

Fertilizer timing affects nitrous oxide, carbon dioxide, and ammonia emissions from soil

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Abstract

The impact of interactions between management and climate on nitrous oxide (N₂O), carbon dioxide (CO₂), and ammonia (NH₃) emissions are not well understood. This study quantified the effect of urea fertilizer application timing on inorganic N movement, immobilization, and the gaseous emissions of N₂O-N, CO₂-C, and NH₃-N. Urea was applied once, at two rates (0 and 224 kg ha⁻¹) on six dates (early fall, 20 Sept. 2017; mid-fall, 11 Oct. 2017; early winter, 1 Nov. 2017; early spring, 1 May 2018; mid-spring, 22 May 2018; and early summer, 12 June 2018). Gaseous emissions, soil temperature, and soil moisture were measured every 4 h for 21 consecutive days following urea application. Changes in soil inorganic N contents were used to determine the amount of inorganic N remaining in the soil, nitrification, immobilization/fixation, and leaching. For all fertilizer application dates, the cumulative fertilizer derived N₂O-N emissions for the 21 days following application were <0.05% of the applied N. Fertilizer-derived N₂O-N emission rates were higher than N₂O-N emission rates in the unfertilized soil in early fall and early summer. Even though the highest net N₂O-N emissions occurred in early spring, the application of fertilizer did not increase emissions. The highest net N₂O-N + NH₃-N emissions occurred in cool soils (early spring) in soils with water filled pore space (>60%). These findings indicate that intergovernmental panel on climate change (IPCC) default value of 1% of applied N for N₂O emissions improved by considering the fertilizer application date.

1 | INTRODUCTION

When applied to soil, urea [(CO(NH₂)₂)] is hydrolyzed by the urease enzyme to produce ammonia (NH₃), carbon dioxide (CO₂), and water (Clay, Malzer, & Anderson, 1990b; MacLean & McRae, 1987). The hydrolysis reaction is relatively fast, that can be slowed by treating the urea granule with a hydrolysis inhibitor (Clay et al., 1990b; Sherlock &

Goh, 1984). Following hydrolysis, a portion of the ammonia (NH_{3(aq)}) which is in equilibrium with ammonium (NH₄⁺_(aq)) can be volatilized into the atmosphere. The temporal pattern of NH₃ volatilization generally follows the soil temperature diurnal cycle because NH₃ water solubility and the pK_a value for the reaction, NH₄⁺_(Aq) → NH₃_(Aq) + H⁺ decrease with increasing temperature (Bates & Pinching, 1949; Clay et al., 1990b; Young, 1981).

Once urea is hydrolyzed, NH₄⁺ can be immobilized into soil organic matter, fixed by soil clays, nitrified to NO₃⁻ by

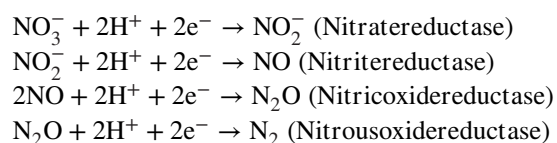
Abbreviations: phospholipid fatty acids, PLFA.

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soil bacteria, or utilized by growing plants (Azum, Simmons, & Mulvaney, 1993; Reddy & Reddy, 1993). The fixation/immobilization processes can be either non-biological or biological. Non-biological N fixation/immobilization, which can be as high as 200 kg NH₄⁻N ha⁻¹ (Broadbent, 1965; Nieder & Benbi, 2008) can occur through several mechanisms including the trapping of NH₄⁺ between the clay layers, reactions between ammonium with organic matter, and the fixation of ammonium as ammonium aluminum phosphates (Allison, 1973; Nieder, Benbi, & Scherer, 2011). These mechanisms can lower the amount of NH₄-N subject to nitrification, which in turn may reduce the loss of nitrate through leaching or denitrification.

Two biological process that reduce inorganic N concentrations are microbial N immobilization and N uptake by plants. In biological N immobilization, soil microbial uptake of inorganic N reduces the amount available to growing plants. The remaining NH₄⁺, be transformed to NH₂OH, nitrite (NO₂⁻), or nitrate (NO₃⁻) through a process called nitrification (MacLean & McRae, 1987). Nitrification requires oxygen and generally decreases with decreasing soil temperatures (Liu et al., 2016). However, this process is not 100% efficient and a small portion (0.03 to 1%) can be lost as nitrous oxide (Farquharson, 2016).

