require the use of high-clearance equipment, we decided to incorporate after every application to reduce the potential for NH_3 losses. In the absence of incorporation, rainfall of at least 0.5 inches (13 mm) occurring in a single event within 24 to 48 h of application is also recommended as a means to incorporate urea and to reduce NH_3 losses (Jones et al., 2013). However, relying on expected rainfall risks delaying the application; in the current study, this practice likely would have resulted in later application and inconsistent timing with respect to growth stage between growing seasons, since prolonged dry periods occurred in 2012 when the crop was at V6 and in 2013 when the crop was at V14. Relying on weather forecasts also risks high NH_3 losses in the event of lower than predicted rainfall amounts. Thus, mechanical incorporation of urea represents a best possible case for minimizing NH_3 losses under the given conditions; nonetheless, significant NH_3 losses may have occurred.

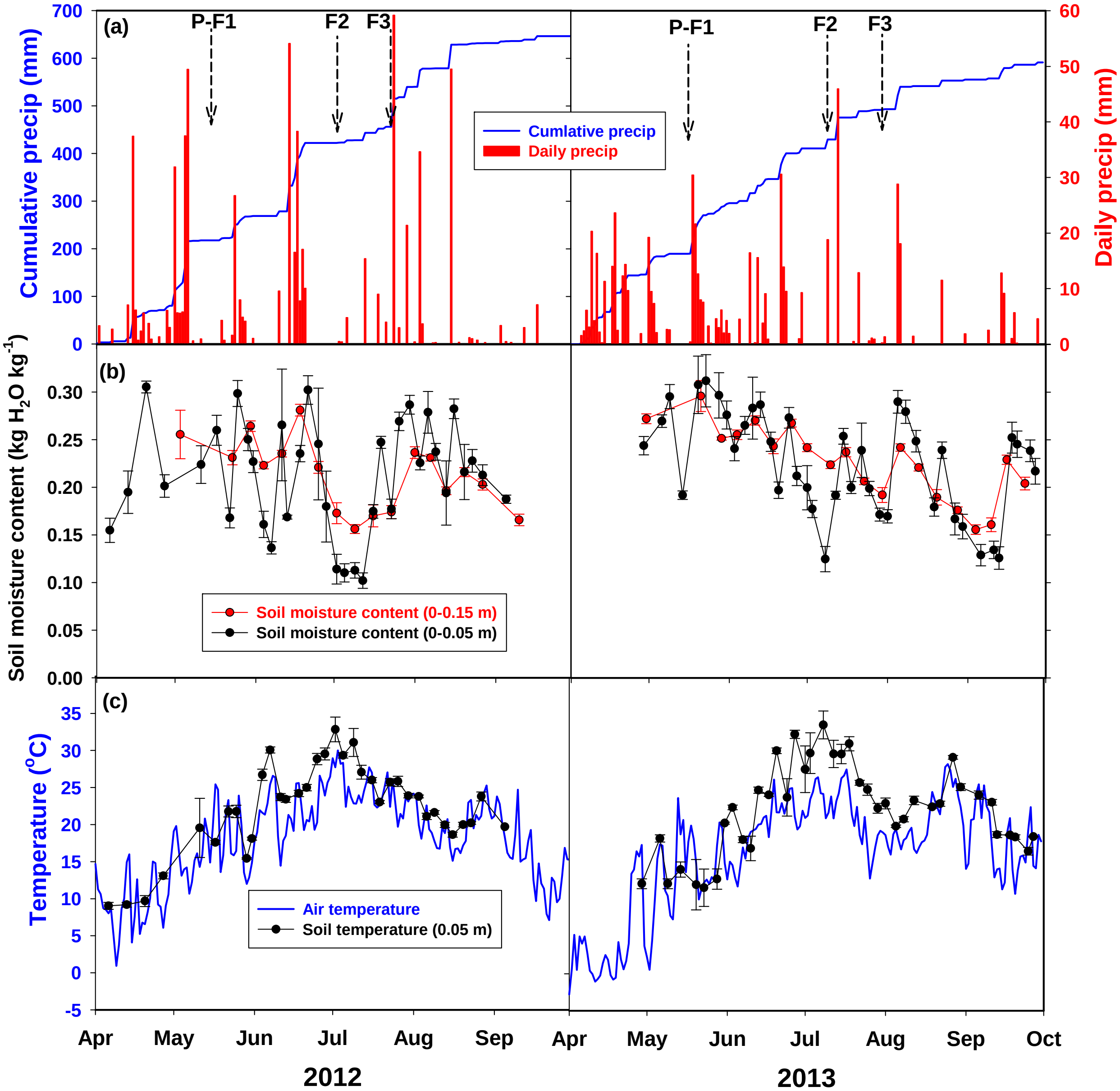

Fig. 1. (a) Daily and cumulative precipitation, (b) soil moisture content, and (c) air and soil temperature. Downward-pointing arrows indicate dates of planting (P) and fertilizer N application (F). Single application treatments received 100% of their N at F1, and the split-application treatments received three equal applications at F1, F2 and F3.

Table 2. Significance of *F*-values for fixed sources of variation†.

