



Nitrous oxide emissions from manured, no-till corn systems

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Abstract Using dairy manure and legumes in crop rotations can reduce inorganic N inputs for corn (*Zea mays* L.), yet these practices can also contribute to nitrous oxide (N₂O) emissions. In two crop rotations we investigated how different (i) organic and inorganic N amendments and (ii) prior legume crops with broadcast manure influenced direct N₂O emissions from silt-loam soils planted to no-till corn. We measured N₂O fluxes from April to December for two years using closed vented chambers from soils planted to corn with no spring residue to compare inorganic N fertilizer (S-UAN) and two liquid dairy manure application methods: surface broadcasted (S-BM) and injected (S-IM). Emissions were also measured to compare the effect of crop residue of

perennial forages, a green manure legume, or soybean (*Glycine max* L. Merr.) from soils all amended with liquid dairy manure. Nitrous oxide emissions were greatest during the 15–45 days after manure was injected compared to broadcasting, and cumulative N₂O emissions were larger from S-IM (2.8–2.5 kg ha⁻¹) than S-BM (1.4–0.7 kg ha⁻¹) and S-UAN (0.3 kg ha⁻¹). Cumulative and yield-scaled N₂O emissions did not differ among the prior legume treatments. A ranking approach based on random forests, identified the most important variable contributing to N₂O emissions in both comparisons as corn growing degree days, indicative of the asynchrony of spring legume termination and manure application with corn planting and N use; and changing environmental conditions for N mineralization and denitrification.

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Introduction

Crop production systems are an important source of the N₂O. This is especially true for systems that include corn, which requires large N fertility inputs and accounts for the highest N₂O emissions among the

major crops in the US (Del Grosso et al. 2005; US EPA 2016). Numerous studies have shown that increasing N amendments to soil can increase N₂O emissions (Millar et al. 2010), especially when N additions exceed crop N needs (McSwiney and Robertson 2005; Hoben et al. 2011). Nitrous oxide emissions have been shown to vary with form and management of N applications. Davidson (2009) estimated that 2.5% of inorganic fertilizer-N and 2.0% of manure-N applied to soils was converted to N₂O between 1860 and 2005. Despite the small portion of applied N lost as N₂O, the N₂O global warming potential is approximately 300 times that of carbon dioxide (CO₂) over a 100-year period (US EPA 2016), and it is currently the dominant ozone-depleting substance (Ravishankara et al. 2009).

Because N₂O is primarily produced by nitrification and denitrification in soils, application of both organic and inorganic N fertility sources can contribute to N₂O emissions. Nitrogen use efficiency from manure and/or fertilizers on dairy farms varies from 16 to 77% (Powell et al. 2010), suggesting that improved N management may present an opportunity to capture more N for crops, improve the economic benefits of manure use, protect water quality (Jokela 1992; Clarke and Beegle 2014), and reduce N₂O emissions. Strategies to increase the utilization efficiency of fertilizer and manure N include adjusting the application rate, timing, and placement of the N source (Schröder 2005). Side-dressing inorganic fertilizer N at the time of rapid corn N uptake was shown to reduce N₂O emissions in no-till soils in eastern Canada (Wagner-Riddle et al. 2007). However, high N₂O emissions have still been reported when side-dress N fertilizer applications coincided with heavy rainfall events (Venterea and Coulter 2015). Shallow-disk manure injection provides N conserving benefits of incorporating manures into soil in no-till production (Aita et al. 2014; Bierer et al. 2017), but manure injection also has the potential to increase N loss via leaching (Dell et al. 2011) and N₂O emissions through denitrification (Aita et al. 2014; Duncan et al. 2017).

Nitrogen inputs in cropping systems typically come from inorganic commercial fertilizers, livestock manures, residues of previous crops, or a combination of these sources. In northeastern US dairy cropping systems, corn harvested for silage is typically grown following three or more years of perennial alfalfa (*Medicago sativa* L.) or alfalfa and grass mixtures. Other legumes, such as clovers, are also included in

dairy rotations either in place of alfalfa as a forage crop or as a winter cover crop. In dairy rotations, corn N requirements are typically provided by a combination of legumes, dairy manure, and inorganic fertilizers (Powell et al. 2010). Inclusion of legumes in a rotation can reduce the need for N applications to subsequent corn crops (Fox and Piekielek 1988; Velthof et al. 1998), potentially reducing N₂O emissions. Including alfalfa in a rotation has also been found to reduce nitrate pollution of water (Peterson and Russelle 1991; Randall and Mulla 2001).

Managing cropping systems to minimize N₂O emissions requires a better understanding of factors controlling emissions. Because N₂O emission rates are influenced by multiple N input sources and multiple factors (such as soil temperature and moisture and microbial community composition and activity), multivariate statistical tools are needed to evaluate drivers of N₂O emissions in complex crop rotations. In recent years, researchers have used Random Forest (RF) analysis to evaluate influences of multiple factors on a range of processes in agroecosystems including N₂O emissions (Villa-Vialaneix et al. 2012; Philibert et al. 2013; Finney et al. 2015; Lugato et al. 2017; Saha et al. 2017a, b; Lush et al. 2018).

In the current study, N₂O emissions and ancillary measures (soil temperature, moisture, and inorganic N concentration) were monitored from corn in both a complex, 6-year dairy forage rotation and a simple corn/soybean rotation as part of long-term cropping system research in central Pennsylvania, USA. These cropping systems represent practices that farmers typically employ or could use and allowed the evaluation of factors controlling N₂O emission following application of multiple N amendments including manure application methods and different crop residue inputs preceding the corn. The objectives of this study were to evaluate differences in N₂O emissions in response to different prior legumes and N amendments following soybean and to identify the most influential factors controlling N₂O emissions in these systems. We hypothesized that cropping systems receiving organic N amendments prior to corn planting would have higher potential for N₂O emissions compared to the treatment receiving inorganic starter and side-dress N fertilizer to coincide with high crop N demand; termination of legumes prior to corn plant would increase N₂O emissions; and, N₂O emissions would be greater from shallow-disk injected manure

than with surface broadcasted manure without incorporation.

Materials and methods

Study site and crop rotations

The experiment was conducted within the Northeast Sustainable Agriculture Research and Education-funded Dairy Cropping Systems project at the Pennsylvania State University's Russell E Larson Agronomy Research Farm, near State College, Pennsylvania, USA (40.72° N, -77.92° W, Elevation 366 m). The parent project aims to sustainably produce the forage, feed, and canola oil fuel for a 65 cow, 97 ha dairy farm in Pennsylvania, and was initiated in 2010 and at 1/20th of the scale on 4.85 ha of land. The soil at the site is primarily Murrill channery silt loam (fine-loamy, mixed, semiactive, mesic Typic Hapludults) with small areas of Buchanan channery silt loam (fine-loamy, mixed, semiactive, mesic Aquic Fraguidults), with 0–3 and 3–8% slopes. Average total C and N were 14.9 and 1.48 g kg⁻¹ in the upper 0–5 cm and 12.0 and 1.24 g kg⁻¹ in the 5–15 cm depth. Average pH of the upper 2 cm of soil was 6.2.