Once in the NO₃⁻ form, it can be taken up by plants, leached to lower soil depths, or denitrified. Denitrification is the process where NO₃⁻ is used as the terminal electron acceptor of respiration (Barnes, Smith, & Aiken, 2012; Blackmer & Bremner, 1978, 1979; Holtan-Hartwig, Dörsch, & Bakken, 2002; Sánchez-Martin, Vallejo, Dick, & Skiba, 2008; Senbayram, Chen, Budai, Bakken, & Dittert, 2012; Weier, Doran, Powers, & Walters, 1993; Włodarczyk, Stepniewska, & Brzezinska, 2003; Maeda et al., 2015). During denitrification, NO₃⁻ is reduced to NO₂, NO, N₂O, and N₂ through a series of half reactions that include:



Denitrification is not 100% efficient, and depending on several factors including temperature and NO₃⁻ concentration, NO, N₂O, and N₂ can be emitted. For example, each half reaction involves a different enzyme with a different Q₁₀ value. The Q₁₀ value is ratio between the reactions rates measured at two temperatures that differ by 10°C (Baer, Connelly, Sipler, Yager, & Bronk, 2014). Philips, Mcmillan, Palmada, Dando, and Giltrap (2015) reported that nitric oxide reductase has a Q₁₀ of 2.0 and nitrous oxide reductase has a Q₁₀ of 1.4. The higher Q₁₀ value for nitric oxide reductase (NO to N₂O) than nitrous oxide reductase (N₂O to N₂) suggests that the ratio between N₂O and N₂ will increase with temperature.

Core Ideas

- Management and climate interactions affected N₂O-N, CO₂-C, and NH₃-N emissions.
- Temperatures <12°C slowed nitrification.
- Ammonia losses from urea were greater in the spring than fall.
- Total N₂O emissions were highest when soil moisture and CO₂ emissions were high.
- For all application dates, fertilizer-derived N₂O-N emission over 21 days were <0.05% of applied N.

Even though the influence of temperature and soil moisture on N₂O emissions has been widely studied (Linn & Doran, 1984; Sainju, Stevens, Caesar-Tonthat, & Liebig, 2012; Wagner-Riddle & Thurtell, 1998), interactions between management and these factors in the field are poorly understood (Abagandura et al., 2019). If management can produce predictable and consistent impacts on emissions, then these findings can provide a justification for adjusting the calculated agricultural carbon footprint (DeKlein et al., 2006; Han, Walter, & Drinkwater, 2017; IPCC, 2007; Snyder, Bruulsema, Jensen, & Fixen, 2009). For example, increasing N use efficiency by synchronizing the N supply with crop demand is a strategy that has been integrated into the 4 R program (Fixen, 2007): apply N at the right rate, at the right time, in the right form, and at the right location. However, synchronization has produced mixed impacts on GHG emissions (Burzaco, Smith, & Vyn, 2013; Venterea & Coulter, 2015; Eagle, Olander, Locklier, Heffernan, & Bernhardt, 2017; Ma et al., 2010). The lack of consistent findings may be attributed to papers that report total losses and not fertilizer derived-N (Lehman, Osborne, & Duke, 2017; Johnson & Barbour, 2019), and sampling methods that do not account for diurnal and seasonal differences in soil temperature, soil moisture, and microbial activity (Liu et al., 2019; McGowan, Roozeboon, & Rice, 2018; Shurpali et al., 2016). Therefore, to resolve why synchronization produces mixed impacts on N₂O-N emissions, research was conducted to determine the effect of fertilizer application timing on N₂O-N, CO₂-C, and NH₃-N emissions from soil-applied urea. This study quantified the effect of urea application timing on inorganic N movement, immobilization, and the gaseous emissions of N₂O-N, CO₂-C, and NH₃-N.

2 | MATERIALS AND METHODS

2.1 | Experimental design and treatments

This research concentrated on the period immediately following the fertilizer application because season-long studies

shows that emissions are event-based and that a large percentage of the total emission occurs within 30 days of applying the N (Fujinuma, Venterea, & Rosen, 2011; Burzaco et al., 2013; Halvorson & Del Grosso, 2013; Shurpali et al., 2016). The dataset used in this experiment is available in Thies (2018) and this paper is the second in a series of paper using this dataset. In the first paper, Thies et al. (2019) showed the linkages between temperature, N_2O emission, and soil moisture across a 24-h cycle. In this paper, the impact of fertilizer application date on average daily N_2O , CO_2 , and NH_3 emissions were investigated.

