Soil N$_2$O emissions as affected by long-term residue removal and no-till practices in continuous corn

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Abstract
The environmental consequences of residue removal practices to support cellulosic biofuel production remain poorly understood. In the U.S. Midwest, corn (Zea mays L.) stover removal combined with no-till practices may increase or decrease soil N$_2$O emissions by influencing soil moisture, temperature, and nutrient dynamics, yet empirical evidence from long-term field experiments is inconsistent. We investigated the effects of residue management (residue retained or removed) and tillage (chisel-till or no-till) on cumulative soil nitrous oxide (N$_2$O) emissions, grain yield, and yield-scaled N$_2$O emissions in a 3-year study initiated 10 years after treatment implementation in a long-term, continuous corn experiment in Illinois, United States. Crop yields were affected by treatment in only one of three study years, with the combination of residue removal and no-till reducing yields compared to both chisel-till treatments. Cumulative N$_2$O emissions, soil inorganic N concentrations, and yield-scaled N$_2$O emissions differed over the 3-year period and were significantly affected by tillage, with no response to residue management. In 2 years, no-till decreased cumulative N$_2$O emissions and yield-scaled N$_2$O emissions by an average of 64% and 60%, respectively. Correlations between daily N$_2$O fluxes and soil moisture, temperature, and inorganic N concentrations suggested that the relative importance of these variables changed depending on year and treatment. While more research across a range of sites and management practices is needed, our findings support previous studies which have challenged IPCC methodology assumptions regarding the effects of residue removal on N$_2$O emissions. We conclude there is inherent difficulty in predicting the impacts of residue removal due to the complexity of soil processes underlying N$_2$O emissions coupled with inter-annual weather variability in this rainfed continuous corn system. Future efforts to evaluate the net greenhouse gas emissions of cellulosic biofuel production may benefit from accounting for this uncertainty.

KEYWORDS
corn stover, Nitrous oxide, no-till, residue removal, tillage, yield-scaled N$_2$O emissions
1 INTRODUCTION

Major efforts are underway to develop sustainable biofuel production systems in the United States with the goal of reducing national dependency on fossil fuels while decreasing greenhouse gas (GHG) emissions. The US Energy Independence and Security Act of 2007 established ambitious targets for biofuel production from different feedstocks, including an initial mandate of 16 billion gallons of cellulosic biofuels by 2022. The Corn Belt has been identified as an important source of crop residues including corn (Zea mays L.) stover to meet the growing needs of an expanding renewable biomass energy industry (Johnson et al., 2007; Jones, Zhang, Reddy, Robertson, & Izaurralde, 2017). Illinois is a leading producer of corn, with an average yield of 12.6 Mg/ha on 4.5 million ha in 2017 (USDA-NASS, 2017). While cellulosic ethanol production is not economically competitive with corn grain ethanol, it is the most feasible option at present and improvements in processing technologies and economic efficiencies are expected (Foust, Aden, Dutta, & Phillips, 2009). As such, there is continued interest in evaluating the environmental and economic performance of stover relative to other cellulosic feedstocks under different policy scenarios (Hudiburg et al., 2016).

Despite these potential benefits, the environmental consequences of residue removal to support cellulosic ethanol production remain poorly understood. Concerns have been raised about impacts on crop productivity (Johnson et al., 2007; Karlen & Johnson, 2014), soil organic carbon (SOC) stocks (Liska et al., 2014), water quality (Acharya & Blanco-Canqui, 2018; Gramig, Reeling, Cibin, & Chaubey, 2013), and soil GHG emissions (Jin et al., 2014; Qin et al., 2018). An important criterion of the Renewable Fuel Standard is that cellulosic biofuel production must reduce GHG intensity by 60% compared to gasoline. Therefore, changes in direct soil emissions of nitrous oxide (N$_2$O), which has roughly 300 times the global warming potential of CO$_2$, must be accounted for when determining net GHG impacts of residue removal practices. However, most studies use approaches that poorly reflect empirical field measurements, which suggest the magnitude and direction of changes in soil N$_2$O fluxes under different residue management practices are highly variable. The carbon footprint of cellulosic biofuels is often calculated using life cycle assessment approaches with fixed N$_2$O emission factors (Sheehan et al., 2004). Another example is IPCC protocols which assume 1% of N contained in crop residue is converted to N$_2$O (IPCC, 2007), indicating that crop residue removal will consistently result in reduced direct N$_2$O emissions due to lower C and N inputs. Changes in N$_2$O emissions have also been simulated using process-based biogeochemical models which can better capture site-specific variability. However, these models also tend to predict a decrease in N$_2$O emissions following residue removal (Gramig et al., 2013; Kim et al., 2018).

Of concern is that previous field results do not always support the assumption that residue removal decreases N$_2$O emissions. Residue removal can alter soil microclimate conditions (e.g., temperature and moisture), N and C availability, and microbial activity, leading to complex impacts on soil N cycling. Consistent with IPCC emission factors, a number of previous studies have found that residue removal decreases N$_2$O emissions (Abalos, Sanz-Cobena, Garcia-Torres, Groenigen, & Vallejo, 2013; Jin et al., 2014; Mutegi, Munkholm, Petersen, Hansen, & Petersen, 2010; Yao et al., 2013). However, studies have also shown N$_2$O emissions can either increase or remain unchanged with crop residue removal (Congreves, Brown, Németh, Dunfield, & Wagner-Riddle, 2017; Hao, Chang, Carefoot, Janzen, & Ellert, 2001; Lehman & Osborne, 2016; Yao et al., 2013). Increased N$_2$O emissions may be due to larger soil temperature fluctuations or changes in moisture/aeration conditions compared to the practice of incorporating crop residues. Variability in precipitation also plays a critical role, with meta-analysis results showing that the most significant reduction in N$_2$O emissions from residue removal occurred at 60%–90% water-filled pore space (Chen, Li, Hu, & Shi, 2013).