Nitrous oxide emissions were measured from corn phases of two crop rotations: a corn–soybean (C–S) rotation and a dairy forage rotation (Fig. S1). The experiment was a randomized, nested split-plot design with 3 replications sampled for N₂O emissions. Main plots were the crop phase in both rotations and the nested split-plots (27 × 9 m) provided comparison of management treatments for that crop. In the C–S rotation, broadcast dairy manure application (S-BM) and shallow disk manure injection (S-IM) and inorganic-fertilizer (liquid urea ammonium nitrate, S-UAN) were compared. The S-BM and S-IM treatments received manure once in the spring before corn was planted in each year that corn was grown since 2010. The corn sampled in the S-BM treatment in 2016, however, did not receive manure in 2012 and 2014.

To compare the influence of prior legume crop residues on emissions, all with surface broadcast manure, we sampled corn for grain following soybeans without winter cover crops in the C–S rotation. In the dairy forage rotation, we sampled split plots that

received broadcast manure in the corn phases following termination of a mixture of alfalfa and orchardgrass (*Dactylis glomerata* L.) (AO-BM) or crimson clover (*Trifolium incarnatum* L.) (CC-BM). The dairy forage rotation was a 6-year rotation with 2-year mixed alfalfa and orchardgrass forage crop, followed by corn grown for grain (2015) or silage (2016). The corn grain was interseeded with a mixture of cover crop species dominated by annual ryegrass (*Lolium multiflorum* L.) and forage radish (*Raphanus sativus* L.). Corn harvested for silage was followed with winter cereal rye (*Secale cereale* L.) that was planted in the fall, overwintered, and harvested the following spring for silage, then planted to crimson clover, followed in spring by corn grown for silage (2015) or grain (2016) interseeded with the cover crop mixture, and followed by an oat cover crop (Fig. S1). Therefore, each corn phase in the dairy rotation was preceded by a different legume or legume/grass mixture.

Both crop rotations were managed under no-till, and perennial and annual legume crops were planted with a Great Plains 1005 solid-stand no-till drill (Great Plains Manufacturing, Inc, Salina, KS). A mixture of alfalfa and orchardgrass was planted at a rate of 9.9 and 24.7 kg ha⁻¹, respectively, in 19-cm rows on 24 April 2013 and 14 April 2014. Crimson clover was planted at 49.42 kg ha⁻¹ in 19-cm rows on 12 September 2014 and 29 August 2015. Alfalfa and orchardgrass and crimson clover were terminated with glyphosate [*N*-(phosphonomethyl) glycine] and 2,4-D [(2,4-dichlorophenoxy) acetic acid] on 8 May 2015 and 27 April 2016. Soybean was planted at a rate of 494,000 seeds ha⁻¹ in 38-cm rows on 2 June 2014 and 22 May 2015 and harvested on 27 October 2014 and 8 October 2015. Corn was planted with a John Deere 1780 no-till planter (Deere & Company, Moline, IL 61265) in 76-cm wide rows between 14 and 19 May in 2015 and 2016. Corn for grain (TA566-31, T.A. Seeds, Jersey Shore, PA in 2015; 105 RDM and MC5663 Kings Agri Seeds, Ronks, PA in 2016; 106 RDM) was planted in the C–S rotation and in the forage rotation (TA566-18, T.A. Seeds, Jersey Shore, PA in 2015; 105 RDM and MC5661, Kings Agri Seeds, Ronks, PA in 2016; 106 RDM) at a rate of 79,070 seeds ha⁻¹. Corn for silage (TA089-00, T.A. Seeds, Jersey Shore, PA, in 2015 and TA290-18, T.A. Seeds, Jersey Shore, PA in 2016; both 89 RDM) was planted in CC-BM in 2015 and AO-BM in 2016 at a rate of 86,500 seeds ha⁻¹. Corn grain and corn silage were harvested on 5

November 2015 and 28 October 2016 and 8 September 2015 and 16 September 2016, respectively.

Nitrogen management

Starter fertilizer was applied to corn at planting (Table 1). Dairy slurry manure was surface broadcast applied or shallow disk injected between 3 and 10 days prior to corn planting on 11 May in 2015 at 44 Mg ha⁻¹ and on 9 May in 2016 at 42 Mg ha⁻¹. Manure was injected to approximately 10 cm with a shallow disk injector (Avenger, Yetter Manufacturing, Inc, Colchester, IL), with injectors spaced at 76 cm intervals. To calculate the amount of N and other nutrients added, manure samples were analyzed by The Pennsylvania State University's Agricultural Analytical Services Laboratory. The N content in the manure was 3.13 kg N Mg⁻¹ in 2015 and 3.26 N kg N Mg⁻¹ in 2016. The total manure N was calculated by multiplying the manure applied per ha by the N content (Table 1). The total percentage of solids in the manure was 8.3% in 2015 and 10.6% in 2016.

Consistent with the recommended practice for farmers in Pennsylvania (Penn State Agronomy Guide 2015), pre-sidedress nitrate tests (PSNT: Magdoff 1991) were conducted at V6 corn growth stage to determine if corn crop could be expected to respond to in-season N application. Soil samples for PSNT were taken from 0 to 15 cm depth. For the yield goal at the

experimental site, the Pennsylvania State University Cooperative Extension recommends inorganic N should be applied to corn when PSNT NO₃⁻ levels are lower than 21 ppm (Penn State 2015; Fox et al. 1989; Beegle et al. 1999). Based on the PSNT tests in 2015 and 2016, only S-BM and S-UAN needed supplemental N and were side-dressed with liquid UAN at 53 kg ha⁻¹ N and 129 kg ha⁻¹ N in 2015, and 100 kg ha⁻¹ N and 122 kg ha⁻¹ N in 2016, respectively.

Spring biomass sampling for the crops prior to corn

Aboveground biomass of the crop grown prior to corn was determined for crimson clover, and alfalfa and orchardgrass through destructive harvest of two 0.25 m² quadrats per plot before crop termination. Aboveground biomass was sorted into legume versus non-legume plants and dried at 45 °C. A sub-sample of the biomass was ground following drying, and total C and N were determined by combustion analysis (Elementar Vario Max N/C; Horneck and Miller 1998). The ratio of C to N was calculated for the RF analysis to account for the influence of aboveground residue decomposition rate on N₂O emissions.

We used Penn State Agronomy Guide (2015) estimates for the residual legume N to account for the N contribution of the crops prior to corn. The N contribution from the soybean was estimated by multiplying soybean grain yields (kg ha⁻¹) by

Table 1 N inputs from prior legume N, manure, and fertilizer in 2015 and 2016 to corn planted after different crops or with different amendments: alfalfa and orchardgrass with broadcast manure (AO-BM), crimson clover with broadcast manure (CC-

BM), soybean with broadcast manure (S-BM), soybean with injected manure (S-IM), and soybean with inorganic fertilizer (S-UAN)

Treatment	Year	N contribution from prior legume kg ha ⁻¹	Total manure N	N starter fertilizer	Side-dress N
AO-BM	2015	90	169	22	0
	2016	90	156	22	0
CC-BM	2015	45	165	9	0
	2016	45	176	9	0
S-BM	2015	68	167	22	53
	2016	65	169	22	100
S-IM	2015	66	186	22	0
	2016	62	180	22	0
S-UAN	2015	63	0	22	129
	2016	70	0	22	122

56 kg N kg⁻¹ soybean (Penn State 2015). Crimson clover was estimated to contribute 45 kg N ha⁻¹ and alfalfa 90 kg N ha⁻¹ (Penn State 2015).