Source of variation	Corn grain yield	Cumulative N_2O emissions (cN_2O)	N_2O emissions factor (EF)	Yield-scaled cumulative N_2O emissions (cN_2O-y)	Nitrate-N intensity (SNI)	Yield-scaled nitrate-N intensity (SNI-y)
		$P > F$				
Year	0.081	0.224	0.200	0.310	0.844	0.204
Crop rotation (rotation)	0.628	0.039	0.029	0.095	0.022	0.350
N rate (rate)	0.003	<0.001	0.542	<0.001	<0.001	<0.001
N application timing (timing)	0.227	0.010	0.054	0.003	0.001	0.009
Year × rotation	0.257	0.215	0.066	0.103	0.052	0.191
Year × rate	0.732	0.073	0.505	0.227	0.336	0.721
Year × timing	0.610	<0.001	<0.001	<0.001	<0.001	<0.001
Rotation × rate	0.645	0.680	0.663	0.562	0.249	0.569
Rotation × timing	0.265	0.485	0.173	0.481	0.769	0.647
Rate × timing	0.539	0.039	0.156	0.162	0.007	0.012
Year × rotation × rate	0.591	0.245	0.035	0.346	0.137	0.606
Year × rotation × timing	0.544	0.265	0.160	0.435	0.105	0.050
Year × rate × timing	0.138	0.534	0.961	0.417	0.132	0.252
Rotation × rate × timing	0.986	0.922	0.789	0.951	0.709	0.531
Year × rotation × rate × timing	0.887	0.720	0.629	0.617	0.555	0.599

† Statistical analysis of cN_2O, EF, cN_2O-y, SNI and SNI-y are based on logarithm base 10 transformed data.

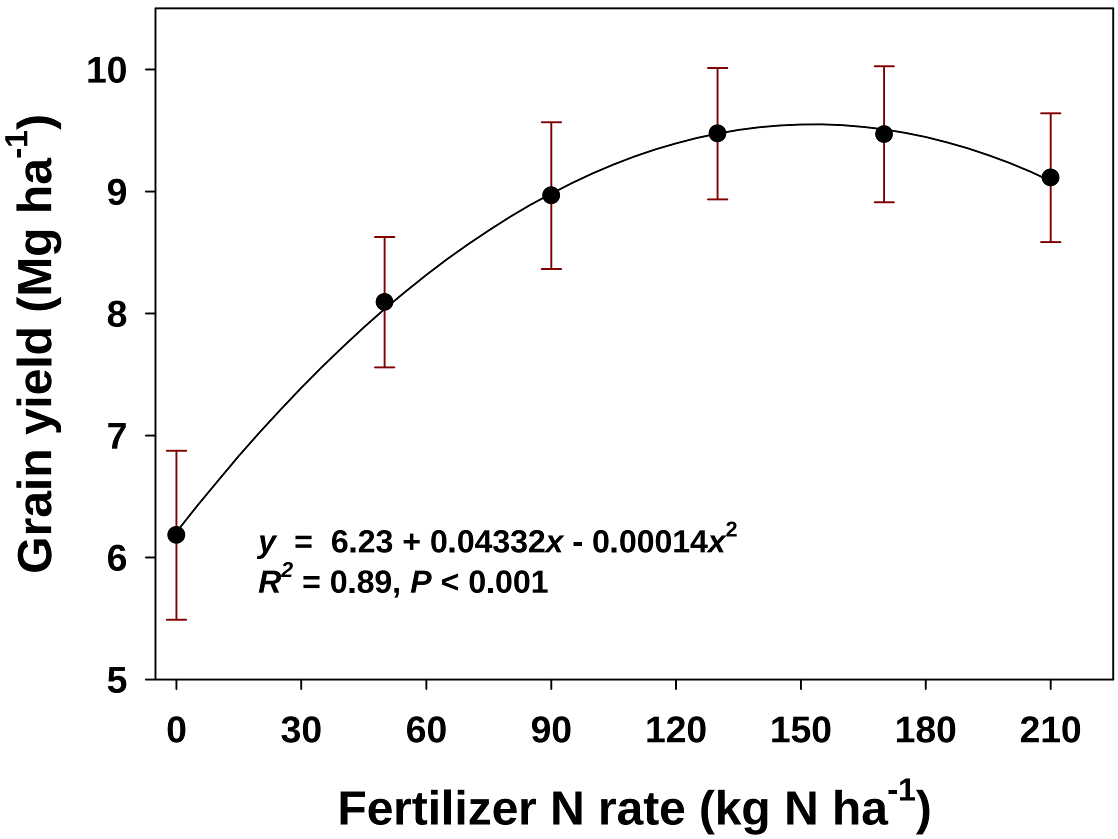

Fig. 2. Response of corn grain yield to fertilizer N rate, across years, crop rotations, and fertilizer N application timings.

N_2O flux (dN_2O) and soil nitrate-N concentration (SN)

Daily mean N_2O fluxes ranged from below 10 to approximately 1000 μg N m^{-2} h^{-1}(Fig. 3). Several episodic increases followed by decreases in dN_2O were observed at different periods during each growing season. Soil nitrate-N concentration ranged from below 1.0 to approximately 90 ug N g^{-1} (Fig. 4). Segregated by year, rotation, and timing, dN_2O was positively correlated with SN in all but one case, positively correlated with SMC, and negatively correlated with ST in some cases (Table 3). Significant regression models using combinations of SMC, ST, and/or SN as predictors were obtained, with R^2 values ranging from 0.08 to 0.42 (Table 3). When included in multiple regression models together with SMC and SN, ST had a positive association with dN_2O.