When assessing the effects of residue removal, no-till (NT) is frequently considered a complementary management practice because of its potential to increase SOC, helping offset possible increases in soil GHG emissions (Gramig et al., 2013; Qin et al., 2018). Although the impacts of tillage have been studied widely, there is little consensus regarding NT effects on soil N$_2$O emissions (Gregorich, Rochette, St-Georges, McKim, & Chan, 2008). Several field studies have reported that soil N$_2$O emissions under conservation tillage (e.g., reduced tillage [RT] and NT) decrease compared with conventional tillage practices (e.g., chisel-till [CT]; Congreves et al., 2017; Dendooven et al., 2012; Guzman, Al-Kaisi, & Parkin, 2015; Rochette, 2008). These reductions are often attributed to improvements in soil structure and/or cooler soil temperatures with RT/NT relative to CT. However, many other researchers have observed greater soil N$_2$O emissions under RT/NT, possibly due to increased carbon and nitrogen inputs, greater soil water-filled pore space (WFPS), or less aeration due to changes in soil bulk density (Guzman et al., 2015; Jin et al., 2014; Snyder, Bruulsema, Jensen, & Fixen, 2009; Venterea, Maharjan, & Dolan, 2011; Yao et al., 2013). Tillage effects can also differ between years within the same study, with NT resulting in similar, reduced, or increased N$_2$O emissions (Gregorich et al., 2008). Study duration has also been identified as an important factor, as changes in soil properties under RT/NT may develop more
strongly over time. A meta-analysis of 239 direct comparisons between RT and NT with CT found that in drier climates, NT/RT decreased N$_2$O emissions in the longer term (>10 years) by 27%, but increased emissions in the short term (<10 years) by 56% compared to CT (van Kessel et al., 2013).

An important consideration is that the N$_2$O response to crop residue removal may interact with tillage practices (Congreves et al., 2017; Dendooven et al., 2012; Jin et al., 2014, 2017; Mutegi et al., 2010). Hence, large uncertainties in the relative impact of different tillage and crop residue management combinations on N$_2$O emissions have been reported. For instance, Mutegi et al. (2010) observed that in residue removal scenarios, N$_2$O emissions were similar for all tillage treatments, but in residue retention scenarios, N$_2$O emissions were significantly higher in CT than RT. Meanwhile, Congreves et al. (2017) found that N$_2$O emissions were higher in residue removal compared to retention scenarios, but the effect of residue removal was more marked under CT than NT. In a multi-location study of nine maize production systems, Jin et al. (2014) found that residue removal decreased overall N$_2$O emissions by approximately 7%, but significant reductions were only observed under RT/NT, whereas significant increases occurred in several years for CT. A number of the above studies were conducted in long-term field experiments, suggesting that interactions between NT and residue removal practices on N$_2$O emissions are not only site- and region-specific, but may also be influenced by the duration of NT management.

Accurately estimating the carbon footprint of cellulosic biofuels derived from corn stover will in part depend on understanding changes in N$_2$O emissions following residue removal, yet high variability in previous field results indicates the need for further research. The objectives of this study were to (a) quantify the effects of tillage and residue management practices on N$_2$O emissions and corn yield, (b) assess the environmental factors regulating soil N$_2$O emissions, and (c) evaluate N$_2$O emissions as a function of crop yield to identify practices that minimize GHG impacts per unit of crop production.

2 | MATERIALS AND METHODS

2.1 | Study site and experimental design

Field experiments were conducted in 2015, 2016, and 2017 at the University of Illinois Crop Sciences Research and Education Center in Urbana, IL (40°2′ N, 88°13′ W). Soil type at the experimental site is classified as a Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls). The region has a temperate climate with a 30-year (1981–2010) average temperature of 10.9°C and average cumulative rainfall of 1,051 mm/year. All slopes ranged from 0% to 2%. Measurements for the present study were conducted in a long-term, rainfed, continuous corn experiment with residue removal and tillage treatments initiated in the fall of 2005 and assigned to the same plots each year. Further details about this field experiment are documented in Villamil, Little, and Nafziger (2015).

Four of the treatments were selected for measuring N$_2$O emissions, resulting in a split-plot randomized complete block design with four replications. Main plots consisted of two levels of residue removal (full [RR—] and none [R+]). Residue removal followed harvest in the fall, with stover chopped 5 cm above the soil surface and raked into windrows prior to removal. It was estimated that 7.6 and 0.8 Mg/ha of residue remained with no and full removal of residue, respectively, when averaged across locations, years, tillage systems, and N rates (Villamil et al., 2015). Subplots consisted of two levels of tillage: conventional tillage using a chisel plow (chisel-till, CT) and left undisturbed (no-till, NT). Conventional tillage included chisel plowing in the fall to a depth of 25 cm and secondary tillage with a field cultivator prior to planting. The experiment was planted each year at a population of 81,500 seeds/ha with a no-till planter in late April. Rows were located approximately 15 cm between rows from the previous year. In all treatments, N fertilizer was injected as urea ammonium nitrate solution at the V4-V5 growth stage at 202 kg N per ha. The N fertilizer application dates were May 14, 2015, May 16, 2016, and June 1, 2017.

2.2 | Nitrous oxide emissions

Soil N$_2$O emissions were measured using nonsteady-state vented closed chambers adapted from Rochette and Bertrand (2008) and Ventera, Coulter, and Dolan (2016). In brief, rectangular chamber bases and lids were constructed from clear acrylic plastic. Chamber bases were installed after N application between corn rows to a depth of 5 cm and remained in place for the remainder of the growing season. Chamber lids (67.3 cm length, 40.6 cm width, 19 cm height) were insulated with reflective double bubble foil insulation (Ecofoil, Urbana, IA) to minimize temperature changes during gas sampling. Lids were fitted with vent tubes and septa to serve as a sampling port for gas extraction. To create an air-tight seal during gas sampling, clamps were used to secure lids in place with closed-cell foam (Lundell Manufacturing Corporation, Minneapolis, MN) lining the connection between lids and bases.