A field study was conducted near Aurora, South Dakota ($44^\circ 18' 20.57'' \text{N}$, $96^\circ 40' 14.04'' \text{W}$). The site was located on the border between the Bsh (semi-arid) and DFa (continental wet all seasons) Köppen climate groups. The soil at the study site was a Brandt silty clay loam (Fine-silty, mixed, superactive, frigid Calcic Hapludolls) (Soil Survey Staff, 2018). The surface soil (15 cm) contained 280 g clay kg^{-1} (28%), 65 g silt kg^{-1} (65%) 7 g sand kg^{-1} (7%) and 36 Mg ha^{-1} (1.8% SOC) of soil organic carbon (SOC). As reported by Clay et al. (2015), the no-tillage first order rate constant and half-life for this soil was $0.00675 \text{ kg (kg-C} \times \text{yr)}^{-1}$ and 103 years, respectively. The soil $\text{pH}_{\text{water } 1:1}$ was 5.8, and the soil parent materials were loess (0–60 cm) over glacial outwash. The surface soil hydraulic conductivity was $k_{\text{sp}} = 0.72 \text{ m d}^{-1}$ (Clay, Clay, Brix-Davis, & Scholes, 1994) and the slope was between 0 to 2%. For this site, Kim, Clay, Carlson, Clay, and Trooien (2008) calculated evapotranspiration (ET) with the model, $\text{ET} = \text{I} + \text{P} - \text{D} - \Delta\theta$, where I was irrigation, P was precipitation, D was deep drainage, and $\Delta\theta$ was the change in soil moisture over the growing season. Testing of this soil showed that due to a soil textural discontinuity between 60 and 80 cm (silty clay loam to gravel) the deep drainage (D) value was near zero during the growing season. The gravimetric water contents at field capacity and the wilting point were approximately 0.315 and 0.177 g g^{-1} , respectively. For the study site, additional findings are reported in Clay et al. (1996, 2005, 2015). The prior history of the site was the seeding of soybean (*Glycine max* L.) in the spring of 2017, and fertilizer was not applied for the prior 1.5 years. To prepare the soil for the experiment, soybeans were terminated and surface residue was removed on 14 Sept. 2017.

The experiment contained six blocks, which represented different fertilizer application dates. Within a block, the experiment contained two N rates (0 and 224 kg N ha^{-1}), that were replicated four times. Within a block, all treatments were within 2 m of each of each other. Fertilizer was applied only once to the soil within the chamber, the fertilizer rate and dates treatments were applied in early fall (20 Sept. 2017), mid-fall (11 Oct. 2017), early winter (1 Nov. 2017), early spring (1 May 2018), mid spring (22 May 2018), and early summer (12 June 2018). All soils were exposed to prevailing climatic conditions. All fertilizer application-date plots were located

within 40 m of each other. For the N treatments, the appropriate amount of urea was dissolved in 10 ml of water and sprinkled onto the soil surface inside a 20.3-cm diameter polyvinyl chloride (PVC) ring, resulting in a 224 kg N ha^{-1} rate. Water, 10 ml without urea, was applied to the non-fertilized treatments. In these treatments, N_2O , CO_2 , and NH_3 emissions and changes in soil inorganic N with soil depth were measured.

To assess the importance of N immobilization/fixation a study was conducted in July 2019. This study had two N rates (0 and 74 kg N ha^{-1}), four replicates, and the experiment was conducted at a constant soil moisture content (field capacity). This soil was not dried or ground and the water-filled porosity was approximately 60%. The soil treatments were placed on a plastic tarp and covered with a rainout shelter. Nitrous oxide, $\text{CO}_2\text{-C}$, and $\text{NH}_3\text{-N}$ emissions were measured (Thies et al., 2019), and soil samples collected at 0, 6, 12, 17, and 21 days were dried, ground and analyzed for pH (1:1 water), $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$.