Soil measurements

Soil temperature (Model HI 145, Hanna Instruments) and volumetric soil water content (VSWC) (Model ML3 ThetaProbe, Delta-T Devices) were measured from the 0 to 10 cm soil depth every time N₂O gas samples were collected. Soil samples were taken once a week from the surface layer (0–5 cm) and analyzed for ammonium (NH₄⁺) and NO₃⁻. Sampling was restricted to the surface layer, because all N inputs in this no-till system were made to the surface without incorporation and past measurements indicated that most added N remained near the surface. Additional soil samples from the 5–15 cm depth would have added useful information, but stone and clay content of the soils makes consistent sampling of that depth difficult in dry weather. Three random soil samples (2.5 cm diameter cores plot⁻¹) were collected in each treatment-plot, except for the S-IM treatment. For S-IM, we followed the soil N sampling protocol developed by Meinen et al. (2015). Five soil samples were collected 15.2 cm apart across the 76 cm injection bands. Inorganic N was extracted with 2 M KCl (Mulvaney 1996), with NH₄⁺ and NO₃⁻ in extracts determined by a flow injection analyzer (Lachat Instruments 2001, 2003).

N₂O measurements

Gas samples were collected in 2015 from 15 April to 7 December, and in 2016 from 12 April to 9 September. Nitrous oxide fluxes were measured from each treatment plot with vented static chambers (78.5 cm × 40.5 cm × 12 cm) (Parkin and Venterea 2011). Two chambers were deployed in each plot. Chamber bases were placed perpendicularly between two corn rows in each plot, for a total of 30 samples (5 treatments × 3 blocks × 2 bases). Bases were inserted 5 cm into the soil. Measurements were taken prior to and during the period of anticipated N₂O fluxes to capture the profile of gas emissions during the growing season. We sampled two times a week for approximately 60 days after legume crops were terminated and manure was applied in spring. The days during the week when samples were obtained varied when needed to allow

for timely sampling in response to rainfall. Beyond 60 days after manure application, measured N₂O fluxes were frequently below detection, and sampling frequency was reduced to 7–31 days. When no detectable fluxes were measured on a sampling date, the next sampling was delayed until 24–48 h after rainfall occurred to potentially drive new N₂O emissions. Fluxes were measured between 9:00 to 12:00 h to account for diurnal variation in the flux pattern (Parkin and Venterea 2011). Samples were collected at approximately 10, 20, and 30 min after placing the covers on the bases. Ambient air samples were collected outside of the chambers and used as the time 0 measurement. Emission rate was determined by linear regression of change in N₂O concentration versus time since chamber deployment, as previous analysis with these chambers and deployment times showed that a non-linear model did not improve flux rate estimation (Dell et al. 2014). The average flux rate of the two chambers in a plot was used for further statistical analysis, and data for an individual chamber was omitted on the limited number of occasions when sample leaks occurred. Gas samples were analyzed using a Varian 3800 gas chromatograph (Scion Instruments, West Lothian, UK) with an electron capture detector and an automated sample injection system. The chromatograph oven and injector were maintained at 50 °C and the detector at 285 °C. Dinitrogen was the carrier gas. The minimum detectable flux with this measurement system was previously determined to be approximately 5 g N₂O ha⁻¹ d⁻¹ (Dell et al. 2014). Cumulative N₂O emissions were calculated by linear interpolation of daily flux rates. The need to assume a linear change in emission rate between sampling dates likely leads to some estimation error if emission rates change between sampling dates. However, we are confident our sampling scheme captured most emission events during the growing season. The cumulative emission estimates were a useful tool for comparison among treatments, since gas samples were collected in all treatments on each sampling date. Cumulative N₂O emissions per unit of N applied were calculated by dividing cumulative N₂O emissions during corn growing season by the total N application from the aboveground spring crop residues, N fertilization, and manure application. The cumulative N₂O emissions per unit of grain yield were calculated by dividing

cumulative N₂O emissions during corn growing season by the grain yield.

Weather data

Daily air temperature and precipitation data were obtained from the USDA NRCS-ARS SCAN site at Rock Springs, Pennsylvania (<http://wcc.sc.egov.usda.gov/reportGenerator>). The weather station was located less than 0.5 km from the experimental site. The air temperature data was used to calculate the growth degree days (GDD) accumulated for corn using a base value of 10 °C. The corn GDD was 2044 for 2015 and 1951 for 2016.

Statistical analysis

Conventional ANOVA was used to evaluate differences among management treatments, while a ranking approach based on Random Forests was used to identify the most influential factors controlling N₂O emissions in these systems. Comparisons focusing on the impacts of N amendments (S-IM, S-BM and S-UAN) and of prior legume crop (AO-BM, CC-BM and S-BM) on N₂O emissions were analyzed separately.

To evaluate differences among management treatments, we performed an analysis of variance (ANOVA) with repeated measures using SAS PROC MIXED (v. 9.4, SAS Institute Inc 2012) on the soil moisture, soil temperature, N₂O, NO₃⁻ and NH₄⁺ in the soil with N amendment treatment as a fixed effect. Block and the interaction N amendment were random effects, and sampling date was a repeated measure. For the prior legume comparison, rotation, prior legume crop nested in rotation were fixed effects; and block and the interaction of block and rotation were random effects with sampling date as a repeated measure. Due to unequally spaced sampling events, covariance was modeled using the spatial power law structure [SP(POW)]. Kenward–Roger method was used to approximate denominator degrees of freedom. The SLICE option of the LSMEANS subcommand was used to test differences among treatment means by day. When there were differences among treatment means, the LSMEANS with Tukey–Kramer adjustments was used to separate means. Treatments were considered statistically different at $P \leq 0.05$.

Differences among treatments in spring legume biomass, cumulative N₂O emissions, yields, and cumulative N₂O emissions per unit grain yield and per unit of N applied, were analyzed by ANOVA using SAS PROC MIXED with N amendment treatment as fixed effects, blocks as a random effect, and the interaction of N amendment by block as random effects. For the prior legume comparison, rotation and prior legume crop nested in rotation were fixed effects; and the interaction of block and rotation were random effects. The 2015 cumulative N₂O emissions data was square-root transformed and the 2016 cumulative N₂O emissions data was log transformed to improve normality. For both 2015 and 2016, the cumulative N₂O emissions per unit grain yield and per unit of N applied data were log transformed to improve normality. A two-sided F test was performed with residual values to compare variances among 2015 and 2016. Since variances were significantly different between years, N amendment or prior crop treatments were analyzed within each year. The LSMEANS with Tukey–Kramer option was used to compare treatment means and considered significantly different at $P \leq 0.05$.