Measurements were performed twice per week during the period of higher anticipated N$_2$O emissions (May–July) and once per week thereafter until harvest (August–September). In total, we sampled gases 21, 27, and 18 times in 2015, 2016, and 2017, respectively. Soil N$_2$O flux
measurements were generally taken between the hours of 9:00 and 11:00 a.m. Gas samples were collected at 0, 10, 20, and 30 min by extracting 20 ml of headspace air using plastic 20-ml luer-lock tip syringes with 25-gauge needles. After withdrawing a sample, 5 ml of gas was ejected and 15 ml of gas was immediately injected into 10-ml evacuated glass vials fitted with butyl rubber stoppers (Voigt Global Distribution Inc., Lawrence, KS). Rubber stoppers were covered with clear RTV silicone adhesive sealant (Dow Corning, Midland, MI) which acted as an additional septum to prevent leakage. Gas samples were analyzed using a Shimadzu 2014 gas chromatograph equipped with an electron capture detector and auto-sampler (Shimadzu Scientific Instruments, Columbia, MD). Helium was used as the carrier gas. Gas standards for N2O (Matheson, Basking Ridge, NJ) ranged from 0.32 to 4.02 ppm in 2015 and from 0.1 to 10.14 ppm in 2016 and 2017.

2.3 | Soil and plant measurements

Surface soil samples (0–10 cm in 2015 and 0–20 cm in 2016 and 2017) were collected at the time of gas measurements every other sampling date, which corresponded to weekly during May through July and bi-weekly from August through September. To obtain a representative estimate of soil N concentration across the inter-row area, five equally spaced soil cores were collected from each plot from a transect running perpendicular to the crop row. This approach took into consideration the placement of N fertilizer, with the middle core located over the injection point and two cores on either side moving toward the crop row. Cores were immediately composited and placed on ice. Inorganic N was extracted using 100 ml of 2 M KCl with approximately 12 g moist soil and 1 hr of shaking. Samples were filtered through #2 Whatman filter paper (Sigma Aldrich, St. Louis, MO). Concentrations of NO3-N and NH4-N were determined using a SmartChem 170 discrete wet chemistry auto-analyzer (Unity Scientific, Milford, MD). Soil moisture content was determined by oven-drying a second sample at 105°C for 48 hr.

Corn grain yield was determined using a small plot combine from the center two rows of each plot. Yields were adjusted to 15% moisture.

Soil temperature and moisture in each plot were measured hourly using two Decagon Device sensors and loggers (Pullman, WA). Soil temperature and moisture were measured at between-row location at 10 cm soil depth. A rain gauge (Rainwise, Trenton, ME) and data logger (Onset HOB0 UA-003-64, Bourne, MA) were used to record daily precipitation for the length of the growing season. Long-term weather records at the site were obtained from the Illinois State Water Survey (ISWS, 2017).

2.4 | Data analysis

Daily N2O flux rates were estimated from the linear increase in headspace gas concentration over time. Cumulative N2O fluxes were calculated by linear interpolation between sampling events. To evaluate soil N availability over time, soil N intensity was calculated by trapezoidal integration of inorganic soil N concentrations between sampling dates following Venterea et al. (2011). Soil N intensity was determined separately for NO3− and NH4+ and for the sum of NO3− and NH4+ as total inorganic N (TIN). This parameter was preferred over soil N concentration alone since it was defined as the time-weighted sum of soil N concentration to represent the cumulative exposure of soil microbial populations to different N forms (Maharjan & Venterea, 2013). Yield-scaled N2O emissions were calculated by dividing cumulative N2O emissions by grain yield.

All statistical analyses were conducted in SAS software version 9.4 (SAS Institute Inc., 2013). Linear models for each studied variable were analyzed using the GLIMMIX procedure. Residue management and tillage treatments were considered fixed effects, while replicates (blocks) were considered random effects. Study year was analyzed using a repeated-measures approach with an unstructured (type = un) variance–covariance selected for each variable based on the lowest Akaike’s information criteria (Littell, Milliken, Stroup, Wolfinger, & Schabenberger, 2006). A lognormal distribution (dist = logn) was used for most variables (except yield) due to the lack of normality of model residuals. Least square means were separated using LSMEANS and LINES option within GLIMMIX with Bonferroni adjustment and α = 0.05. The statistical model and SAS codes are available upon request from the authors.

To assess the impacts of soil moisture, temperature, and soil N availability on daily N2O flux rates across different tillage and residue management treatments, Pearson correlation coefficients were determined using the PROC CORR procedure. This analysis focused on relationships between variables during the peak flux period, which we established as the range of sampling dates accounting for 70% of cumulative N2O emissions during each growing season. Correlations between soil moisture, temperature, soil NO3−, NH4−, and total inorganic N vs. daily N2O flux rates were evaluated for each of the four tillage and residue management treatments as well as averaged across the treatments from 2015 to 2017.

3 | RESULTS

3.1 | Environmental factors

Average monthly precipitation and air temperature during June, July, and August from 2015 to 2017 were compared
to the 20-years average (1996–2016; Figure 1). June 2015 and 2016 had higher than average rainfall, with June 2015 representing the wettest June on record (Figure 1a). In contrast, 2017 was much drier during all 3 months compared to the 20-years average (Figure 1a). The 2015 growing season was cooler than average, while 2016 was warmer than average, especially in June and August (Figure 1b).

Soil water content fluctuated with rainfall events throughout the season (Figure 2a–c). The effects of tillage and residue removal on soil water content were generally consistent across years. Significantly lower soil water content was observed in CT treatments than NT ($p < 0.05$) on 28%–76% of the sampling dates in the 3 years. Averaged across the season, CT had 0.28, 0.31, and 0.27 m$^3$/m$^3$ soil water content, respectively, in 2015 to 2017, while soil water content was 0.02–0.03 m$^3$/m$^3$ lower than this for NT treatments.