2.2 | Nitrous oxide, carbon dioxide, and ammonia emissions measurements

A complete description of the methodology used in $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$ and $\text{NH}_3\text{-N}$ flux measurement are available in Thies et al. (2019). In each experiment, eight LI-COR LI-8100-104 long-term opaque chambers (8100-104 LI-COR, Lincoln, NE) were placed onto treatment areas. Each chamber consisted of a base and cover that pivots to cover the treated area during sample collection. The PVC ring was inserted 5 cm into the soil. The surface area of each ring was 314 cm^2 . Gas samples were collected from 0000 to 0230 h, from 0400 to 0630 h, from 0800 to 1030 h, from 1200 to 1430 h, from 1600 to 1830 h, and from 2000 to 2230 h. The sampling intervals were aligned with the average (0930 to 1030 h), minimum (0530 to 0630 h), and maximum air temperatures (1330 to 1430 h) (Thies et al., 2019). In the following discussion, $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$, and $\text{NH}_3\text{-N}$ emissions for the six measurement over a 24-h period were averaged to determine average emission rates or summed to determine cumulative losses.

Chambers were sampled in a designated sequence, and corrections were applied to each individual chamber to account for air volume differences. Air temperatures were measured with a thermistor and a vent was used to equalize the chamber and atmospheric pressures. The gas within the chamber was mixed during the 15-min sampling period. During each chamber sampling interval, a total of 900 gas samples were collected and analyzed for $\text{N}_2\text{O-N}$, $\text{CO}_2\text{-C}$, and $\text{NH}_3\text{-N}$ by a Picarro Cavity Ringdown Spectrometer (model G2508; Picarro Inc., Santa Clara, CA). Nitrous oxide and ammonia fluxes were calculated with data obtained between 45 to 900 s after chamber closure, whereas CO_2 fluxes were determined with data obtained between 45 and 165 s. The $\text{N}_2\text{O-N}$,

CO₂-C, and NH₃-N flux values were calculated using version 4.01 LI-COR SoilFluxPro software (v. 4.01; LICOR, Lincoln, NE).

The Picarro factory calibrations were checked with N₂O, CO₂, and NH₃ standards at the beginning and end of the experiments. All standards were purchased from Airgas Specialty Gases (Airgas USA LLC, Cinnaminson, NJ). The N₂O-N standards had concentrations of 0.378 and 149 ppm, whereas the CO₂-C standards had concentrations of 99.91 and 2998 ppm. The equation between the N₂O-N standard and the factory calibrations, conducted prior to and following the experiments was $y = 0.02 + 1.013 \times (\text{standard})$, $r^2 = 0.99$. The equation between the CO₂-C standards and the factory calibration was $y = -0.288 + 0.994$, $r^2 = 1.00$. For NH₃-N, the standard (1804 ppb) and machine reading (1806 ppb) were virtually identical. Following the fertilizer application, emissions were measured every 4 h for 21 days. However, for fertilizer applied on 1 Nov. 2017 the experiment was terminated on November 15 due to soil freezing. Adjacent to the chambers, soil moisture and temperatures for the surface 5 cm were measured using the LI-COR LI-8150-205 Soil Moisture Probe (LI-COR, Lincoln, NE) and the LI-COR LI-8150-203 Soil Temperature Probe (LI-COR, Lincoln, NE), respectively. During the experiments, power outages or machine failures resulted in short gaps in the dataset (September 21 to 22 and May 1 to May 2). Missing information was replaced with time appropriate information collected from each chamber.

2.3 | Soil nutrients

Prior to each experiment, soil samples (0- to 15-, 15- to 30-, 30- to 45-, and 45- to 60-cm depths) were collected from an area adjacent to the chambers and final samples were collected from within the PVC rings. Each composite sample consisted of eight soil cores that were frozen until analysis. A subsample was analyzed for gravimetric moisture content by drying the soil samples to a constant weight at 105°C. The bulk densities for the 0- to 15-, 15- to 30-, 30- to 45-, and 45- to 60-cm depths in the fall of 2017 were 1.29, 1.22, 1.22, and 1.22 g cm⁻³, respectively. The bulk densities for the 0- to 15-, 15- to 30-, 30- to 45-, and 45- to 60-cm depths in the spring of 2018 were 1.34, 1.30, 1.30, and 1.30 g cm⁻³, respectively. Based on the measured bulk densities and volumetric water contents, the water filled pore space was determined. This calculation assumed that the soil particle density was 2.65 g cm⁻³. Soil samples were dried at 40°C, ground (<2 mm) and analyzed for NH₄⁺-N and NO₃⁻-N (Clay, Clay, Lyon, & Blumenthal, 2005; Kim et al., 2008). The bulk densities were used to convert gravimetric values to volumetric values.