We identified the most important variables contributing to N₂O emissions by using a conditional Random Forest (RF) which is a non-parametric, machine learning approach. A considerable advantage of using this method is that data do not need to be rescaled, transformed, or modified and missing values are not problematic. The conditional RF analysis was conducted using the “party” package of R statistical software (R Development Core Team 2013). The suite of environmental and management variables used for the conditional RF analysis that we expected to influence N₂O emission, are listed in Table 2. In the past years, random forests have been used to rank the importance of predictor variables by constructing classification and regression trees (CART) (Breiman 2001). However, because the variable importance measures from the random forests showed bias towards correlated predictors, Strobl et al. (2008) developed a new conditional permutation for determining variable importance. Contrary to the original random forest, in the conditional forest the trees are constructed using random restricted subsets of the predictors variables for each split to avoid correlation among the trees (Strobl et al. 2008). Then, the values of a given variable are permuted while all others are

Table 2 Response variable and input variables evaluated across the corn cropping systems with Random Forest analysis in 2015 and 2016

Name	Type	Description
N ₂ O	Q	Nitrous oxide emission (g N ha ⁻¹ day ⁻¹)
Rotation	C	Dairy forage or corn-soybean rotation
Daylength	Q	Daylength (hours)
GDD	Q	Growth degree days accumulated for corn
Soil nitrate	Q	Soil nitrate on the day of observation at 5 cm depth (mg N kg ⁻¹)
Soil ammonium	Q	Soil ammonium on the day of observation at 5 cm depth (mg N kg ⁻¹)
Soil moisture	Q	Soil volumetric water content on the day of observation at 10 cm depth
WFPS	Q	Soil water-filled pore space on the day of observation at 10 cm depth
Precipitation 1	Q	Precipitation one day prior the observation (mm)
Precipitation 2	Q	Precipitation two days prior the observation (mm)
Precipitation 3	Q	Precipitation three days prior the observation (mm)
Soil temperature	Q	Average soil temperature (°C) on the day of observation at 10 cm depth
Avg air temperature	Q	Average air temperature on the day of the observation (°C)
Max air temperature	Q	Maximum air temperature on the day of the observation (°C)
Min air temperature	Q	Minimum air temperature on the day of the observation (°C)
N application method	C	Broadcast/Injected
Total manure N	Q	Total manure N application rate (kg N ha ⁻¹)
Total N applied	Q	Total N application (kg N ha ⁻¹)
Temporal N application	Q	Total N applied through given day (kg N ha ⁻¹)
Slope	Q	0–3% or 3–8%
Starter fertilizer	Q	N application rate (kg N ha ⁻¹)
Fertilizer	Q	N application rate (kg N ha ⁻¹)
C/N	C	C:N ratio of aboveground spring residue biomass
N spring residue biomass	Q	N content of spring residue biomass (kg N ha ⁻¹)

Q quantitative variable, C categorical variable

left unchanged and then passed down each tree in the forest to obtain new predictions (Strobl et al. 2009). The individual importance of the predictor variable is explained by the % Mean Square Error (MSE) increase, which is calculated by subtracting the MSE before and after the permutations, averaged over all trees. If the predictor variable is strongly associated with the response variable, permutation will greatly increase the MSE. This measure allows comparison of the predictor variables with respect to their impact in N₂O emissions. To assure stability of the importance measure for the highest-ranked variable, we verified the results by constructing 10 forests (each with random restricted subsets of 4 and 1000 trees) from 10 random seeds; importance scores were averaged over these 10 repetitions of the algorithm. For cases in

which important variables from the conditional importance measure were strongly correlated ($r > 0.7$), only the highest-ranking variable was considered most important.

Separate RF analysis was performed for comparisons of N amendment type (S-IM, S-UAN, and S-BM) and for impacts of prior legume crops (AO-BM, CC-BM, and S-BM).

Results

Environmental factors

Precipitation, soil temperature, and soil moisture during the 2015 and 2016 sampling periods are shown

in Figs. 1a–f and 2a–f. Precipitation during the growing season varied widely between 2015 and 2016. May was a drier in 2015 (64 mm) compared to 2016 (86 mm), and the amount of precipitation was higher in June and July of 2015 (294 mm) compared to 2016 (164 mm). Soil temperature and soil moisture differed significantly across sampling dates ($P < 0.0001$), but were not affected by cropping systems on individual sampling dates. In both years, soils were cooler in April (avg. temperature of 9 °C) and warmer in June (20 °C).

Aboveground spring biomass of the prior legume crop

Aboveground spring biomass of the crops prior to corn in the dairy forage rotation differed between years but not between the crops prior to corn in the dairy rotation. The biomass of the alfalfa and orchardgrass mixture had 45% alfalfa and 55% orchardgrass in 2015 and 56% alfalfa and 44% orchardgrass in 2016. Average aboveground biomass of the mixture was 1.08 Mg ha⁻¹ in 2015 and 2.75 Mg ha⁻¹ in 2016,