Cumulative N_2O emissions (cN_2O and cN_2O-y)

Crop rotation had a significant effect on cN_2O (Table 2). Across years, application timings, and fertilizer N rates, cN_2O in the CC rotation (1.57 kg N ha^{-1}) was significantly greater than in the CS rotation (1.05 kg N ha^{-1}). There were significant rate-by-timing and year-by-timing interaction effects on cN_2O (Table 2). Across crop rotations and N rates, the SpA treatments in 2012 had greater cN_2O compared with the SA treatments in 2012 and compared with the SpA treatments in 2013 (Table 4). Across growing seasons and crop rotations in treatments receiving the maximum N fertilizer rate (210 kg N ha^{-1}), cN_2O was greater with SpA (2.47 kg N ha^{-1}) compared with SA (1.57 kg N ha^{-1}). However, N application timing had no significant effect on cN_2O across growing seasons and crop rotations in treatments receiving < 210 kg N ha^{-1}. Accordingly, the response of log-transformed cN_2O to fertilizer N

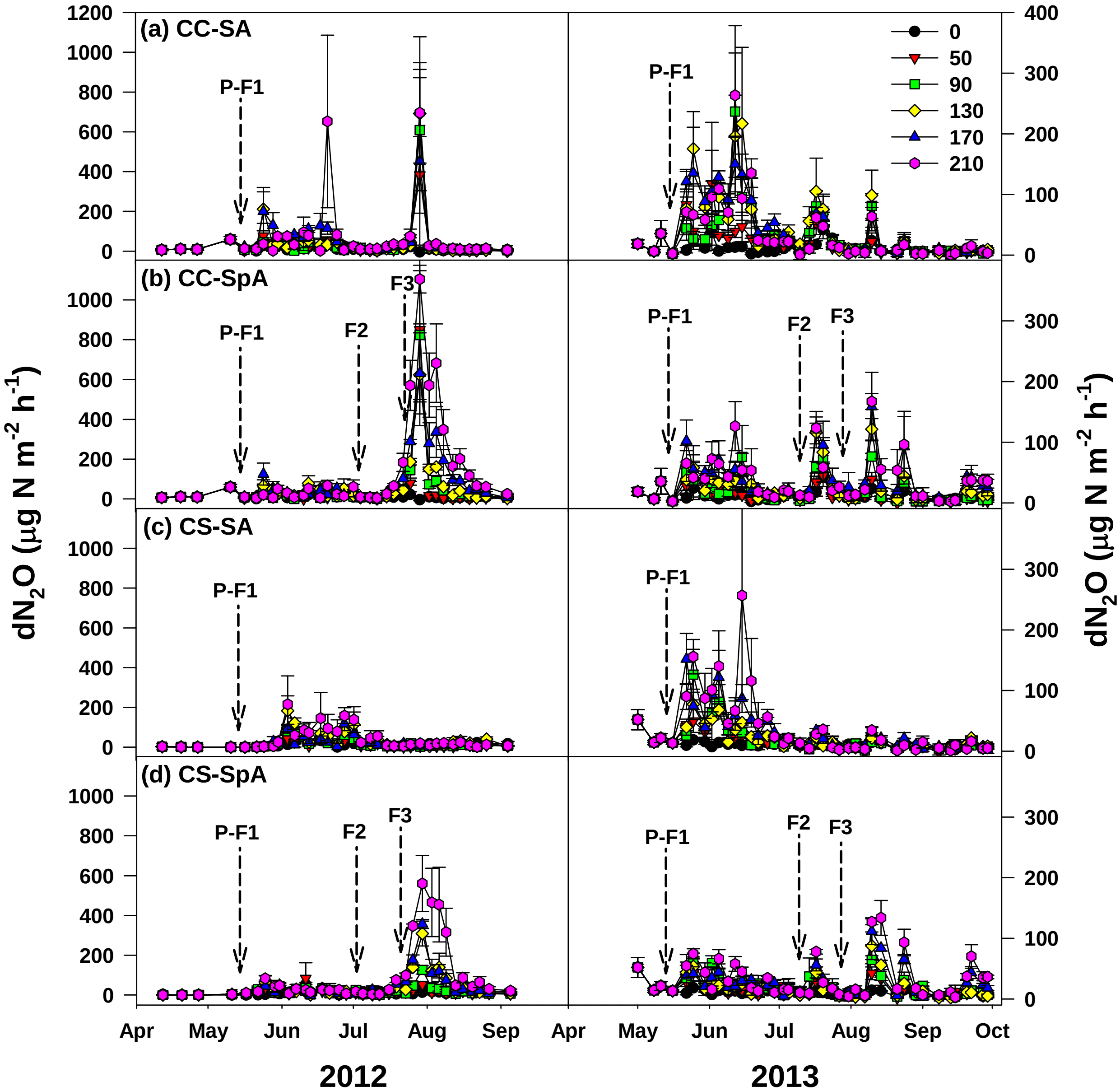

Fig. 3. Daily N_2O fluxes (dN_2O) in continuous corn (CC) and corn-soybean (CS) rotations receiving single (SA) and split (SpA) fertilizer applications. Downward-pointing arrows indicate dates of planting (P) and fertilizer N application (F). Single application (SA) treatments received 100% of their N at F1, and split application (SpA) treatments received three equal applications at F1, F2 and F3. Note different scales on right and left vertical axes.

