Impact of Kura Clover Living Mulch on Nitrous Oxide Emissions in a Corn–Soybean System

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Abstract

Nitrous oxide (N₂O), produced primarily in agricultural soils, is a potent greenhouse gas and is the dominant ozone-depleting substance. Efforts to reduce N₂O emissions are underway, but mitigation results have been inconsistent. The leguminous perennial kura clover (Trifolium ambiguum M. Bieb.) (KC) can grow side-by-side with cash crops in rotational corn (Zea mays L.)soybean (Glycine max L.) systems. With biological nitrogen fixation, KC provides land managers an opportunity to reduce external fertilizer inputs, which may diminish problematic N₂O emissions. To investigate the effect of a KC living mulch on N₂O emissions, automated soil chambers coupled to a N₂O analyzer were used to measure hourly fluxes from April through October in a 2-yr corn-soybean (CS) rotation. Emissions from the KC treatment were significantly greater than those from the conventional CS treatment despite the fact that the KC treatment received substantially less inorganic nitrogen fertilizer. A seasonal tradeoff was observed with the KC treatment wherein emissions before strip-tillage were reduced but were surpassed by high losses after strip-tillage and postanthesis. These results represent the first reported measurements of N₂O emissions from a KCbased living mulch. The findings cast doubt on the efficacy of KC for mitigating N₂O loss in CS systems. However, if KC reduces nitrate leaching losses, as has been reported elsewhere, it may result in lower indirect (offsite) N₂O emissions.

Core Ideas

• Kura clover living mulch increased total N₂O emissions.

- Nitrogen scavenging by the kura clover living mulch may have reduced spring N₂O emissions.
- Emissions in the kura clover treatment were affected by soil disturbance and plant stress.
- Corn and soybean yield were only marginally affected by kura clover living mulch.

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ITROUS OXIDE (N₂O) is an important greenhouse gas (Hartmann et al., 2013) and is the primary ozonedepleting substance (Ravishankara et al., 2009). In the United States, approximately 74% of the anthropogenic N₂O source is linked to agriculture (USEPA, 2015), placing tremendous pressure on the agricultural community to reduce N₂O emission while simultaneously increasing grain yields. Most often, the discussion to reduce N₂O emission revolves around modifying traditional crop and N management practices. Efforts have included optimizing fertilizer timing (Venterea and Coulter, 2015), source (Halvorson et al., 2014), and depth of application (Maharjan and Venterea, 2014); amendments of biochar (Lin et al., 2014); and alternative tillage techniques (Bavin et al., 2009; Jin et al., 2014). However, the success rates of these practices have been inconsistent and conflicting, in part because of the complex site-specific soil and meteorological interactions governing N₂O production (Decock, 2014).

Because N fertilizers stimulate N₂O production, the N rate is often the best predictor of N₂O emissions (Stehfest and Bouwman, 2006). Notably, recent work suggests that emissions may respond exponentially to increasing N application rates (Hoben et al., 2011; Shcherbak et al., 2014), especially beyond the agronomic optimum application rate (Van Groenigen et al., 2010). Correspondingly, N₂O emissions can be reduced most effectively by simply reducing the N rate, but this practice can reduce yield. To minimize yield loss in response to N rate reduction, a legume can be used to supplant N through biological nitrogen fixation (BNF). This process releases N over time and at lower concentrations than those initially observed after fertilizer application, which could improve synchronization with crop N needs (Crews and Peoples, 2004) while affecting N₂O emissions. Commonly grown legumes include soybeans and cover crop (CC) species such as alfalfa (*Medicago sativa* L.), red clover (Trifolium pretense L.), and hairy vetch (Vicia villosa Roth). A long-term alternative is a perennial living mulch management system. This approach provides the BNF benefit of a leguminous CC, minimizing the need for external N fertilizers, which could concurrently reduce N₂O emissions without harming yields.

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Abbreviations: BNF, biological nitrogen fixation; CC, cover crop; CS, conventional corn–soybean; DOY, day of year; KC, kura clover.