Early in the growing season when the crop canopy had not completely covered the soil surface, specifically before June 24 in 2015, June 21 in 2016, and July 17 in 2017, soil temperature in R + NT was significantly lower compared to the other three treatments ($p < 0.05$) on 32%–48% of the sampling date in the 3 years (Figure 2d–f). Averaged across this period each year, soil temperatures in R + NT were 2.1, 2.7, and 1.2°C lower than the mean of the other three treatments, respectively, in 2015, 2016, and 2017.

3.2 | Soil N$_2$O emissions

Soil N$_2$O emissions followed a similar temporal pattern during each of the 3 years (Figure 3a–c). Emissions generally remained low during the beginning and end of the growing season, with the highest emissions occurring from the middle of June through early July. Periods of elevated N$_2$O emissions coincided with high daily rainfall events (Figure 3a–c), though large variability was observed between tillage and residue management treatments. In 2015, the highest flux rate was observed on June 17 for RR-NT, while R + NT was associated with higher emissions earlier in the season (Figure 3a). In 2016, the two highest N$_2$O peaks occurred in mid-June under RR-CT, with slightly elevated emissions observed from late May to early June for R + CT (Figure 3b). Similar to 2016, the greatest flux was also found in RR-CT in 2017, but high rates persisted for a longer period lasting from mid-June to mid-July, with elevated emissions also occurring with R + CT during this time (Figure 3c).

Cumulative soil N$_2$O emissions were significantly influenced by year, tillage (T), and a year by tillage interaction (Year × T), while no residue management effect was detected (Table 1). The 2015 season produced the highest N$_2$O emissions, followed by intermediate emissions in 2016 and relatively low emissions in 2017 (Table 2). Across the 3 years, NT reduced N$_2$O emissions by 37% compared to CT (Table 2). The year × tillage interaction revealed that cumulative N$_2$O emissions were not influenced by tillage practices in 2015, but higher emissions occurred in CT compared to NT during both 2016 and 2017 (Table 2).

3.3 | Soil inorganic N dynamics

High levels of total inorganic N (NO$_3^−$ + NH$_4^+$) were observed from late May through late June in 2015 and 2016 and from early June to early July in 2017, which generally coincided with elevated N$_2$O emissions except for 2015 (Figure 3d–f). Similar to soil N$_2$O emissions, concentrations of total inorganic soil N started to decline after early July throughout the season across all treatments in the 3 years (Figure 3d–f); however, the treatment response was different from that of soil N$_2$O emissions.

Soil NO$_3^−$, NH$_4^+$, and total N intensity were affected by year and a year × tillage interaction, with soil NH$_4^+$ intensity also being influenced by tillage (Table 1). The three parameters were significantly lower in 2015 due to the different sampling method (i.e., 0–10 cm sampling depth in 2015 relative to 0–20 cm in 2016 and 2017). For each parameter, the highest soil N intensities were found in 2017, with intermediate and low soil N intensities observed in 2016 and 2015, respectively (Table 2). Across the
Soil water content (m$^3$/m$^3$, a–c) and soil temperature (°C, d–f) measured between rows at 10 cm depth from 2015 to 2017 as affected by two tillage (chisel-till [CT] and no-till [NT]) and residue management (residue retained [R+] and residue removed [RR−]) treatments. Soil temperature data were missing for R+ CT and RR− NT from May 22nd to June 4th in 2015 (Figure 2d), and for RR− CT from May 19th to June 19th in 2016 (Figure 2e).

Daily soil N$_2$O fluxes (g N$_2$O/ha per day, a–c) and total inorganic N concentrations (NO$_3^− +$ NH$_4^+$, mg/kg, d–f) from 2015 to 2017 affected by two tillage (chisel-till [CT] and no-till [NT]) and residue management (residue retained [R+] and residue removed [RR−]) treatments. Bars represented standard errors. Surface soil samples were collected at 0–10 cm depth in 2015 and 0–20 cm depth in 2016 and 2017.)
3 years, soil NH$_4^+$ intensity was higher for NT than CT. While soil NO$_3^-$ intensities were similar between CT and NT in all years, soil NH$_4^+$ and total N intensities responded to tillage differently in 2015 (with NT being higher than CT) compared to 2016 and 2017, resulting in a year × tillage interaction (Table 2).

### 3.4 Grain yield and yield-scaled N$_2$O emissions

Year and tillage had significant effects on grain yield, and there was also a year × tillage × residue removal interaction (Year × T × R, Table 1 and Figure 4). The 2015 season had the highest yield, followed by 2016 and 2017 (Table 2). Chisel-till (CT) produced 8.8% higher yield than NT averaged across the three seasons. Residue management by itself did not affect grain yield, but it interacted with year and tillage. In 2016, R + CT and RR-CT yielded more than R + NT, whereas RR-NT showed intermediate values. Corn yields in 2015 and 2017, on the other hand, were not affected by residue removal (Figure 4).

When expressing N$_2$O emissions on a yield-scaled basis, a similar response was observed as cumulative N$_2$O emissions. Yield-scaled N$_2$O emissions were significantly influenced by year, tillage (T), and a year × tillage interaction (Tables 1 and 2). Across years, NT reduced yield-scaled N$_2$O emissions by 31% (Table 2), but these effects were not consistent each year. No differences occurred between NT and CT in 2015, whereas NT significantly reduced yield-scaled N$_2$O emissions by 60% in 2016 and 2017 (Table 2).