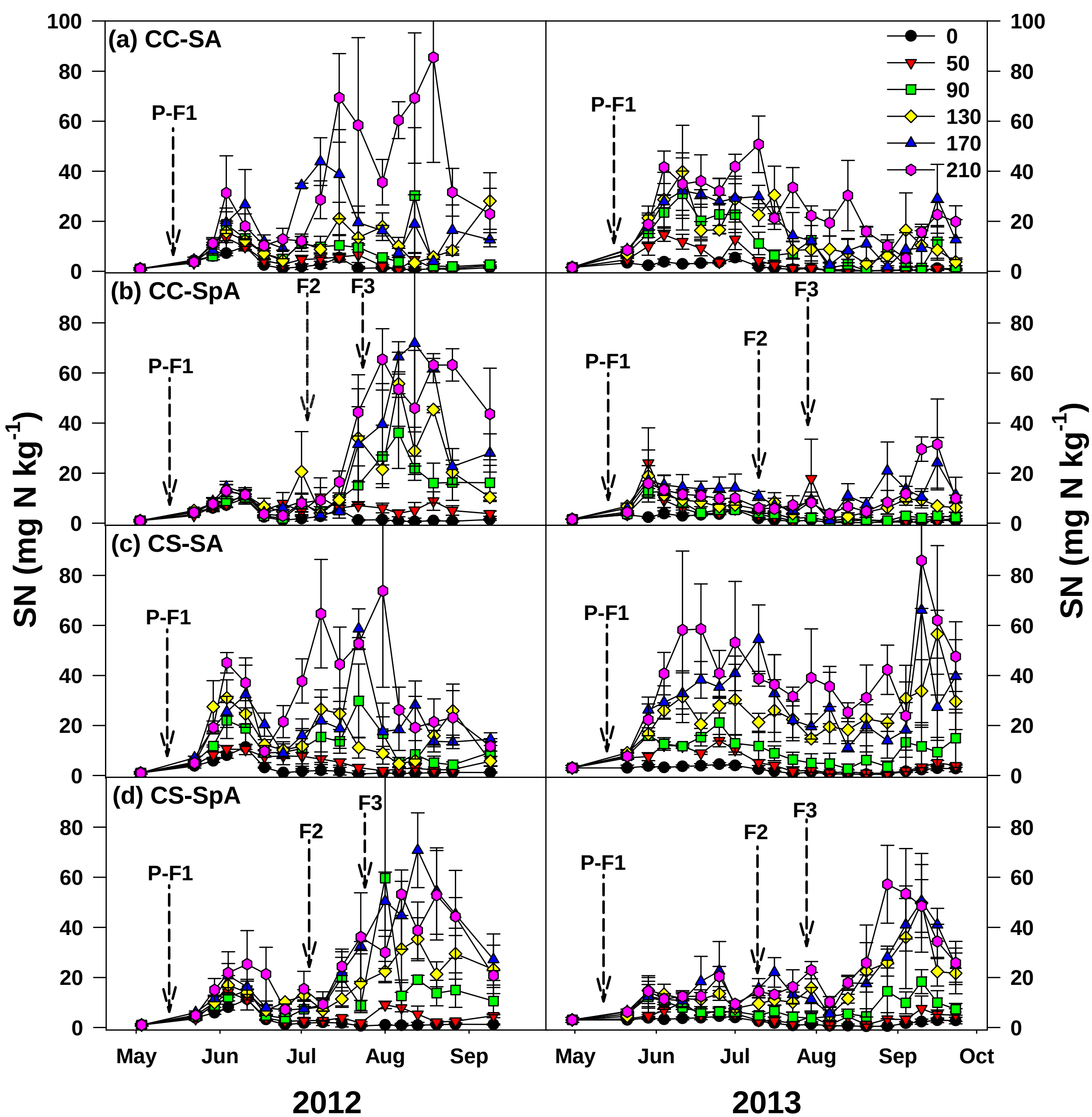

Fig. 4. Soil nitrate-N concentrations (SN) in continuous corn (CC) and corn-soybean (CS) rotations receiving single (SA) and split (SpA) fertilizer applications. Downward-pointing arrows indicate dates of planting (P) and fertilizer N application (F). Single application (SA) treatments received 100% of their N at F1, and split application (SpA) treatments received three equal applications at F1, F2 and F3.

rate was fit to a linear regression model (r^2 = 0.74) for the SA treatments, and to a quadratic regression model (R^2 = 0.62) for the SpA treatments (Fig. 5a). When cumulative N_2O emissions were expressed on a yield-scaled basis, there was a significant year-by-timing interaction effect on cN_2O-y. Consistent with the cN_2O result, cN_2O-y was greater with SpA compared with SA only in 2012, and cN_2O-y in the SpA treatments was greater in 2012 than 2013 (Table 4). In contrast with cN_2O, crop rotation had no significant effect on cN_2O-y, and there was no rate-by-timing interaction effect. The response of log-transformed cN_2O-y to fertilizer N rate across all treatments was fit to a quadratic regression model (R^2 = 0.62) (Fig. 5b).