A living mulch is an alternative cropping system in which a CC grows year round with a commodity crop planted into it. Living mulches provide numerous environmental benefits to the community and to land managers by reducing soil erosion (Wall et al., 1991), increasing soil organic matter (Farahbakhshazad et al., 2008), reducing nitrate (NO_3^-) leaching (Ochsner et al., 2010), and improving the field's pest resilience (Enache and Ilnicki, 1990). The leguminous perennial KC can grow alongside corn and, when managed correctly, may only minimally affect grain yield (Zemenchik et al., 2000). We have successfully grown soybeans interseeded into KC as well (Baker, 2012), so it has the potential to be a perennial living mulch for corn–soybean (CS) rotational systems. However, for farmers to make informed management decisions relative to total environmental impact, the N₂O advantages or disadvantages of living mulches must also be considered.

The impacts of CCs on agricultural N₂O emissions are poorly understood because studies have been sparse and conflicting (Basche et al., 2014). Leguminous CCs have been reported to either increase (Gomes et al., 2009) or to have no effect (Alluvione et al., 2010; Barton et al., 2011) on the N₂O budget. Leguminous production of N₂O is driven by the decomposition of N-rich residues (Rochette and Janzen, 2005) rather than the process of BNF (Zhong et al., 2009). Residues of legume CC species generally have low (<25:1) C/N ratios (Gomes et al., 2009) that permit rapid decomposition and nitrification of biotic N, creating an abundance of NO₃⁻ for N₂O production. Because living mulch systems are not terminated, proper management of these perennial systems could curtail the N₂O losses observed after the termination of a CC (Basche et al., 2014), which are amplified at low C/N residue ratios (Huang et al., 2004). However, the effects of KC management and root turnover on N₂O emissions are unknown. There is hope that agricultural producers can play an important role in climate change mitigation, but that requires a better understanding of the impact of alternative management practices on greenhouse gas emissions. Toward that end, our objective was to compare N₂O emission budgets from a conventional CS system with one that included a KC living mulch in a strip-tilled, irrigated field over two growing seasons.

Materials and Methods

Site Description and Experimental Design

The experiment was conducted in a 17-ha field on the University of Minnesota Rosemount Research and Outreach Center near Rosemount, MN ($44^{\circ}42'$ N 93°06' W) over the 2013 and 2014 growing seasons. The soil is a Waukegan silt loam consisting of a silt loam surface layer of 0.4 to 0.7 m depth overlying a layer of outwash sand and gravel >20 m thick. It is well drained, and the surface layer has an organic matter content of 5.2% and a pH of 6.4 (Baker, 2012). This location has been under nearly continuous cultivation for 125 yr, primarily in corn and soybeans since the 1950s (Griffis et al., 2007).

Kura clover was established vegetatively in the summer of 2010 with the primary intent of comparing corn and soybean production in a living mulch versus conventional production (Baker, 2012). The field is equipped with a center pivot. The field was split into four blocks (quartered), and each block was subdivided into four 1-ha plots: irrigated CS, irrigated KC, rain-fed conventional CS, and

rain-fed KC. Our instruments were housed on the border between an irrigated CS plot and an irrigated KC plot. Measurements reported here were taken only from the irrigated plots. For the past 4 yr, the entire field has been in a corn–soybean rotation, with soybean in even years and corn in odd. Each year the entire field is strip-tilled immediately before planting using an Othman six-row unit. This implement has a single shank with fluted row cleaners in front of the shank and wavy coulters on either side.

Glyphosate [N-(phosphonomethyl)glycine]-resistant seed was planted on day of year (DOY) 154 (corn; Pioneer P9917R) and 151 (soybean; Pioneer 22T69R) in 2013 and 2014, respectively. In late spring after strip-tillage and planting, during the crucial germination and early growth period for corn and soybeans, the KC remaining in the inter-row grows vigorously, competing with the young crop for light and resources (Baker, 2012). To minimize these impacts, the KC was mowed to a height of 0.05 m shortly after planting on DOY 165 and 163 in 2013 and 2014, respectively, with the residue left to decompose (Baker, 2012). Weed control in both treatments was accomplished with a glyphosate application at a rate of 1.04 kg ae ha⁻¹ on DOY 176 and 175 in 2013 and 2014, respectively. This rate has been tailored to suppress rather than to kill the KC (Zemenchik et al., 2000) while also controlling weeds. To minimize water stress, the field received 12.5 mm from the center pivot irrigator on seven and three occasions in 2013 and 2014, respectively.