2.4 | Microbial community measurements

The microbial community structures were measured on surface soil samples (0- to 15-cm) collected on 11 Oct. 2017 and 12 June 2018. Protocols for collecting and storing these samples are provide by Veum, Lorenz, and Kremer (2019). These samples were analyzed for the microbial community structure using the Phospholipid Fatty Acid (PFLA) approach (Reese et al., 2014). For this analysis, the soil samples were submitted to WARD Laboratories, Inc. (WARD Labs Inc, Kearney, NE). WARD Laboratories Inc. uses a modification of Hamel et al. (2006). In this analysis, 2 g of soil were mixed with 9.5 ml of dichloromethane for 1 h. Following mixing, 2.5 ml of dichloromethane (DCM) and 10 ml of saturated KCl were added, and the test tubes were shaken and centrifuged. The aqueous portion was placed into clean vials and 5 ml of dichloromethane/methanol (1:1 v/v) was added, shaken, and centrifuged. The aqueous fraction was dried, dissolved in 2 ml of dichloromethane, placed onto silica gel columns, and washed with dichloromethane. The neutral, glycol, and phospholipid fractions were collected by sequential leaching with 2 ml of DCM, acetone, and methanol, respectively. Of these fractions, the neutral and glycol fractions were discarded. These samples were dried, dissolved in methanol and the fatty acid methyl esters were created by mild acid methanolysis. Neutral and phospholipids fractions were dried under a N₂ flow at 37°C in the fume hood. Methanol/H₂SO₄ (25:1 v/v) was added to the vials, which were placed in an 80°C oven for 10 min, and cooled to room temperature before adding approximately 2 ml of hexane. Vials were vortexed for 30 s and left to settle for 5 min before the lower fraction was discarded. Vials were vortexed a second time for 30 s, and allowed to separate 5 min before the aqueous fraction was discarded. The samples were dried, resuspended in 50 µl of hexane, placed inside a gas chromatograph (GC) vial, and analyzed on a GC. Based on the PLFA biomarkers, the gram (+) bacteria, gram (-) bacteria, actinomycetes, arbuscular mycorrhizal fungi, saprophytic fungi, rhizobia, and protozoa organisms were identified (Rankoth et al., 2019) using the MIDI Sherlock Software system (Veum et al., 2019). This approach is useful at providing information on the living biomass and community structure. The microbial biomass was the sum of the PLFA biomarkers, and this value does not reflect total biomass.

2.5 | Nitrogen calculations

The cumulative emissions of N₂O-N, CO₂-C, and NH₃-N were determined by assuming that the point measurements were estimates of the emissions over a 4-h period. Total emissions was determined by summing these values. The NH₄-N

TABLE 1 Average 7-d soil and air temperatures, soil moisture, water filled pore space contents for the surface 5 cm and precipitation. The time intervals shown are 0 to 7, 8 to 14, and 15 to 21 d. The values in parenthesis represent the range in values over the study period. Values in parentheses represent the range in values during a study period