### 3.5 Correlations

Correlation coefficients between soil variables and N$_2$O emissions differed widely among treatments during the

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**TABLE 1** Probability values and numerator degrees of freedom (df) associated with the different sources of variation in the analysis of variance for cumulative N$_2$O emissions (g N$_2$O/ha), soil NO$_3^-$ intensity (g N/kg per day), soil NH$_4^+$ intensity (g N/kg per day), soil total inorganic N (TIN) intensity (g N/kg per day), grain yield (Mg/ha), and yield-scaled N$_2$O emissions (g N Mg/yield)

| Source of variation | df | Cumulative N$_2$O | Soil NO$_3^-$ intensity | Soil NH$_4^+$ intensity | Soil TIN intensity | Yield | Yield-scaled N$_2$O |
|---------------------|----|-------------------|-------------------------|------------------------|------------------|-------|-------------------|
| Year                | 2  | <0.0001           | <0.0001                 | <0.0001                | 0.0015           | 0.0002 |
| Residue (R)         | 1  | 0.2664            | 0.2184                  | 0.9393                 | 0.462            | 0.391  | 0.3672 |
| Year × R            | 2  | 0.2579            | 0.301                   | 0.1834                 | 0.3812           | 0.2593 | 0.2350 |
| Tillage (T)         | 1  | 0.0002            | 0.5698                  | 0.0364                 | 0.1027           | 0.0005 | 0.0016 |
| Year × T            | 2  | 0.0311            | 0.0314                  | 0.0054                 | 0.0034           | 0.4001 | 0.0311 |
| R × T               | 1  | 0.2249            | 0.4645                  | 0.7576                 | 0.9581           | 0.1956 | 0.1614 |
| Year × R × T        | 2  | 0.9653            | 0.4107                  | 0.7171                 | 0.8997           | 0.0051 | 0.9712 |

**TABLE 2** Main effects and interactions of year and tillage (chisel-till [CT] and no-till [NT]) on cumulative N$_2$O emissions (g N$_2$O/ha), soil NO$_3^-$ intensity (g N/kg per day), soil NH$_4^+$ intensity (g N/kg per day), soil total inorganic N (TIN) intensity (g N/kg per day), grain yield (Mg/ha), and yield-scaled N$_2$O emissions (g N Mg/yield)

| Treatments | Cumulative N$_2$O | Soil NO$_3^-$ intensity | Soil NH$_4^+$ intensity | Soil TIN intensity | Yield | Yield-scaled N$_2$O |
|------------|-------------------|-------------------------|------------------------|------------------|-------|-------------------|
| Year Tillage (T) | g N$_2$O/ha | g N/kg per day | g N/kg per day | g N/kg per day | Mg/ha | g N Mg/yield |
| 2015 CT 9,349 | 0.57 | 0.40 | 0.07 | 12.15 | 773 |
| 2016 CT 5,309 | 1.76 | 1.01 | 2.77 | 12.11 | 432 |
| 2017 CT 3,022 | 2.20 | 3.84 | 1.81 | 5.64 | 324 |
| 2015 NT 7,232 | 2.20 | 1.04b | 3.24 | 12.18 | 588 |
| 2016 NT 4,555 | 1.91 | 0.10a | 0.30 | 11.15 | 405 |
| 2017 CT 9443a | 0.43b | 0.14a | 0.57d | 12.70 | 738a |
| 2016 NT 9255a | 0.71b | 0.65b | 1.37c | 11.61 | 807a |
| 2017 NT 7856ab | 1.87b | 1.02ab | 2.89b | 12.84 | 619ab |
| 2017 CT 2761bc | 1.64b | 1.00ab | 2.64bc | 11.38 | 245bc |
| 2017 NT 4395ab | 4.29a | 1.96a | 6.26a | 11.02 | 405ab |
| 2017 CT 1649c | 3.39a | 1.65a | 5.03a | 10.48 | 162c |

Note. Mean separation groupings are not shown for the main effects of year and tillage due to a significant year × tillage interaction. Within a column and given combination of factors (i.e., tillage within year), means followed by the same letter or no letter are not statistically different (α = 0.05).
3 years (Supporting Information Table S1). Correlation coefficients between soil water content and daily \( \text{N}_2\text{O} \) fluxes were \( \geq 0.5 \) for all treatments in 2015, but only for \( R + NT \) in 2017. In NT treatments, correlation coefficients between soil temperature and daily \( \text{N}_2\text{O} \) emissions ranged from 0.50 to 0.91 in 2017 (Supporting Information Table S1). Soil \( \text{NO}_3^- \), \( \text{NH}_4^+ \), and total N concentrations were correlated with daily \( \text{N}_2\text{O} \) fluxes for several treatments each year; however, the specific treatment and direction of the relationship (positive or negative) were not consistent across years. For example, in CT treatments, soil \( \text{N}_2\text{O} \) emissions were correlated with \( \text{NO}_3^-\)-N concentrations in 2016 and 2017 (correlation coefficients ranged from 0.66 to 0.82, except for \( R + CT \) in 2016). Soil \( \text{NH}_4^+ \) concentrations were related (correlation coefficients \( \geq 0.5 \)) to daily \( \text{N}_2\text{O} \) fluxes for \( R + CT \) in all three seasons (Supporting Information Table S1), but the correlation was negative in 2015 and positive in 2016 and 2017.

4 | DISCUSSION

4.1 | Tillage and residue management effects on \( \text{N}_2\text{O} \) emissions

In this study, cumulative \( \text{N}_2\text{O} \) emissions were influenced by tillage as well as a year \( \times \) tillage interaction (Table 1). However, there were no significant effects of residue management or a tillage \( \times \) residue management interaction. Net \( \text{N}_2\text{O} \) emissions reflect the combined effects of several microbial processes that are highly sensitive to changes in chemical and physical soil properties; thus, it is understandable that soil management practices which strongly influence the biophysical soil microenvironment such as NT and residue removal can positively or negatively impact \( \text{N}_2\text{O} \) emissions in different contexts. In a comprehensive multi-location study in the US Corn Belt, Jin et al. (2014) observed that residue removal effects on total GHG emissions ranged from \(-36\%\) to \(-54\%\) compared to residue retention, with decreases in \( \text{N}_2\text{O} \) emissions more often occurring under conservation compared to conventional tillage systems.