To capitalize on the BNF benefit provided by the KC, the N rate was reduced (-43%) during the corn phase (2013) in the KC relative to the CS treatment. We anticipated that labile KC residues created by tillage and mowing would release mineralizable N in the root zone to support crop requirements. On DOY 154, a broadcast pre-plant N, P, K starter fertilizer (9–18–9) provided 6.7 kg N ha⁻¹ to the entire field. The whole field was side-dressed with a 28% urea and ammonium nitrate solution at a rate of 57.1 kg N ha⁻¹ on DOY 171. The remaining 76.2 (DOY 200) and 15.7 (DOY 203) kg N ha⁻¹ was applied to the CS and KC treatment, respectively, as 28% urea and ammonium nitrate through the center pivot irrigator. Fertilizer was not applied to the soybean crop in 2014.

N₂O Emissions

Soil N₂O fluxes were measured with automated soil chambers (Model LI8100–104, Li-Cor Inc.) controlled by a datalogger (Model 23X, Campbell Scientific) connected to a multiplexer controlling two sets of solenoids (Clippard Inc.) (Baker et al., 2014; Fassbinder et al., 2013). All soil chambers (n = 8) were vented, finished with white enamel to minimize solar heating, and installed onto PVC collars. The collars were placed at the center of the interrow and inserted 0.05 m into the soil. A rubber gasket and weather stripping prevented ambient dilution during chamber closure. Each chamber was activated for 7 min at 1-h intervals, during which headspace air was pulled through a column of desiccant and soda lime before entering a N₂O analyzer (Model M320EU2, Teledyne Instruments API). A nafion dryer within the Teledyne removes remaining H₂O vapor before the sample air enters the measurement cell. Fluxes (n = 31,539 and 28,972 for 2013 and 2014, respectively) were calculated using:

$$F = \frac{PV\Delta}{ART}$$

where P is air pressure (Pa), V is the chamber volume (0.004 m³), A is the chamber footprint (0.032 m²), R is the molar gas constant (J mol⁻¹ K⁻¹), T is the air temperature at the time of measurement (K), and Δ is the slope of N₂O concentration change over time in the chamber headspace. Before slope calculation, the raw N₂O concentration data were passed through a wavelet denoising algorithm to improve the signal to noise ratio (Fassbinder et al., 2013). The slope was calculated from a 90-s window beginning 150 s after chamber closure. All data were processed using Matlab software (Version R2013b, Mathworks).

Climate, Soil, and Plant Analyses

Precipitation was measured at a micrometeorology tower located 1.6 km east of the experimental field. Soil temperature and volumetric water content were measured in each plot at 0.05, 0.1, 0.2, 0.3, and 0.4 m (5TM, Decagon Devices Inc.) at 30-min intervals (EM-50, Decagon Devices Inc.). Each chamber was equipped with a thermocouple (Type E) to measure soil temperature at 0.05 m depth. In August 2015, aboveground KC biomass was collected, and samples (n = 21) were dried and pulverized before total carbon and nitrogen were calculated with an elemental analyzer (VarioMax, Elementar). At crop maturity, corn and soybean grain from 6-m row sections in each plot were hand harvested, dried, and weighed to obtain grain yields.

Data Analysis and Statistics

The 24-h cumulative area-scaled N_2O emissions were determined by integration of the daily mean flux for each plot. The cumulative emission budget was calculated for each plot as the sum of the daily area-scaled emissions during the measurement periods (196 and 151 d in 2013 and 2014, respectively). Missing data due to instrument downtime (3 and 17% in 2013 and 2014, respectively) were not gap filled, and therefore the cumulative emissions represent a conservative estimate. Yield-scaled emissions were determined by dividing the annual cumulative area-scaled emissions by the dry grain yield. Normality was evaluated using a Kolmogorov–Smirnov test. The daily cumulative area-scaled N_2O emissions were log transformed to improve normality (McSwiney et al., 2010). Treatment significance was assessed using a two-factor ANOVA (Jarecki et al., 2009) of log-transformed cumulative area-scaled daily N_2O emission data, treatment, and year. Automated chambers provide a temporally rich dataset that was used to evaluate emissions associated with different field activities (e.g., strip-tilling, mowing, and spraying) and periods (before strip-tilling and postanthesis) using paired *t* tests ($\alpha = 0.01$) of log-transformed daily area-scaled N_2O emissions. Photographs from 2013 were used to estimate the onset of crop anthesis (DOY 226). For consistency, DOY 226 was used in our 2014 analyses.