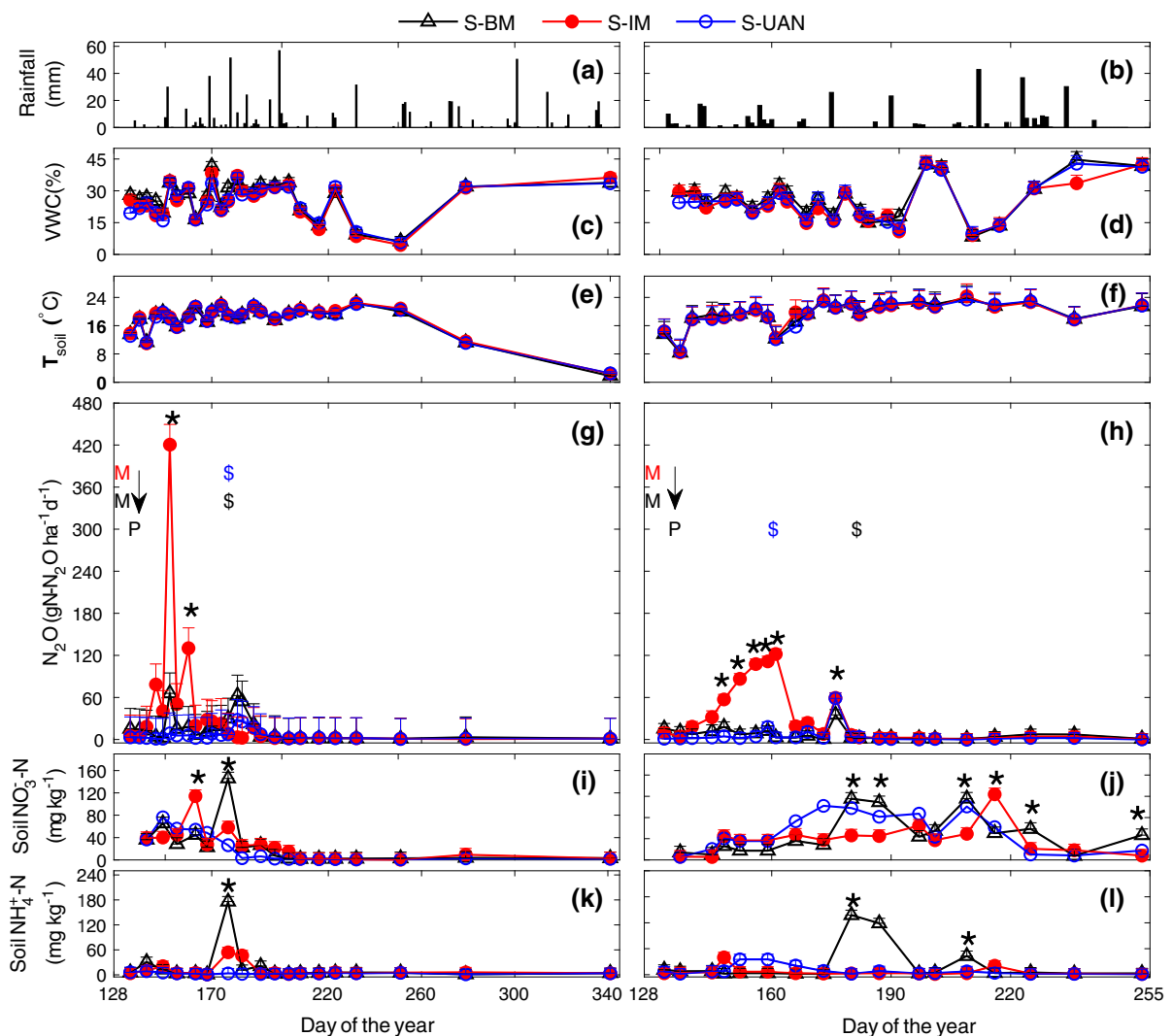


Fig. 1 2015 and 2016: **a, b** precipitation, **c, d** volumetric water content, **e, f** soil temperature, **g, h** nitrous oxide emissions, **i, j** nitrate, and **k, l** ammonium levels in the soil (0–5 cm) during soil gas measurements in the corn following: soybean with broadcast manure (S-BM), soybean with injected manure (S-

IM), and soybean with inorganic fertilizer (S-UAN). M indicates when manure was applied, P indicates when corn was planted, ↓ indicates when starter fertilizer was applied, \$ indicates when side-dress N was applied, and * significant difference among treatments at P value < 0.05

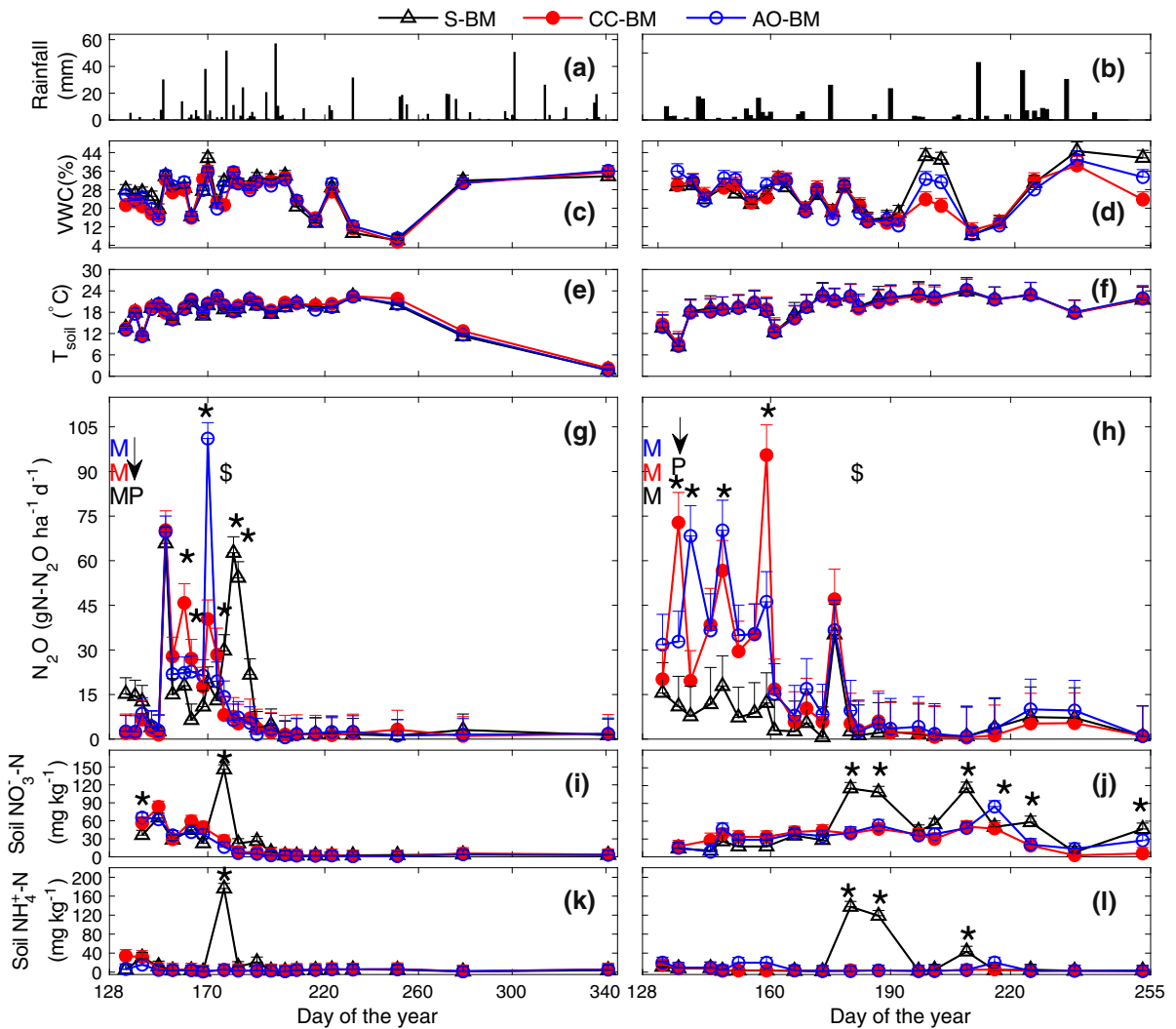


Fig. 2 2015 and 2016: **a, b** precipitation, **c, d** volumetric water content, **e, f** soil temperature, **g, h** nitrous oxide emissions, **i, j** nitrate, **k, l** ammonium levels in the soil (0–5 cm) during soil measurements in the corn following: soybean with broadcast manure (S-BM), crimson clover with broadcast manure (CC-

BM), and alfalfa and orchardgrass with broadcast manure (AO-BM) M indicates when manure was applied, ↓ indicates when starter fertilizer was applied, P indicates when corn was planted, \$ indicates when side-dress N was applied, and * significant difference among treatments at P value < 0.05

with a weighted average C:N ratio of the alfalfa and orchardgrass aboveground biomass of 11.6 in 2015 ($38\ kg\ N\ ha^{-1}$) and 11.5 in 2016 ($113\ kg\ N\ ha^{-1}$). Crimson clover aboveground biomass was $1.56\ Mg\ ha^{-1}$ in 2015 and $1.80\ Mg\ ha^{-1}$ in 2016, with a C:N ratio of 12.1 in 2015 ($55\ kg\ N\ ha^{-1}$) and 11.0 in 2016 ($72\ kg\ N\ ha^{-1}$). The C-S treatments had no living crop biomass in the spring, only a small amount of residue from soybeans that were harvested the previous autumn and yielded $3.60\ Mg\ ha^{-1}$ of grain.