Fertilizer-induced emissions factors (EF)

There was a significant year-by-crop rotation-by-N rate interaction effect on EF (Table 2). Across timing treatments, significant differences in EF by crop rotation and fertilizer N rate were present in 2012 but not in 2013 (Table 5). In 2012, EF was greater in the CC than in the CS rotation at all N rates except 130 kg N ha^{-1}; differences by fertilizer N rate were observed but were not consistent across crop rotations. Regression analysis of EF versus N rate did not generate significant models for any combination of year and crop rotation. There was also a significant year-by-timing interaction effect on EF. Consistent with cN_2O and cN_2O-y, across crop rotations and N rates, the SpA treatments in 2012 had greater EF than the SA treatments in 2012 and the SpA treatments in 2013 (Table 4). In 2013, EF was greater with SA than SpA.

Soil nitrate-N intensity (SNI and SNI-y)

Crop rotation had a significant effect on SNI (Table 2). Across years, timings, and fertilizer N rates, SNI in the CS rotation (2.45 mg N d g^{-1}) was significantly greater than in the CC rotation (1.80 mg N d g^{-1}). There was also a significant rate-by-timing interaction effect. Across growing seasons and crop rotations in treatments receiving the maximum N fertilizer rate (210 kg N ha^{-1}), SNI was greater with SA (4.51 mg N d g^{-1}) compared with SpA (2.93 mg N d g^{-1}). Timing had no significant effect on SNI in treatments receiving less than 210 kg N ha^{-1}. Accordingly, the response of log-transformed SNI to fertilizer N rate was fit to a linear regression model (r^2 = 0.89) for the SA treatments, and to a linear-plateau regression model for the SpA treatments (Fig. 6a). There was also a significant year-by-timing interaction effect on SNI. In 2012, SNI was greater with SpA compared with SA, while in 2013 the opposite pattern of differences by timing was observed; and within the SA treatments, SNI was greater in 2013 than 2012, but within the SpA treatments, there was no difference by year (Table 4). There was a significant correlation between SNI and cN_2O for all combinations of year, timing, and crop rotation (Table 3). When SNI was expressed on a yield-scaled basis, there was a significant year-by-crop rotation-by-timing interaction effect on SNI-y (Table 4). In 2012, SNI-y was greater with SpA compared with SA in the CC rotation only. In 2013, SNI-y was greater with SA compared with SpA in both rotations; and SNI-y was greater in the CS compared to CC for both the SA and SpA treatments. There was a significant rate-by-timing interaction effect on SNI-y consistent with the SNI results. Accordingly, the response of SNI-y to fertilizer N rate was fit to a linear regression model (r^2 = 0.90) for the SA treatments, and to a linear-plateau regression model for the SpA treatments (Fig. 6b).

Previous studies examining N application timing effects on N_2O emissions have observed varying results, including no effects of delayed or split application (Zebarth et al., 2008; Phillips et al., 2009); reduced N_2O emissions with delayed application under some but not all conditions (Drury et al., 2012); or reduced N_2O emissions with split application in some but not all growing seasons (Burton et al., 2008). This is the first study to report an increase in cumulative N_2O emissions with split compared to a single early-season N application, which was observed in 2012 (across crop rotations and N rates) and in the highest fertilizer N rate (across rotations and growing seasons). Greater cN_2O with SpA across rotations and N rates in 2012 was likely caused by wide fluctuations in SMC and rainfall occurring prior to and following the V6 and V14 applications. A prolonged dry period persisted from about 2 wk prior to until 1 wk following the V6 application, which (as discussed previously) may have inhibited the movement of applied N through the soil profile and limited crop N uptake. Soil N availability was then supplemented by additional N from the V14 application, which was followed by a series of rainfall events, including the largest event of the season (60 mm) the following day, and three events exceeding 20 mm occurring 5, 10

Table. 3. Correlation of daily N_2O flux (dN_2O) with soil moisture content (SMC), soil temperature (ST), and soil nitrate-N concentration (SN), correlation of cumulative N_2O emissions (cN_2O) with soil nitrate-N intensity (SNI), and multiple regression of dN_2O versus SMC, ST, and SN. Pearson's *r* and *P* values are shown for correlation analyses. Model coefficients (b_0, b_1, b_2 and b_3) and R^2 are shown for multiple regression analyses.†

			Correlation				Multiple regression§				
			dN_2O vs.			cN_2O vs.					
			SMC	ST	SN	SNI		SMC	ST	SN	R^2
Year	Rotation	Timing	*r*				b_0	b_1	b_2	b_3	
		SA	0.26***	-0.05ns‡	0.21***	0.64**	-0.14	3.23	0.028	0.227	0.19
2012	CC	SpA	0.34***	-0.07ns	0.54***	0.83***	-0.58	3.63	0.031	0.588	0.42
		SA	0.22***	-0.06ns	0.14**	0.59**	0.97	1.40	ns	0.127	0.08
	CS	SpA	0.38***	-0.14***	0.42***	0.65**	0.63	2.40	ns	0.353	0.29
		SA	0.47***	-0.08*	0.40***	0.71***	0.61	2.91	ns	0.259	0.33
2013	CC	SpA	0.45***	-0.13**	0.23***	0.70**	0.75	2.58	ns	0.173	0.24
		SA	0.47***	-0.19***	0.13**	0.73***	0.75	2.65	ns	0.076	0.24
	CS	SpA	0.46***	-0.18***	-0.06ns	0.61**	0.75	2.62	ns	0.091	0.22

† dN_2O, cN_2O, SN and SNI were logarithm base 10 transformed prior to analysis.
‡ ns, not significant.
§ Multiple regression results are for models in the form: $dN_2O = b_0 + b_1SMC + b_2ST + b_3SN$. All models were significant at P <0.001 and all parameter estimates (b_1, b_2 and b_3) were significant at $P < 0.001$.
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

and 22 d later (these four events accounted for 26% of total growing season precipitation). It is likely that these rainfall events combined with high levels of soil N created conducive conditions for the large and prolonged pulses in dN_2O that occurred following the V14 application in 2012.