Results and Discussion

Overall, daily average N₂O fluxes were significantly influenced by treatment (KC > CS; p < 0.001) and year (2013 > 2014; p < 0.005). The year effect is most likely a result of fertilizer application that only occurred during the corn phase (2013) rather than a meteorological difference. Average air temperature and precipitation during the measurement period in each year were 17°C and 606 mm and 17.5°C and 818 mm in 2013 and 2014, respectively (data not shown). Corn (2013; p = 0.08) yields were 11.1 ± 0.9 and 9.7 ± 0.9 Mg (dry) ha⁻¹, and soybean (2014; p = 0.03) yields were 2.51 ± 0.11 and 2.11 ± 0.18 Mg ha⁻¹ in the CS and KC treatments, respectively.

2013: Corn

Presence of the KC living mulch significantly affected N_2O emissions during the corn phase (Fig. 1). Cumulative area-scaled and yield-scaled emissions were 2.3 ± 0.1 kg N ha⁻¹ and 233 ±



Fig. 1. Daily area-scaled N_2O fluxes (symbols) and cumulative emissions (lines) for (A) 2013 and (B) 2014 averaged (n = 4) by treatment. Missing data due to instrument downtime were not gap filled. Cumulative emissions represent the sum of treatment daily averaged fluxes throughout the measurement period. Dotted vertical lines and numbers correspond with emission intervals described in Table 1.

112 g N Mg⁻¹ grain, respectively, in KC and 1.3 ± 0.1 kg N ha⁻¹ and 118 \pm 137.6 g N Mg⁻¹ grain, respectively, in CS. During the entire measurement period, the average volumetric water content and soil temperature at 0.05 m depth were 0.24 \pm 0.02 and 16.5 \pm 6.6°C, respectively, from the KC treatment and 0.22 \pm 0.02 and 14.5 \pm 6.5°C, respectively, from the CS treatment (data not shown).

2014: Soybeans

The KC living mulch increased N_2O emissions during the soybean phase (Fig. 1). Area-scaled and yield-scaled emissions were 1.6 ± 0.1 kg N ha⁻¹ and 765 \pm 65 g N Mg⁻¹ grain, respectively, from the KC treatment and 0.7 \pm 0.1 kg N ha⁻¹ and 291 \pm 58 g N Mg⁻¹ grain, respectively, from the CS treatment. Intermittent problems with our soil moisture and temperature probes prevented a full assessment of the treatment effects on the microclimate during 2014.

Before Strip-Tillage

The available data suggest that the KC living mulch may have marginally reduced N_2O emissions in the spring before strip-tillage (Table 1). Although the treatment effect was not significant, the CS treatment emitted 9.3 and 12 mg N_2O –N m⁻² more than the KC treatment in 2013 and 2014, respectively (Table 1). This trend may have resulted from greater residual soil N in the CS treatment, which the presence of a CC has been shown to reduce (Nair and Lawson, 2014). This benefit, known as "N scavenging," is the result of greater plant N demand, especially outside of the growing season. The KC living mulch removes N that would otherwise be available for N_2O production in the spring, contrary to the CS plots that were left fallow between growing seasons. The presence of an over-winter CC can result in lower spring N_3O emissions relative to fallow conditions (Wagner-Riddle and

Thurtell, 1998). The amount of residual N scavenged by a CC is variable and depends on the CC species (Nair and Lawson, 2014) and management decisions (Komatsuzaki and Wagger, 2015). In Iowa, uptake estimates of 20 kg N ha⁻¹ are common from rye CCs (Nair and Lawson, 2014), whereas rates as high as 80 kg N ha⁻¹ under legume CCs have been observed in the Philippines (George et al., 1994). Importantly, N scavenging can improve the field's overall N use efficiency by assimilating and recycling N (George et al., 1994) that could be lost through leaching and runoff. In addition to N scavenging, the additional surface residue alters the KC soil microclimate by insulating the surface from solar radiation. However, we did not observe significant differences in soil temperature or volumetric water content between treatments during this period in 2013 (temperature, p = 0.8; moisture, p = 0.5) or 2014 (temperature, p = 0.9; moisture, N/A).