Nitrous oxide emissions in response to N amendment type (S-IM, S-UAN, and S-BM)

Nitrous oxide emissions were typically near the lower detection limit, except for periods of about 14–45 days after manure application and a few days following side-dress N application (Fig. 1g, h). Following manure application in both years, N_2O emissions from S-IM were significantly greater than from S-BM and S-UAN ($P < 0.0001$) (Table 3). Type of N amendment, sampling date, and their interaction significantly

Table 3 Cumulative corn growing season N₂O emissions, total N applied, grain yield, and N₂O emissions per unit grain yield from corn planted following different crops or with different

amendments: soybean with injected manure (S-IM), soybean with broadcast manure (S-BM), and soybean with inorganic fertilizer (S-UAN)

Year	Treatment	Corn growing season N ₂ O emissions (kg N ha ⁻¹)	Total N applied (kg N ha ⁻¹)	Grain yield (Mg ha ⁻¹)	N ₂ O emissions per unit grain yield (g N Mg ⁻¹ grain ⁻¹)
2015	S-IM	2.8 ± 0.1 a	274	8.8 ± 1.2 a	294.2 ± 2 a
	S-BM	1.4 ± 0.1 ab	310	8.7 ± 1.2 a	152.7 ± 2 ab
	S-UAN	0.3 ± 0.1 b	214	9.6 ± 1.2 a	24 ± 2 b
2016	S-IM	2.5 ± 0.02 a	264	10 ± 1.1 a	244.6 ± 1.7 a
	S-BM	0.7 ± 0.02 b	356	11 ± 1.1 a	63.7 ± 1.7 ab
	S-UAN	0.3 ± 0.02 b	214	11 ± 1.1 a	18.8 ± 1.7 b

Mean ± least squared means. Different letters (a, b) indicate a statistical significance at $P < 0.05$ within a year

affected N₂O emissions in both years ($P < 0.0001$). Cumulative N₂O emissions from N amendment treatments were significantly different ($P = 0.0028$) in 2015 and 2016 ($P < 0.0001$). In both years, S-IM had greater cumulative N₂O emissions than S-BM and S-UAN (Table 3). Total emissions from S-BM and S-UAN were not significantly different. In 2015 and 2016, the cumulative N₂O emissions per unit of grain yield were significantly larger in S-IM than S-UAN (Table 3). Since the corn grain yields didn't differ among treatments (Table 3), the larger grain yield-scaled and N-scaled N₂O emissions from S-IM was due to the smaller cumulative N₂O emissions of S-UAN compared to the S-IM.

Nitrous oxide emissions in response to prior legume crop (AO-BM, CC-BM, and S-BM)

In 2015 and 2016, daily mean N₂O fluxes from AO-BM and CC-BM increased after manure application and termination of legumes (Fig. 2g, h). As with amendment type comparisons in C-S, notable emissions in dairy forage rotation were observed only during the 14–45 days after manure application and for a short period following side-dress N application in 2015. In both 2015 and 2016, the interaction between sampling date and prior crop treatment was significant for N₂O emissions ($P < 0.0001$), and emissions from the prior legume crop treatments differed among some sampling dates ($P < 0.0001$). Emissions were especially elevated in May 2015 shortly after a large precipitation event, when wet soils likely favored denitrification (Fig. 2a, g). Nitrous oxide emissions

from AO-BM were somewhat larger early in the season, compared to the S-BM with no spring residue (Fig. 2g, h), therefore mineralization from the N rich residues (C:N about 11.5) may have contributed to greater early season N₂O emissions. However, the specific contribution of legume residues to N₂O emissions could not be determined, because no unmanured controls were established. Spring termination of alfalfa/orchard grass or clover did not significantly increase cumulative N₂O emissions compared to those when corn was grown following soybean with no winter cover crop (Table 4).

Main drivers contributing to N₂O fluxes

The predictor variables identified by conditional RF differed slightly between the two comparisons as indicated in Fig. 3a, b. For comparisons of N amendment types (Fig. 3a), RF ranked GDD, N application method, total manure N, and daylength as the most important predictors of daily N₂O emissions. For the comparison of prior legume crops that all received broadcast manure (Fig. 3b), GDD, C:N ratio of aboveground spring residue biomass and precipitation were the most important predictors of daily N₂O emissions. The variance explained by the conditional RF was 41% for the N amendment comparison, and 39% for the prior legume crops comparison. Because only the highest ranked predictor variable was designated as most important when variables were highly correlated, the correlation matrix of the predictor variables used in the random forest analysis for comparisons of N amendment type and of prior

Table 4 Cumulative corn growing season N₂O emissions, total N applied, grain yield, and N₂O emissions per unit grain yield from corn after the following crops and amendments: alfalfa

and orchardgrass with broadcast manure (AO-BM), crimson clover with broadcast manure (CC-BM), and soybean with broadcast manure (S-BM)

Year	Treatment	Corn growing season N ₂ O emissions (kg N ha ⁻¹)	Total N applied (kg N ha ⁻¹)	Grain yield (Mg ha ⁻¹)	N ₂ O emissions per unit grain yield (g N Mg ⁻¹ grain ⁻¹)
2015	AO-BM	1.3 ± 1.2	281	10.1 ± 1.1	124.9 ± 1.2
	CC-BM	1.2 ± 1.2	219	NA	NA
	S-BM	1.3 ± 1.2	313	8.7 ± 1.1	158.5 ± 1.2
2016	AO-BM	1.8 ± 1.4	268	NA	NA
	CC-BM	1.6 ± 1.4	230	11.7 ± 1.1	124.2 ± 1.4
	S-BM	0.7 ± 1.4	356	11.1 ± 1.1	63.7 ± 1.4

Mean ± least squared means. There was no statistical significance at *P* < 0.05 within a year