Season	Fertilizer application date	Days after application	Soil Temp. 0-5 cm	Air Temp.	Volumetric soil moist 0-5 cm	Water-filled pore space 0-5 cm	Rainfall
		d	°C	°C	cm ³ cm ⁻³	cm ³ cm ⁻³	cm
Early fall	20 Sept.	0-7	17.5	15.6	0.44 (0.12-0.52)	0.857	11
Early fall	20 Sept.	8-14	15.2	13.6	0.45 (0.36-0.51)	0.877	6.5
Early fall	20 Sept.	15-21	11.8	9.16	0.45 (0.34-0.49)	0.877	0.2
Mid-fall	11 Oct.	0-7	10.9	9.36	0.32 (0.28-0.39)	0.624	0.3
Mid-fall	1 Oct.	8-14	12.2	10.7	0.32 (0.25-0.45)	0.643	0
Mid-fall	11 Oct.	15-21	3.99	0.05	0.28 (0.18-0.45)	0.643	0.4
Early winter	1 Nov.	0-7	3.31	-2.12	0.24 (0.23-0.26)	0.468	0.7
Early winter	1 Nov.	8-14	2.19	-0.03	0.24 (0.17-0.32)	0.468	0
Early spring	1 May	0-7	12.6	17.5	0.37 (0.36-0.41)	0.749	1.3
Early spring	1 May	8-14	10.4	13.2	0.45 (0.28-0.41)	0.91	2.3
Early spring	1 May	15-21	14.4	18	0.33 (0.30-0.42)	0.668	0
Mid-spring	22 May	0-7	21.9	25.6	0.27 (0.20-0.33)	0.546	0.3
Mid-spring	22 May	8-14	18.9	23.3	0.27 (0.19-0.32)	0.546	1.2
Mid-spring	22 May	15-21	18.5	21.6	0.26 (0.19-0.34)	0.526	0.9
Early summer	12 June	0-7	21.1	22.9	0.29 (0.22-0.37)	0.546	2.3
Early summer	12 June	8-14	18.9	21.2	0.34 (0.12-0.56)	0.465	4.2
Early summer	12 June	15-21	21	23.9	0.36 (0.26-0.68)	0.607	3.8

and NO₃-N concentration in surface 30 cm was determined by summing the 0- to 15- and 15- to 30-cm soil depths. The amount of nitrified N fertilizer was calculated by subtracting the kilograms of NO₃⁻-N + N₂O-N per hectare in the unfertilized plot from kilograms of NO₃⁻-N + N₂O-N per hectare in the fertilized plots. However, in the actual calculations, N₂O emissions were assumed to approach zero because these losses were 1,000 to 10,000 times less than the changes in soil NO₃-N. The amount of fertilizer N that leached below the surface 30 cm was determined by subtracting the amount of nitrate-N in the 30- to 60-cm depth in the unfertilized plots from the nitrate-N in the fertilized plots. Leaching below 60 cm was not considered because previous analysis showed that a soil texture discontinuity slowed water movement into the gravel (Kim et al., 2008).

The percentage of NH₄-N in the soil samples were determined by dividing NH₄-N by total inorganic N (NO₃⁻-N + NH₄⁺-N). The amount of immobilized/fixated N in the soil profile was determined with the equation: Immobilized or fixed = N rate - [(Inorganic_{soil, fert} - inorganic_{soil, no-fert}) + Emitted N].

2.6 | Statistical analysis

With the exception of block 3 (early winter), each of the fertilizer application dates were separated into three periods: 0 to

7, 8 to 14, and 15 to 21 days. Treatment differences for the 7-day intervals and loss over the 21-day experiment were determined using ANOVA (R Core Team, 2017) where fertilizer application dates were blocks and N rates were treatments. Within each block, the treatments were replicated four times. Due to interactions between the blocks (fertilizer application date) and treatments (N rates), the fertilizer application dates were analyzed separately. The emissions from the six fertilizer application dates were compared using ANOVA. Unless noted, a Duncan's test at an $\alpha = .10$ level was used to separate treatment differences.

3 | RESULTS AND DISCUSSION

3.1 | Rainfall, soil temperatures, and moisture

The mean 7-d average soil temperatures decreased from 17.5°C for fertilizer applied in early fall to 2.19°C for fertilizer applied in early winter (Table 1). For fall-applied fertilizers, the average 7-d water filled pore spaces were greater than 60% for fertilizer applied in early fall and mid-fall. Based on Linn and Doran (1984) the 60% water filled porosity was the point where the soil switched from aerobic to anaerobic. In spring 2018, temperatures generally increased from 1 to 22 May 2018, and the average water filled pore space was greater than 60% for fertilizer applied on 1 May 2018.

TABLE 2 Amount of inorganic N ($\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$) contained in the surface 30 cm and the percentages of the ammonia in the soil samples at the beginning and completion of each fertilizer application experiment. The initial rates are provided for comparison and the statistical analysis was between the two N rates at the completion of the experiments