Strip-Tilling and Planting

Area-scaled N_2O emissions increased immediately after striptillage (Fig. 1). Emissions from the KC treatment were up to 2.6fold higher than the CS treatment in 2013 (Table 1). An even stronger N_2O emission response was observed in 2014 after tillage and planting, where total N_2O losses from the KC treatment were 3.6-fold greater than the CS treatment (Table 1). Because strip-tillage and planting are disruptive activities, damage to the KC root system, coupled with incorporation of above-ground vegetation, may have caused the release of mineralizable N.

Mowing

Immediately after KC mowing, emissions were not significantly different between treatments in 2013 or 2014 (Table 1). However, cumulative N_2O emissions from the KC treatment were 1.5- and 1.6-fold larger than the CS treatment during 2013 and 2014, respectively (Table 1). This may represent a direct response

Table 1. Cumulative emissions and results from statistical analyses from each activity period during the two sample years. The total emissions during a measurement interval are reported.

Treatment†	Before field activity 1‡	Tillage, planting 2	Mowing 3	Side-dressing 4	Spraying 5	Fertigation 6	Postanthesis 7	Cumulative
	2013							
DOY range	114–151 (<i>n</i> = 37)§	152–164 (<i>n</i> = 11)	165–170 (<i>n</i> = 6)	171–175 (n = 2)	176–199 (<i>n</i> = 23)	200–226 (<i>n</i> = 27)	227–316 (<i>n</i> = 90)	114–316 (<i>n</i> = 196)
KC, mg N m ⁻²	37.8	26.2	9.4	7.1	51	30.6	64.4	226.5
CS, mg N m ⁻²	47.1	10	6.4	4.8	26.2	10.2	26.6	131.3
Treatment difference, mg N m ⁻²	-9.3(<i>p</i> = 0.15)¶	16.2 (<i>p</i> < 0.01)	3 (<i>p</i> = 0.4)	2.3 (<i>p</i> = 0.02)	24.8 (p < 0.01)	20.4 (<i>p</i> < 0.01)	37.8 (<i>p</i> < 0.01)	95.2 (<i>p</i> < 0.01)
	2014							
DOY range	99–150 (<i>n</i> = 32)	151–162 (<i>n</i> = 9)	163–174 (<i>n</i> = 9)	N/A	175–226 (n = 52)	N/A	227–279 (n = 49)	99–279 (<i>n</i> = 151)
KC, mg N m $^{-2}$	27.7	15.5	6.4		32.9		78.8	161.3
CS, mg N m ⁻²	39.7	4.3	4		12.7		12.1	72.8
Treatment difference,# mg N m ⁻²	-12 (<i>p</i> = 0.3)	11.2 (<i>p</i> < 0.01)	2.4 (<i>p</i> = 0.08)		20.2 (p < 0.01)		66.7 (<i>p</i> < 0.01)	88.5 (p < 0.01)

+ CS, corn-soybean; DOY, day of year; KC, kura clover.

‡ The numbered intervals correspond to Fig. 1.

§ Sample size (n) excludes instrument downtime.

¶ Significance was determined using paired t tests performed on the log-transformed emissions over the listed DOY range.

Treatment difference determined as kura clover minus corn-soybean.

of mowed KC residue mineralization because others have also observed high N₂O emissions coinciding with CC decomposition (Basche et al., 2014; Brozyna et al., 2013; Mitchell et al., 2013). The application of residue on the surface has resulted in a positive effect on N₂O emissions (Baggs et al., 2003) that are amplified at low C/N residue ratios (Huang et al., 2004). Leaving the clippings in place can alleviate the denitrification carbon limitation, creating an opportunity for high N₂O production (Mitchell et al., 2013). Kura clover biomass samples show that the C/N ratio was 14.4:1, which is considerably lower than corn stover (>50:1) (Baker et al., 2014), with an average of 2.8% N. The low C/N ratio of the KC clippings would allow for rapid decomposition and nitrification, boosting soil NO₃⁻ content for N₂O production. Further, rapid decomposition would deplete oxygen, creating a more conducive environment for denitrification. Although CC residues may also immobilize soil N (McSwiney et al., 2010), our results suggest that the KC clippings had a positive but minor effect on N2O emissions (Table 1).