Our study was conducted in a long-term experiment; thus, the finding that NT reduced emissions is supported by a previous synthesis showing that the \( \text{N}_2\text{O} \) mitigation benefits of NT become more evident when practiced over the long-term (Six et al., 2002). Although cumulative \( \text{N}_2\text{O} \) emissions under CT and NT were similar in 2015, NT reduced emissions in 2016 and 2017 (Table 2). The following mechanisms are cited as critical factors governing the response of soil \( \text{N}_2\text{O} \) emissions to management practices (Knowles, 1978). First, soil N pools act as substrate for nitrification and denitrification process. Second, readily available C can induce a high O\(_2\) demand in soil (Chantigny, Angers, & Rochette, 2002), leading to formation of O\(_2\) limited microsites that could trigger \( \text{N}_2\text{O} \) formation from both nitrification and denitrification processes. Labile C is also necessary to support denitrifying enzyme activity. Third, soil aeration and drainage conditions also determine \( \text{O}_2 \) status and gas diffusivity of soil, further influencing nitrification/denitrification processes. Fourth, soil temperature is an important driver of biological activity in soil. Finally, soil physical and chemical properties influence microbial activity and soil moisture dynamics including soil pH, texture, and structure (Chen et al., 2013).

Several of these dynamics may explain why NT reduced cumulative \( \text{N}_2\text{O} \) emissions relative to CT in the present experiment. Studies have reported that NT generally increases surface soil moisture content compared to chisel or moldboard plow treatments (Malhi & Lemke, 2007; Muteigi et al., 2010; Ussiri, Lal, & Jarecki, 2009). Soil moisture is closely related with soil aeration which in turn influences nitrifier and denitrifier activity, with increasing anoxic conditions at higher soil water content leading to more complete denitrification (i.e., reduction of \( \text{N}_2\text{O} \) to \( \text{N}_2 \)) (Bouwman, 1998). Moreover, NT may increase denitrifier activity in surface soil layers but has been shown to have the opposite effect below the 15 cm depth (Linn & Doran, 1984). It has also been found that NT may reduce soil temperatures (Ussiri & Lal, 2009), while improving soil structure (Dendooven et al., 2012; Villamil et al., 2015; Zhang, Guo, Liu, Li, & Cao, 2015). Across years in our study, NT had 7.5\%–16.3\% higher soil water content than CT during the peak \( \text{N}_2\text{O} \) emission periods. Venterea, Burger, and Spokas (2005) also found higher soil moisture in NT compared to CT, and this was associated with decreased \( \text{N}_2\text{O} \) emissions, but only for injected N fertilizer treatments. These
lower denitrification activity in NT soils at depth coupled with increased soil moisture supporting the reduction of $N_2O$ into $N_2$ during upwards diffusion of gas through the soil profile. In addition, soil temperature in our study tended to be lowest in R + NT during the early period of each growing season (Figure 2), likely due to the insulating effect of residue on the soil surface. Only when residue was retained did NT reduce soil temperature, whereas residue removal resulted in bare soil and warmer temperatures, which is important during this period because of the large pool of soil $N$ available for microbial activity and subsequent $N_2O$ losses. These results agree with observations by Dendooven et al. (2012). Whereas cooler soil temperatures early in the season may have contributed to the lowest cumulative $N_2O$ emissions for R + NT numerically when averaged across the three seasons (Supporting Information Table S2), no significant residue × tillage interaction was observed (Table 1). As the crop canopy continued to develop, soil temperature became similar across treatments (Figure 2).

In contrast to tillage, we found that residue removal had no effect on cumulative area- or yield-scaled $N_2O$ emissions (Table 1). The following mechanisms can contribute to short-term positive or negative impacts of residue removal, yet it is generally assumed that residue removal leads to a net reduction in $N_2O$ emissions. Enhanced emissions can occur when the addition of crop residue stimulates denitrification (Burford & Bremner, 1975; Groffman, 1985) by providing readily available $C$ for denitrifying bacteria. Decomposition of crop residues also consumes oxygen, which can further stimulate denitrification and $N_2O$ emissions. Moreover, $N$ contained in crop residues can represent additional substrate for microbial reactions and stimulate $N_2O$ emissions, as assumed in IPCC protocols (2007). On the other hand, corn residue with a high $C:N$ ratio can also cause $N$ immobilization and reduce available $N$ for denitrification and nitrification, thus reducing $N_2O$ production. Crop residue retention has also been found to reduce soil temperature and increase moisture under NT, in turn decreasing soil $N_2O$ emissions (Dendooven et al., 2012). In addition to these factors, the effects of residue management depend on the timing of residue incorporation and fertilizer application (Hao et al., 2001), particularly in relation to changing environmental conditions (e.g., fall vs. spring). In this study, residue removal may have had little impact on $N_2O$ emissions if $N$ availability and $C$ availability were already non-limiting factors for soil $N_2O$ production as a result of the experimental conditions (i.e., $N$ fertilizer application rate of 202 kg per $N$ per ha and relatively high soil C content), contributing to environmental conditions such as soil water content and temperature having a larger influence on $N_2O$ emissions for NT compared to CT treatments.

The lack of a residue removal effect disagrees with previous results for this region. Across 38 site-years, Jin et al. (2014) found that residue removal reduced $N_2O$ emissions by 7%, with this reaching up to 16% under medium residue removal levels in conservation tillage systems. In contrast, Congreves et al. (2017) found that residue removal increased $N_2O$ emissions, primarily during the winter period in CT systems in Ontario, Canada. The lack of an effect in the present study challenges the assumption that crop residue removal will reduce $N_2O$ emissions based on IPCC methodology (IPCC, 2007). A wide variety of responses to residue removal have been observed and the fact that field results do not always support top-down GHG accounting protocols has been argued in previous studies (Congreves et al., 2017; Hao et al., 2001; Lehman & Osborne, 2016; Yao et al., 2013). Together these findings suggest the impacts of residue removal cannot easily be predicted for maize production in this region, which presents a challenge for accurately estimating the net GHG impacts of cellulosic biofuel production.