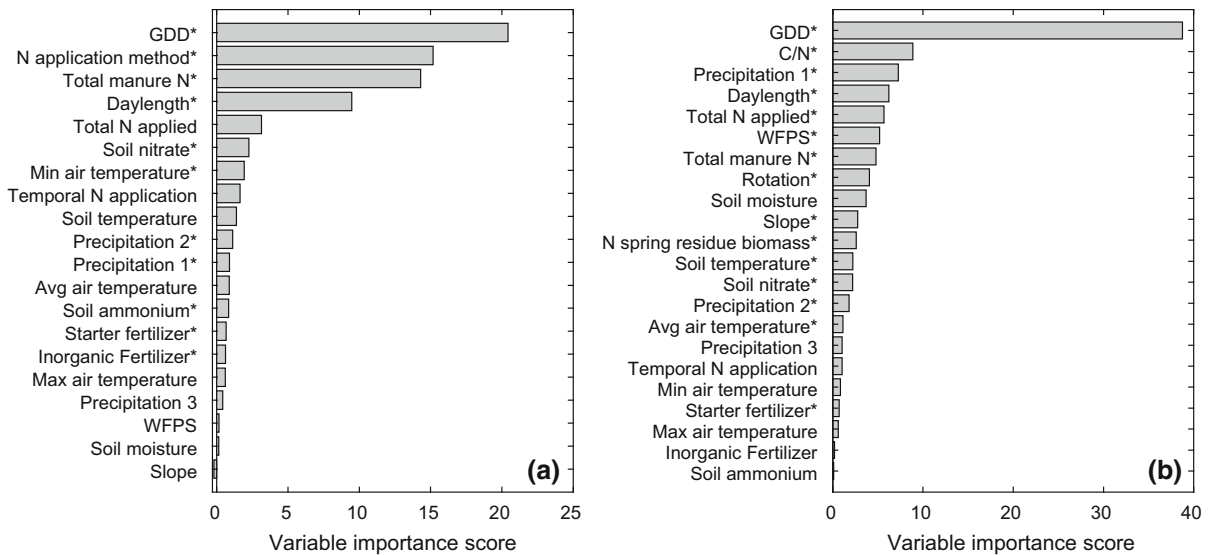


Fig. 3 Mean importance score calculated of the input predictor variables for N₂O from 10 repetitions of the conditional Random Forest (RF) algorithm, as measured by determining the mean absolute percentage increase in mean square error (MSE). **a** N amendment comparison includes S-BM, S-IM, and S-UAN, **b** prior crop comparison includes AO-BM, CC-BM, and S-BM.

The variables are presented in the figure in decreasing order of the importance value. Predictors marked with * were considered most important. When variables from the conditional importance measure were strongly correlated (*r* > 0.7), only the highest-ranking variable was considered most important

legume crops are presented in Table 1S and 2S, respectively.

Discussion

Effect of N amendment on N₂O emissions

N₂O emissions from injected manure was 2–3 times greater than broadcast manure, which is consistent

with other studies that also reported greater N₂O emissions from manure injection compared to unincorporated surface application (Flessa and Beese 2000; Velthof et al. 2003; Velthof and Mosquera 2011; Duncan et al. 2017; Dittmer et al. 2020). Duncan et al. (2017) attributed much of the increased N₂O emission to greater conservation of ammonium–N and the subsequent production of N₂O through nitrification and denitrification. Additionally, we suspect that greater water content and increased microbial

oxygen consumption within the manure injection zone created anaerobic microsites with increased denitrification activity. The greater cumulative N_2O emissions of S-IM compared to S-UAN was also consistent with other studies that observed enhanced conditions for denitrification with manure injection compared to surface mineral fertilizer application (Velthof et al. 1996, 2003; Ball et al. 2004; Velthof and Mosquera 2011; Davis et al. 2019). Despite the greater N_2O emission associated with shallow disk injection, the negative impact of N_2O increase should be weighed against the value of other benefits; especially ammonia emission reduction (Morken and Sakshaug 1998; Dell et al. 2012), phosphorus loss reduction (Jahanzad et al. 2019), odor reduction (Brandt et al. 2011), and the potential for increased profits (Rotz et al. 2011).

With the exception of S-UAN in 2016, cumulative N_2O emissions were within the 0.4 to 19.3 kg N_2O-N ha⁻¹ year⁻¹ range of total annual N_2O emissions reported from cropping systems in the central and eastern US (Cavigelli and Parkin 2012). The smaller cumulative emissions from S-UAN relative to S-IM in both years and S-BM in 2016 may have occurred both because the total N applied was greater in the manured treatments than the S-UAN, and UAN was applied to corn only at the V6 growth stage. This likely allowed for better synchronization between corn N demand and supply, and reduced excess inorganic N and potential N_2O losses compared to applying manure prior to corn planting. Moreover, the large loss of ammonia-N with broadcast manure application caused the need for additional side-dress N and greater overall quantity of N that could be converted to N_2O . The larger total N application and lack of synchrony of N application with corn uptake could also explain the greater N_2O emissions per grain yield of the S-IM compared to the S-UAN in both years. Furthermore, similar to a study by Ma et al. (2010), fluctuations in precipitation and soil moisture contributed to different timing of elevated N_2O after side dress N application, as well as to the cumulative N_2O emissions.

In a meta-analysis of multiple N source and management practices, Han et al. (2017) reported that multiple studies also applied more total N with livestock manure than with fertilizer treatment comparisons, confounding N source and rate as the driver of greater N_2O emissions from manure. Since most nutrient management plans account for similar N losses and slower mineralization from livestock

manure due to asynchronous application timing, larger total N application with manure is likely representative of most farm manure management. While the recommendation for greater application of total N with manure results from the understanding that only a portion of the organic N in manure will become available to the crop during the growing season it is applied, estimates of N mineralization are conservative to ensure that enough manure is applied to meet crop N requirements. Therefore, if mineralization rates exceed the estimate, quantities of soil NH_4^+ and NO_3^- could be greater than anticipated in manured soil.

Effect of prior legume crop on nitrous oxide emissions

Although nitrous oxide emissions from AO-BM and CC-BM residues were larger than S-BM with no spring residue early in the season on multiple days, the experimental design did not allow us to quantify the contribution of each N source individually. The lack of a significant difference in cumulative emissions with the addition of spring-terminated legume residues suggests manure was the main driver of emissions in the system. While we expected that N mineralized from alfalfa or clover residues would add to the soil inorganic N pool and potentially increase N_2O production through nitrification and denitrification, decomposition of those residues may have been slow enough that the impact on emissions during the entire growing season was not apparent. Significant temporal variability also may have contributed to the lack of statistical significant difference among the prior legume crop treatments. Total emissions for these treatments (direct emissions from N inputs as well as background emissions from mineralization) were within the broad range reported for the region. Yield-scaled N_2O emissions did not differ significantly among the treatments that received organic N inputs from manure and/or crop residues (Table 4). As in our study, Osterholz et al. (2014) found that crops with the largest yields had the smallest yield-scaled emissions within grain and dairy forage-based production in Wisconsin.