Treatment	Day after application	Early fall		Mid-fall		Early winter	
		Total inorganic N	NH ₄ -N/Inorganic	Total inorganic N	NH ₄ -N/Inorganic	Total inorganic N	NH ₄ -N/Inorganic
	d	kg N ha ⁻¹	%	kg N ha ⁻¹	%	kg N ha ⁻¹	%
Initial	0	48.9	29.8	58.3	35.7	61	57.8
Urea	21	152.6	26.2	181.6	76	213	74.4
No-urea	21	46.8	69.4	55.1	27.6	39.7	49.8
<i>P</i> value		0.028	0.003	0.013	0.007	0.003	0.0613
Treatment	Day after application	Early spring		Mid-spring		Early summer	
		Total inorganic N	NH ₄ -N/Inorganic	Total inorganic N	NH ₄ -N/Inorganic	Total inorganic N	NH ₄ -N/Inorganic
	d	kg N ha ⁻¹	%	kg N ha ⁻¹	%	kg N ha ⁻¹	%
Initial	0	45.0	20.0	33.4	90	60.6	30
Urea	21	107.4	30.7	145.3	33.2	149.4	26.8
No-urea	21	38.6	66.2	45.0	53.7	56.5	76.7
<i>P</i> value		0.002	0.030	0.009	0.260	0.030	0.069

However, water filled porosities were generally less than 60% for fertilizer applied in mid-spring and early summer.

3.2 | Nitrogen budgets

3.2.1 | Soil inorganic N concentrations

The initial amount of inorganic N ($\text{NO}_3 + \text{NH}_4$) in the surface 30 cm was less than 61 kg N ha⁻¹ for all fertilizer application dates (Table 2). Based on these amounts, nitrate-N concentration in the surface 30 cm in the early fall, mid-spring, early winter, early spring, mid-spring, and early summer were 9.09, 10.24, 6.76, 6.55, 0.91, and 10.66 mg $\text{NO}_3\text{-N}$ kg⁻¹, respectively. For some of the application dates, the $\text{NO}_3\text{-N}$ concentrations may have been low enough in the unfertilized treatments to increase the conversion of N_2O to N_2 (Blackmer & Bremner, 1979; Thomas et al., 2017).

3.2.2 | Amount of nitrified N

The calculated amounts of nitrified fertilizer-N that was applied in the early fall, mid-fall, early winter, early spring, mid-spring, and early summer were 98.1 (44% applied N), 3.69 (1% applied N), 34.6 (15% applied N), 96.1 (43% applied N), 76.2 (34% applied N), and 96.2 (43% applied N) kg N ha⁻¹, respectively. The low nitrification amounts for fertilizer applied in mid-fall and early winter were attributed to reduced biological activity with decreasing soil temperature (Table 1). These findings support the recommendation of waiting to apply fall ammonium based fertilizers until the surface 10 cm of soil decreases below 10°C.

3.2.3 | N fixation, immobilization, and leaching

At the completion of each 21-d experiment, the amount of inorganic N that was derived from the applied urea was 111 ± 32.8 kg N ha⁻¹. Given that 224 kg N ha⁻¹ was applied, these values suggest that approximately 50% of the applied urea-N was not extractable with 1 M KCl. The missing N could be the result of non-measured gas emissions (NO, and N_2), NO_3^- leaching, N mineralization inhibition, or $\text{NH}_4\text{-N}$ immobilization/fixation. We attributed the lost N to immobilization/fixation for several reasons. First, large emissions of NO were discounted because Fujinuma, Venterea, and Rosen, 2011 reported that NO losses were about 50% of N_2O losses. In addition, large emissions of N_2 were discounted because Del Grosso et al. (2000) reported that at 60% water filled porosity in a silt loam soil, the N_2 to N_2O ratio was <1. Del Grosso et al. (2000) also reported that the highest $\text{N}_2/\text{N}_2\text{O}$ ratios were observed in nitrate-limited soil.

Second, ammonium inhibition of N mineralization (Mahal et al., 2019) was discounted because based on the experiment conducted in July 2019, N mineralization was 1.22 mg N d⁻¹, and if NH_4^+ completely inhibited N mineralization, then NH_4 inhibition would only account for 3.1% of the missing N over 6 d. Third, the loss of approximately half of the fertilizer through NO_3^- leaching was discounted because the amount of fertilizer-derived inorganic N that was transported with percolating water below 30 cm for all fertilizer application dates was 11.63 ± 11.9 kg N ha⁻¹ ($p = .05$). Others have reported similar leaching losses of fertilizer-derived N in field experiment (Tran & Giroux, 1998). Forth, the loss of nitrate below the surface 60 cm was discounted because this soil had textural discontinuity between 60 to

80 cm that slows and/or prevents water movement below this layer.