Contrary to the mixed responses reported in previous research (Congreves et al., 2017; Jin et al., 2014; Mutegi et al., 2010), an interaction between tillage and residue management was not observed in the present study for cumulative or yield-scaled $N_2O$ emissions (Table 1). When comparing findings between studies, it is important to consider the period of monitoring emissions. For instance, the interaction between tillage and residue removal for $N_2O$ emissions found in Congreves et al. (2017) occurred over winter under colder soils but not during the growing season, making it incomparable to our study. These authors offered the possible explanation that in colder climates where soil experience a freeze–thaw cycle in winter, NT and residue removal would more dramatically influence soil temperature and moisture dynamics than during growing season. Similarly, Mutegi et al. (2010) reported more distinct tillage by residue removal interactions in winter, spring, and fall than during the growing season. Yet, the lack of a tillage by residue management interaction in the present study was in accordance with earlier research (Baggs et al., 2003; Malhi and Lemke, 2007). Although we observed that tillage had more of an impact on $N_2O$ emissions than residue removal practices, this finding cannot be extrapolated to other sites. Recent work in both rainfed and irrigated corn systems highlights that residue removal can have significant effects on GHG emissions (Congreves et al., 2017; Jin et al., 2017). As noted above, the degree to which tillage and residue management practices interact to modify the biophysical factors controlling $N_2O$ emissions is a dynamic process that depends on sensitive soil microbial responses and continuously evolving environmental conditions. Therefore, we stress the need for future studies to identify the underlying mechanisms behind
potential interactions, not only allowing for better prediction of management effects on \( \text{N}_2\text{O} \) emissions, but also more accurate estimation of the C footprint of cellulosic ethanol under NT and residue removal practices.

### 4.2 Environmental factors influencing \( \text{N}_2\text{O} \) emissions

In this study, cumulative \( \text{N}_2\text{O} \) emissions, yield-scaled \( \text{N}_2\text{O} \) emissions, grain yield, and soil N intensity were all significantly influenced by year (Table 1), indicating that seasonal variations in precipitation and temperature regulated the responses of both \( \text{N}_2\text{O} \) emissions and crop production. High rainfall in June 2015 and 2016 resulted in more frequent occurrences of soil water content ranging between 0.3 and 0.4 m\(^3\)/m\(^3\), which was equivalent to 60%–80% water-filled pore space (WFPS; Figure 2a,b). However in 2017, the drier June and July than the 20-years average resulted in fewer days with soil water content higher than 0.3 m\(^3\)/m\(^3\), or 60% WFPS (Figure 2c). As small changes in WFPS can greatly influence emissions, soil moisture differences among years likely contributed to the differences in cumulative \( \text{N}_2\text{O} \) emissions. For example, Bateman and Baggs (2005) observed that moving from 60% to 70% WFPS resulted in a sixfold increase in \( \text{N}_2\text{O} \) emissions. Across nine corn production systems in the US Corn Belt, increasing total growing season precipitation was found to have a positive impact on cumulative \( \text{N}_2\text{O} \) emissions in experiments investigating tillage and residue management effects (Jin et al., 2014).

Although NT generally had higher soil water content compared to CT, it is intriguing that this did not translate into higher \( \text{N}_2\text{O} \) emissions. The three seasons (Figure 2a–c), soil water content in R + CT and RR-CT was <0.3 m\(^3\)/m\(^3\) (or WFPS ≤ 60%) on 42%–83% of days. Meanwhile, the highest cumulative \( \text{N}_2\text{O} \) emission was reported with RR-CT in all 3 years, and R + CT with the second highest \( \text{N}_2\text{O} \) emissions in 2016 and 2017 (Supporting Information Table S2). These findings contrast with many studies that have shown NT typically has higher \( \text{N}_2\text{O} \) emissions and attribute this to greater denitrification activity under high soil moisture conditions compared with CT (Guzman et al., 2015; Jin et al., 2014; Venterea et al., 2011; Yao et al., 2013). Denitrification is considered to be the dominant process of \( \text{N}_2\text{O} \) production at WFPS of 60%–90%, whereas nitrification is the major contributor at WFPS < 60% (Chen et al., 2013). Considering that CT in our study often had <60% soil WFPS and a stronger correlation between \( \text{N}_2\text{O} \) emissions and soil NO\(_3\)-N concentrations during peak flux periods in the drier study years of 2016 and 2017 (Supporting Information Table S1), we speculate that higher \( \text{N}_2\text{O} \) emissions for CT may have been associated with enhanced nitrification and coupled nitrification–denitrification activity. Research using isotopes in controlled laboratory conditions found that at low oxygen concentrations, the contribution of nitrifier denitrification to total \( \text{N}_2\text{O} \) production ranged from 48% to 66% and 34% to 57% in soils amended with urea and ammonium sulfate, respectively (Zhu, Burger, Doane, & Horwath, 2013). Another potential factor to consider is changes in microbial biomass and enzyme activity under NT (Zuber & Villamil, 2016). Understanding how such changes might in turn influence nitrification and denitrification activity and the ratio of \( \text{N}_2\text{O} \) to \( \text{N}_2 \) production warrants further investigation. It was recently found that differences in microbial communities were more important for explaining variation in high \( \text{N}_2\text{O} \) emissions across four sites in France compared to abiotic soil properties which better explained lower emissions, with tillage being one of the few management practices that affected the diversity of \( \text{N}_2\text{O} \)-reducing microbial communities (Domeignoz-Horta et al., 2018).