Increased ST later in the season may have contributed to some extent to enhanced N_2O emissions following the midseason N applications. Phillips et al. (2009) found a trend (P = 0.103) for greater N_2O emissions when urea was applied 3 d prior to planting compared to 6 wk prior to planting and noted that greater temperatures as well as SMC following the later application may have promoted greater microbial activity and N_2O production compared to the earlier application. The negative correlation observed here between cN_2O and ST is possibly an artifact of a consistent negative correlation (r = -0.55) between SMC and ST, i.e., when soils were wetter and more conducive for N_2O production, they were also cooler as the result of increased evaporative cooling. This explanation is consistent with the observation that when ST was included in multiple regression models together with SMC and SN, there was a positive association between ST and dN_2O. Including ST as a third predictor variable explained an additional 5.7 and 4.9% of the variance in dN_2O for the 2012 CC/SA and 2012 CC/SpA treatments, respectively (Table 3); ST was not included in the final selected model for the remaining treatment combinations because it did not substantially improve the overall model, explaining < 2.5% of additional variance in dN_2O.

The models describing log-transformed cN_2O as a function of fertilizer N rate (Fig. 5) are equivalent to models describing non-transformed N_2O emissions (y) as functions of fertilizer N rate (x) in the forms $y = b_0 10^{b_1 x}$ and $y = b_0 10^{b_1 x + b_2 x^2}$ where b_0, b_1, and b_2 are positive constants; we refer to these models as 'first-order' (1^o) and 'second-order' (2^o) exponential, respectively. Previous studies have reported 1^o as well as linear and other types of non-linear responses of cumulative N_2O emissions to N rate (e.g., Van Groenigen et al., 2010; Kim et al., 2013). Increasing exponential responses similar to the 1^o model imply that with each marginal increase in N fertilizer input, there is a disproportionately larger marginal increase in N_2O emissions. The 2^o model found here for cN_2O with SpA is nearly identical to the 1^o model for cN_2O with SA at N rates ≤ 90 kg N ha^{-1}, but diverges from the 1^o model at higher N rates. As far as we know, 2^o exponential responses of cN_2O or cN_2O-y to N rate have not been previously reported. Increasing exponential responses of N_2O emissions to N rate also imply that EFs will increase with N rate (Shcherbak et al., 2014). However, an increasing response of EF to N rate was not found in this study. The EFs for each individual treatment were calculated by subtracting cN_2O values obtained for the control treatment in each main plot from the cN_2O values of each treatment within that plot. This procedure is required in order to calculate variances that allow for examination of treatment effects (Venterea et al., 2012). Thus, the EF data set has a different content and structure than the original cN_2O data set used to generate the exponential response curves. Thus, an exponential response of cN_2O to N rate does not necessarily imply that EFs increase monotonically with N rate.

Table 4. Cumulative N_2O emissions (cN_2O), N_2O emissions factor (EF), yield-scaled N_2O emissions (cN_2O-y), nitrate-N intensity (SNI), and yield-scaled nitrate-N intensity (SNI-y) as affected by year, crop rotation, and fertilizer N application timing†.

		N application timing	
Year	Crop rotation	Single	Split
		cN_2O, kg N ha^{-1}	
2012	Avg.‡	1.20 aB§	1.86 aA
2013		1.18 aA	1.01 bA
		EF, %	
2012	Avg.	0.54 aB	0.98 aA
2013		0.55 aA	0.37 bB
		cN_2O-y, g N Mg^{-1}	
2012	Avg.	110 bA	180 aB
2013		181 aA	153 aA
		SNI, mg N day g^{-1}	
2012	Avg.	1.91 bB	2.12 aA
2013		2.79 aA	1.69 aB
		SNI-y, µg day g^{-1} Mg^{-1}	
2012	CC	178 aB	225 aA
	CS	169 aA	178 aA
2013	CC	264 bA	155 bB
	CC	694 aA	406 aB

† Statistical analysis of cN_2O, EF, cN_2O-y, SNI, SNI-y are based on logarithm base 10 transformed data, and back-transformed means are reported.

‡ Averaged across continuous corn (CC) and corn-soybean (CS) rotations.

§ Within a column for cN_2O, EF, cN_2O-y, and SNI, and within a column and year for SNI-y, means followed by the same lowercase letter are not significantly different at the 0.05 probability level. Within a row, means followed by the same uppercase letter are not significantly different at the 0.05 probability level.

Table 5. N_2O emissions factor (EF) as affected by year, crop rotation, and fertilizer N rate†.

Fertilizer	2012		2013	
N rate	CC	CS	CC	CS
kg N ha^{-1}	EF, %			
50	1.63 aA‡	0.12 cB	0.41 aA	0.41 aA
90	1.03 abA	0.21 bcB	0.55 aA	0.41 aA
130	0.90 bA	0.46 abA	0.64 aA	0.25 aA
170	0.98 abA	0.28 abcB	0.60 aA	0.39 aA
210	1.26 abA	0.70 aB	0.45 aA	0.47 aA

†Statistical analysis is based on logarithm base 10 transformed data, and back-transformed means are reported.