Suppression Spraying

After herbicide application, N_2O emissions from the KC treatments were elevated (1.9-fold) above the CS treatments in 2013 (Table 1). Likewise, KC N_2O emissions were substantially greater (2.6-fold) in the period after suppression and before anthesis in 2014. The amplification of KC emissions after herbicide application may have been in response to KC mineralization. Suppression spraying can release N from the root system (Zemenchik et al., 2000), which would be an important source of inorganic N for the crop. Moreover, the mineralization of KC root exudates may partially explain the N_2O emission pulse observed on DOY 180 in 2013.

Fertilizer Application

The N₂O emissions from the KC treatment were 1.8-fold greater than the CS treatment after side-dressing, which was possibly bolstered by plant stress caused by KC mowing or the recent decomposition of mowed KC residue (Table 1). Similarly, plant stress brought about by the recent application of herbicides may partially explain why KC emissions were 3-fold greater than the CS treatment after fertigation (Table 1). By increasing N and C availability while depleting O₂, the rapid decomposition of residues and plant stress may enhance KC N₂O emissions, a response that becomes even more pronounced when N fertilization follows these events. Further, the comparatively low N2O emission response from the CS treatment could indirectly signal optimal N application timing that facilitated rapid uptake by actively growing corn. A reduction in N₂O emissions has been observed in fertigated vegetable systems (Kennedy et al., 2013) because N is applied more in line with crop needs.

Postanthesis Mineralization

In 2013 and 2014, a substantial proportion of the cumulative KC emissions occurred after anthesis (Fig. 1). Emissions from the KC treatment were 2.4-fold greater than the CS treatment from DOY 226 through the end of the 2013 measurement campaign, likely in response to postsenescence kura mineralization (root turnover + exudates). This late-season pulse was responsible for

28% of the cumulative KC N₂O budget; 20% of the CS budget was lost during the same period (Table 1).

The postanthesis burst in 2014 was sufficiently large to offset the modest early-season N_2O reductions provided by KC N scavenging. Kura clover losses here accounted for 49% of the cumulative N_2O budget but for only 17% of losses from the CS (Table 1). Because we did not observe a similar pulse from the CS treatment, this would indicate that postharvest mineralization of N from soybean residues must have been minimal. However, because measurements were seasonally limited to spring, summer, and fall, it is possible that low winter emissions from the KC treatment could counterbalance our observations (Basche et al., 2014).

Irrigation

There were emission pulses after irrigation events in 2013 on DOY 186 (first irrigation event) and DOY 233 (fifth irrigation event), where the CS system lost 63 and 43% more N_2O , respectively, than the KC treatment (data not shown). Greater levels of available N earlier in the season that are progressively depleted through the growing season may have been responsible for the pulse observed on DOY 186. The remaining five irrigation events in 2013 exhibited a negligible effect on emissions, possibly because either the standard 12.5-mm irrigation event was insufficient to saturate soils and promote denitrification or because of low residual N. Further, the emission response after irrigation on DOY 233 may have been made possible because an irrigation event on DOY 228 "primed" the soil microbe community. There was not an N₂O emission response to irrigation from either treatment during the 2014 (soybean) period.

Indirect Emissions

We did not consider the role of indirect N_2O emissions (i.e., through subsequent denitrification of NO_3^- lost in leaching and runoff) in our budget. Offsite N_2O emissions associated with leaching and runoff have been identified as strong sources (Z. Chen et al., personal communication, 2016), especially in agricultural regions (Griffis et al., 2013; Turner et al., 2015). If perennial crops can consistently reduce NO_3^- leaching (Ochsner et al., 2010), a necessary precursor for aquatic N_2O production (Turner et al., 2016), it is conceivable that groundwater and riverine emissions would be mitigated by increased use of KC living mulch systems, although indirect emissions are highly uncertain and much additional research is needed.

Conclusions

Perennial cover crops offer many environmental benefits to agricultural systems. However, reduction of N_2O emissions may not be one of them. We observed that even with a 43% reduced N application rate compared with a conventional CS system, the KC living mulch system increased direct N_2O emissions. Our data suggest an important trade-off in the KC system whereby emissions before strip-tilling may be lowered due to N scavenging, but strong emissions driven by postanthesis mineralization offset this benefit. Lastly, because differences in corn grain yield were not significant, it is possible that the N application rate could be reduced further to minimize N_2O emissions.

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