The interactions among mechanisms underlying soil \( \text{N}_2\text{O} \) emissions are complex and can be difficult to elucidate in field experiments. When evaluating the relative importance of soil moisture, temperature, and N concentrations in this study, several conclusions emerged (Supporting Information Table S1). First, precipitation was the overriding factor influencing \( \text{N}_2\text{O} \) emissions in 2015. This year had the highest overall \( \text{N}_2\text{O} \) emissions, and soil water content was positively correlated with \( \text{N}_2\text{O} \) fluxes across treatments during the period of peak emissions (correlation coefficients ranging from 0.54 to 0.92). Correlations between soil moisture and \( \text{N}_2\text{O} \) flux were low in 2016 and 2017 (except for R + NT in 2017), and the relative importance of soil temperature and N concentrations was higher in these years. However, effects were not consistent across tillage systems. In NT systems, which experienced higher soil water content during the period of peak emissions each year as discussed above, an increase in soil temperature appeared to be more important to support \( \text{N}_2\text{O} \) production than increased soil N availability. On the other hand, in CT systems where soil moisture was found to be lower during the period of peak emissions each year, increased NO\(_3\) concentrations appeared to be more important to support \( \text{N}_2\text{O} \) production than relative changes in soil temperature. The fact that soil NO\(_3\) concentrations had a stronger effect on \( \text{N}_2\text{O} \) emissions compared with NH\(_4\)+ and total mineral N concentrations agrees with the results reported by Mutegi et al. (2010). While it is not common to perform regressions separately by treatment when evaluating the effects of environmental variables on \( \text{N}_2\text{O} \) emissions, these results emphasize that different mechanisms may be influencing \( \text{N}_2\text{O} \) emissions in NT compared with CT treatments. Therefore, how future changes in environmental conditions (e.g., climate change) or management practices will affect soil GHG fluxes will likely be distinct for NT and CT systems,
which should be accounted for when designing policies and approaches to mitigate N$_2$O emissions.

4.3 | Tillage and residue management effects on yield

We observed a significant year $\times$ tillage $\times$ residue management interaction for maize yield (Table 1), with RR-NT reducing yield compared with both CT treatments in 2016. Similar yield reductions with NT have been reported for continuous corn systems in the US Midwest (Griffith, Kladivko, Mannering, West, & Parsons, 1988; Karlen, Kvar, Cambardella, & Colvin, 2013; Venterea et al., 2011). This is often attributed to cooler and wetter soil conditions early in the growing season which can negatively impact stand establishment and early crop development. Tillage practices are also thought to affect crop yields by changing soil properties, particularly soil C and N availability (Malhi & Lemke, 2007; Zhang, Zheng, et al., 2015). However, previous research at this site shows few changes have occurred in soil C and N under NT management (Villamil et al., 2015). An important finding is that despite lower yield with NT compared to CT, soil N$_2$O emissions were reduced to an even greater extent, meaning that yield-scaled soil N$_2$O emissions were significantly lower for NT treatments. This finding is in agreement with Venterea et al. (2011) and emphasizes the need to simultaneously account for potential changes in crop productivity and environmental impacts when evaluating alternative crop management strategies. While yields are difficult to predict on a site-specific basis, the potential for negative yield impacts with NT has implications for adoption of this practice. As such, changes in yield should be considered by studies aiming to quantify the net C footprint of cellulosic biofuels based on corn stover removal, possibly through sensitivity analyses or other scenarios.

Crop yield was only affected by residue as part of the three-way year $\times$ tillage $\times$ residue management interaction (Table 1). Prior studies have also observed inconsistent crop yield responses to tillage and residue management practices. Jin et al. (2015) showed no detectable differences in grain yield between residue retention and partial removal treatments in a rainfed continuous corn system under NT in the western Corn Belt. Similarly, a small positive or no effect of residue removal on grain yield was reported by Kenney et al. (2015) in a continuous corn system in Kansas. However, Linden, Clapp, and Dowdy (2000) reported a gradual decrease in corn grain yield after long-term adoption of intermediate residue removal in dry years, but under normal or extremely dry years treatment differences diminished. Retaining crop residue on the soil surface in dry years can enhance yield by improving soil water conservation and moderating soil temperature fluctuation compared with residue removal. In this way, the negative effects of residue removal tend to be minimized in years with adequate rainfall (Jin et al., 2015). On the other hand, residue retention can lead to wet and cold soils, uncontrolled weed development, and poor seed and stand establishment in early spring which can decrease yields (Swan et al., 1994). The fact that residue management was only significant as part of an interaction in our experiment might be attributed to the absence of drought conditions occurring during the study period, combined with the relatively high soil water-holding capacity of soils at this site. As the effects of residue management on crop yield are complex and variable depending on climate, soil fertility, and the duration of conservation tillage practices (Brennan et al., 2014; Zhang, Zheng, et al., 2015), we encourage researchers investigating GHG emissions to also determine impacts on crop productivity. Changes in grain and stover yields not only alter C and N inputs and other biophysical drivers of N$_2$O emissions, but are an important factor for farmers considering alternative management practices that have associated environmental benefits.

5 | CONCLUSIONS

In this study, a year $\times$ tillage interaction significantly influenced cumulative N$_2$O emissions, resulting in 64% lower N$_2$O emissions in NT compared to CT when averaged across 2016 and 2017. This N$_2$O reduction was observed despite NT having higher soil water content than CT, which would generally be expected to increase denitrification activity and N$_2$O emissions, as concluded in previous studies. We did not observe any effect of residue management or a tillage $\times$ residue interaction on N$_2$O emissions. Seasonal variations in environmental factors significantly impacted cumulative and yield-scaled N$_2$O emissions, grain yield, and soil N intensity. Yields were influenced by a year $\times$ tillage $\times$ residue management interaction, where RR-NT reduced yield compared to both CT treatments in 2016, but no significant yield differences among treatments were observed otherwise. Aside from 2015 where a strong relationship between soil water content and N$_2$O emissions was observed for all treatments, there was greater evidence of correlations between soil temperature and N$_2$O emissions for NT treatments and soil N concentrations and N$_2$O emissions in CT treatments. Thus, the high degree of variability in previous field research may in part be explained by different mechanisms influencing N$_2$O emissions in NT and CT systems. Overall, findings from this study provide more evidence in support of previous studies which have challenged the assumption based on IPCC methodology that crop residue removal will consistently result in reduced N$_2$O emissions. While it is commonly estimated that harvesting crop residues such as corn stover to support
cellulosic biofuel production will reduce GHG emissions in this region, our results support a growing evidence-base suggesting that the benefits of this practice may be overestimated if soil N$_2$O emissions do not decrease as predicted.

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SUPPORTING INFORMATION

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