‡ Within a column, means followed by the same lowercase letter are not significantly different at the 0.05 probability level. Within a row for a given year, means followed by the same uppercase letter are not significantly different at the 0.05 probability level.

Across growing seasons, application timings, and N rates, cN_2O was 50% greater in CC compared with the CS rotation. In both years, the CC/SA treatments exhibited increases in dN_2O above baseline levels following rainfall events in early Aug 2012 and mid-Aug 2013, but this was not observed in the CS/SA treatments (Fig. 3). Soil nitrate-N concentrations were relatively high in both the CC and CS treatments during these events (Fig. 4). Across growing seasons, application timings and N rates, SNI was greater with CS than CC, which could have resulted from greater amounts of N released from the previous year's crop (N-fixing soybean) residue. Thus, the SN and SNI data suggest that some factor other than soil N availability was responsible for greater cN_2O in CC compared to CS. It is possible that the greater mass of crop (i.e., corn) residue inputs from the previous growing season in the CC rotation compared with the soybean residue inputs in the CS rotation resulted in greater levels of soluble organic C (SOC) in the CC rotation. Differences in tillage regime between rotations (i.e., fall tillage occurred following the previous crop in CC but not in CS) may have also promoted greater levels of SOC in the CC rotation. It has been shown that SOC can promote N_2O production via denitrification (Burford and Bremner, 1975) and/or nitrification (Venterea, 2007). A previous study at the same site as the current study observed greater soil CO_2 emissions with CC than CS, which supports the hypothesis that labile SOC was greater in CC than CS. It is also possible that variation in SOC availability during the growing season may have differed between rotations; i.e., SOC may have been more available in CC compared to CS during July and August. Previous studies have found significant differences in N_2O emissions, but the differences were in opposite directions; Drury et al. (2008) found greater N_2O emissions with CC and Mosier et al. (2006) found greater N_2O emissions with CS. Differences in microbial community structure or function may also have been involved. Further study is needed to explain crop rotation effects on cN_2O and SNI.

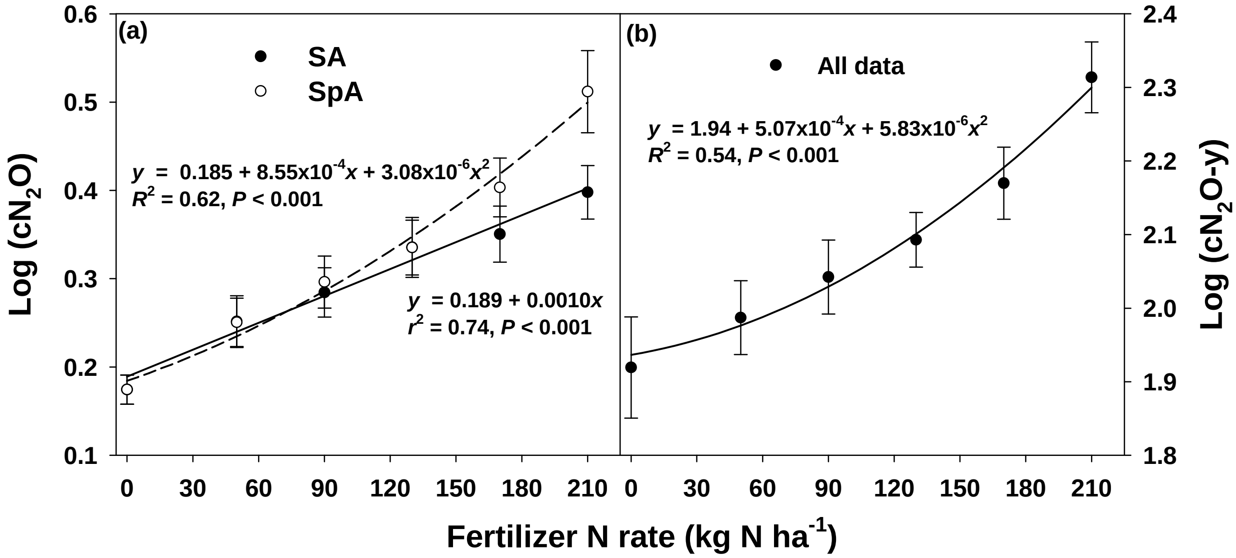

Fig. 5. Response to fertilizer N rate for (a) cumulative N_2O emissions (cN_2O) for single (SA) and split (SpA) application timings across years and crop rotations, and (b) yield-scaled cumulative N_2O emissions (cN_2O-y) across years, crop rotations, and fertilizer application timings. Regression analyses were conducted using individual observations. Note that *y*-axes use log scale.