While total N_2O emissions were not significantly changed by the inclusion of alfalfa and orchardgrass or crimson clover in the rotation, a complete life cycle analysis of all aspects for cropping systems and all N

inputs is needed to better quantify the impact the crop sequence has on the rotation's greenhouse gas emission footprint. A meta-analysis of cover crop effects on N_2O emissions concluded that legume cover crops typically increase emissions relative to no cover crop (Basche et al. 2014), while non-legume cover crops usually reduce emissions. In our no-till dairy systems, the legume residues were not incorporated, and Basche et al. (2014) found that direct N_2O emissions were significantly lower in most studies where residues were left on the soil surface compared to those when residues were incorporated. In addition, cover crop residues in locations with total precipitation greater than 500 mm and significant variability also tended to produce greater N_2O emissions (Basche et al. 2014). However, the specific combination of factors addressed in our study was not assessed in the Basche et al. (2014) meta-analysis. Another meta-analysis conducted by Han et al. (2017) found no significant effect of cover crops on N_2O emission during the cash crop growing season, similar to our study.

Drivers contributing to N_2O emissions

The importance of GDD in both RF analyses indicates increasing temperatures and conditions for corn growth predicted emissions and aligns with our observation of the largest N_2O emissions in the 15–45 days after manure application. Since manure was applied 3–10 days prior to corn planting, in the absence of growing corn, there was initially no corn N uptake. As GDD accumulated, microbial N mineralization increased and the subsequent nitrification contributed some N_2O , and some of the accumulated nitrate was likely converted to N_2O through denitrification. By contrast, later in growing season N_2O emissions were typically reduced, when corn was actively growing and taking up available N. Duncan et al. (2017) also observed high N_2O emissions in the spring when manure was applied prior to corn planting, and Wagner-Riddle and Thurtell (1998) reported greater N_2O emissions when manure was applied in the absence of a growing crop in fall compared to a manure application to growing perennial crops in spring.

The RF analysis of the C–S treatments identified the N application method as a major source of daily variation in N_2O emissions. With manure injection,

the combination of NO_3^- accumulation, limited oxygen (due to high moisture and microbial respiration), and the large concentration of readily available organic C in the 10 cm deep manure band likely promoted more denitrification activity than in the surface broadcast manure and the UAN treatments. Others have also found that subsurface manure placement injection resulted in greater N_2O emissions than surface applied manure (Flessa and Beese 2000; Wulf et al. 2002; Duncan et al. 2017) or UAN (Davis et al. 2019).

Another major driver for N_2O emissions in the C–S treatments was “total manure N”. This variable likely reflects both the role of a large N addition in the manured systems prior to plant uptake and stimulation of soil microbial activity due to addition of labile organic C. For instance, S-UAN had no manure application prior to planting, and the side dress UAN application was better synchronized with corn uptake. Consequently, total N application in the UAN treatment was reduced compared to the manure treatments. Total N applied did not rank as highly as a predictor of N_2O emissions as total manure N in both RF analyses (Fig. 3a, b). Some others have also reported that manure N application often increased N_2O emissions compared to inorganic N fertilizers and suggested that the manure carbon likely promoted microbial activity and contributed to the greater N_2O emissions (Decock 2014; Han et al. 2017). However, in a meta-analysis of factors driving N_2O emissions in agricultural fields, Han et al. (2017) observed that manure versus fertilizer treatment comparisons were often confounded due to the larger total N applications of manure N than with fertilizer. This is likely in part because equipment and technology typically limit when farmers can apply manure prior to corn planting versus after the crop is established and rapidly taking up N. In addition, greater microbial activity associated with manure N and labile carbon likely promoted mineralization and subsequent nitrification, which contributes N_2O , and oxygen can become deleted within soil aggregates, causing denitrifying bacteria to switch respiratory pathways and use nitrate in place of oxygen (Hernandez-Ramirez et al. 2009).

For the prior legume crop treatments compared in the study, the C:N of the prior crop terminated in the spring was a major driver of the daily variations in N_2O emissions. The average 11–12.1 C:N of the residue contribution from AO-BM and CC-BM

compared to the S-BM, without spring residue inputs, could explain the larger daily N_2O emissions from the AO-BM and CC-BM early in the season (Fig. 2g, h). Drinkwater et al. (1998) found that in legume-based cropping systems, organic inputs from crop residues and/or manure contributed to increased C and N pools in the soil. While microbial activity and the mineralization of organic N materials can be stimulated by warmer temperatures (Kirschbaum 1995) later in the season when day length was greater and corn GDD accumulated, N_2O emissions were reduced likely because corn was actively growing and taking up the available N.

Surprisingly, precipitation and WFPS were only identified as key predictors of emission rates in the prior legume treatments and not in the C–S treatments. It is widely recognized that precipitation can influence denitrification and N_2O emission rates, since gas diffusivity is low and aeration is poor in wet soils (Linn and Doran 1984; Clayton et al. 1997; Davidson et al. 2000; Hernandez-Ramirez et al. 2009). Sixty percent WFPS is generally considered the lower limit for denitrification activity to occur (Linn and Doran 1984). However, the response of emissions to rainfall and moist soil was not consistent throughout the growing seasons, with some rainfall events later in the two growing seasons not resulting in noticeable increases in emissions. This suggests that other factors, such as crop uptake, may have limited nitrate availability for N_2O emissions in the mid- and later-growing seasons even when WFPS was large in response to precipitation. Additionally, soils dry more rapidly in hot summer weather and periods when conditions favored denitrification may have been short. Therefore, manual sampling could have missed some short-duration emissions following rainfall.

Soil nitrate concentration was expected to be a strong predictor of N_2O production, since nitrate is the precursor of N_2O (Weier et al. 1993). While the RF analysis of both the N amendment type comparisons in C–S rotation and the prior legume crop treatments did not rank nitrate concentration as one of the top predictors, the RF analyses suggest that it was still a contributing variable, particularly for the N amendment treatments. The cause for differing levels of importance of soil NO_3^- in the two comparisons is not clear, but comparison of Figs. 1i, j and 2i, j shows greater variability early in the growing season among the three treatments for the comparison of manure

amendment types compared to the comparison with prior legumes treatments. This greater variation may have resulted in the relatively greater importance of soil NO_3^- for the N amendment treatments.

Conclusion

The largest N_2O emissions during the corn growing season in manured systems happened shortly after pre-plant manure application. Manure and crop residues are essential sources of N for dairy forage rotations, but their use can create asynchrony between the availability of N and corn N uptake and can limit management options. The predominance of GGD as a predictor indicates that delaying manure application until the crop is actively growing and taking up N could reduce the amount of nitrate available for conversion to N_2O , but equipment for side-dressing manure into a growing corn crop is not yet widely available. Given that changing manure application timing is typically not practical, avoiding manure over-application may be the best approach available to limit N_2O emissions. Nutrient management tools should be used to optimize manure and side-dress fertilizer application rates. Nitrogen provided by residues of previous legume crops should also be accounted for when determining manure and fertilizer application rates. Use of moderate manure application rates in the spring with supplemental sidedress N fertilization, when needed as indicated by the PSNT or other tool, can inform N fertilizer management and reduce excess NO_3^- that could be lost as N_2O . Injecting manures increases N_2O emission, therefore benefits of injection must be weighed against the additional emissions when selecting the practice. Continuing research is needed to more fully explain the contribution of legume residues to N_2O emissions in diverse cropping systems. Additional emission measurements following legume termination, in the absence of manure and fertilizer application, and further quantification of N mineralization rates should be conducted.

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