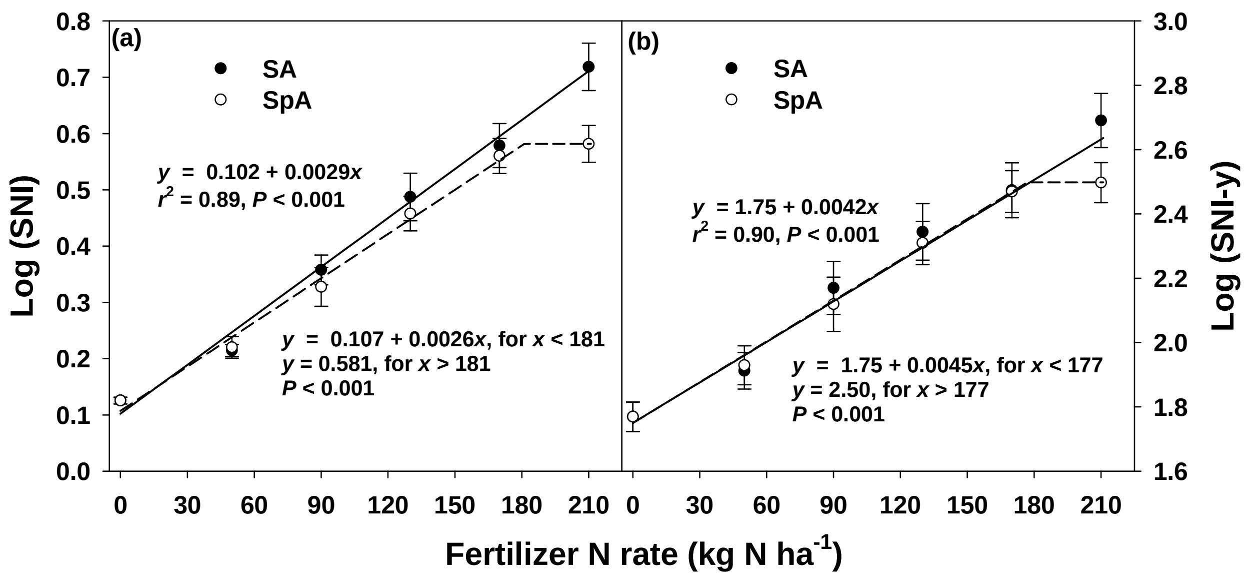

Fig. 6. Response to fertilizer N rate for (a) soil-nitrate N intensity (SNI) and (b) yield-scaled soil-nitrate N intensity (SNI-y) for single (SA) and split (SpA) application timings across years and crop rotations. Regression analyses were conducted using individual observations. R^2 values are not shown for linear-plateau models because residuals do not always sum to zero for nonlinear regression models (Kutner et al., 2004). Note that *y*-axes use log scale.

The strong correlation found here between SNI and cN_2O has been observed in other studies (e.g., Burton et al., 2008). The strength of the correlation between SNI and cN_2O was consistently greater (r = 0.59-0.83) than the correlation between SN and dN_2O (r < 0.54). Similarly, Maharjan and Venterea (2013) observed that correlations between soil nitrite-N intensity and cN_2O were stronger than correlations between soil nitrite-N concentration and dN_2O; and they also remarked on possible explanations for this observation. In contrast to N_2O, SNI and SNI-y were lower with SpA than with SA at the maximum N rate and there was a leveling off of the N rate response in the SpA treatment. Explanations for this result are speculative; it may have resulted from elevated NH_3 losses following the mid-season N applications which might have increased with N rate resulting in disproportionately less N remaining in the soil to be converted to nitrate-N at the highest N rate.

CONCLUSIONS

Applying granular urea using split applications to better coincide with crop N demand does not necessarily reduce and may actually increase N_2O emissions. In the current study, it is likely that persistently dry soil conditions inhibited the mobilization of urea-derived N and its availability for crop uptake, even when urea was applied at a time of high crop N demand. Subsequent rainfall events then stimulated microbial conversion of this N into N_2O before it could be utilized by the crop. These conditions cannot be controlled nor can they be easily predicted in rainfed production systems. On the other hand, applying urea early in the growing season also carries the risk of N losses via direct soil-to-atmosphere N_2O emission as well as nitrate leaching below the undeveloped root zone (Errebhi et al., 1998). Nitrate losses can not only impact water quality but provide a potential source of indirect N_2O emissions, although the extent of off-site conversion to N_2O is highly uncertain (Venterea et al., 2011). Thus, current options available to utilize N fertilizer timing as a tool to reduce total (direct plus indirect) N_2O emissions appear limited, and the calculations involved in determining the least risky option depend upon climate and other factors that are highly uncertain. Injecting post-plant N fertilizer in dissolved form (e.g. as urea-ammonium nitrate solution) might enhance crop uptake especially if applied in close proximity to roots, although this practice does not always enhance yields and in some cases has decreased yields (e.g. Jaynes and Colvin, 2006). Combining split applications with stabilized N sources and/or chemical inhibitors that resist leaching and microbial transformation might also be effective. Some studies have examined combinations of such practices (e.g., Burzaco et al., 2013), but more are needed to identify effective strategies for reducing N_2O emissions while maintaining or enhancing crop production.

EDUCATION, OUTREACH, AND PUBLICATIONS

Findings from this study were presented at the following meetings and conferences:

1. Crop Nutrient Management Conference, February 11, 2014, Mankato MN, organized by the Minnesota Agricultural Water Resources Center. Estimated over 150 farmers and crop consultants in attendance.

2. Joint meeting of the American Geophysical Union, Canadian Geophysical Union, Geological Association of Canada, and Mineralogical Association of Canada on May 6, 2015.

3. Annual meeting of the Tri-Societies (ASA/SSSA/CSSA), November 2015, Minneapolis, MN.

Findings from this study were published in the following article:

Venterea, R.T. and J.A. Coulter. 2015. Split application of urea does not decrease and may increase N2O emissions in rainfed corn. Agronomy Journal, 107:337–348. doi:10.2134/agronj14.041.

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