To confirm that N immobilization/fixation could be responsible for the missing N, an additional experiment was conducted in July 2019. In this experiment, losses through leaching and plant uptake were prevented and the combined gaseous loss of $\text{N}_2\text{O-N} + \text{NH}_3\text{-N}$ over 21 d was $<0.1 \text{ kg N ha}^{-1}$, respectively. In this experiment, soil pH_{water} values in the fertilized and unfertilized soil was 5.8 ± 0.14 and 5.61 ± 0.11 , respectively, and after 6 and 21 d, the soil contained $46 \pm 21\%$ and $44 \pm 7.6\%$ of the fertilizer-derived inorganic N. These values indicate that at 60% water filled porosity, 54% of the applied fertilizer was either immobilized or fixed.

Nieder and Benbi (2008) had similar results and reported that NH_4^+ fixation can range from 10 to 60 mg N kg^{-1} in sandy soils and from 90 to 460 mg kg^{-1} in clay soils. Foster, Beauchamp, and Corke (1985) reported that 3 months after the urea application, 25% was immobilized into organic N.

Reddy and Reddy (1993) reported that following harvest, 50.4% of the 50 $\text{kg NH}_4\text{NO}_3\text{-N ha}^{-1}$ that was applied, remained in the surface 75 cm. Of the 50.4% of the applied N, 94% was contained in organic pools, and most of the immobilized N (60.7%) was derived from NH_4^+ . Azum et al. (1993) had similar results and reported that when organic C limited growth, NH_4^+ was preferentially immobilized over NO_3^- . Okereke and Meints (1985) showed that 12 h after applying N to organic soil, 9 to 10% of the applied N was contained in the organic matter, whereas Kelly and Stevenson (1987) reported that maximum immobilization occurred within 6 d of being treated with glucose.

In a study that considered immobilization/fixation, plant uptake, and mineralization of fixed or immobilized N, Clay, Malzer, and Anderson (1990a) reported that 18 days after applying ^{15}N labeled urea to a sandy loam soil, 35% was either immobilized/ fixed into pools that were not extracted by 1 M KCl. Of the immobilized/ fixed N, 13.9 and 66% was in 6 M HCl hydrolyzable amino acid and NH_4 pools, respectively. The immobilized/ fixed N mineralized 5 to 10 times faster than the non-labeled organic N, which the plants subsequently utilized. These findings have short and long-term implications on N cycling. Over the short term, the immobilization/fixation of NH_4^+ reduces the amount of N that can be lost through leaching or denitrification. Over the long-term, immobilized/ fixed N can effect N cycling for decades. Sebilo, Mayer, Nicolardot, Pinay, and Mariotti (2013) reported that after 30 years, 61 to 65% of the applied ^{15}N labeled fertilizer was taken up by the plant, and that 12 to 15% of the applied N was still contained in the organic matter. Each year the contribution that the plant receive from the single application of ^{15}N labeled fertilizer decreased. These data suggest that the failure to account for immobilization/fixation and subsequent release

results in underestimate N fertilizer efficiency and overestimating N losses.

3.2.4 | N_2O , CO_2 , and NH_3 emissions

For fertilizer applied in the early fall (20 Sept. 2017) and early summer (22 June 2018) the application of urea increased $\text{N}_2\text{O-N}$ emissions. For fertilizer applied in early summer, N_2O emissions may have been limited by organic-C substrate availability. Others have seen high emissions following spring freeze thaw cycle (Liu et al., 2019). However, the amount of fertilizer-derived N that was emitted as N_2O was less than 1% of the applied fertilizer. For fertilizer applied in mid fall, early winter, early spring and mid spring $\text{N}_2\text{O-N}$ emissions from the fertilized and non-fertilized plots were similar (Table 3; Figure 1). Other have also seen emissions rates that are less than 1% of the applied N in frigid soils (Niraula et al., 2019).

In the fall of 2017, $\text{CO}_2\text{-C}$ emissions decreased as the season progressed and for urea applied in the early fall (20 Sept. 2017), mid-fall (11 Oct. 2017), mid-spring (22 May 2018), and early summer (22 June 2018), the fertilizer treatments did not influence $\text{CO}_2\text{-C}$ emissions (Table 4; Figure 2). However, for urea applied in the early winter (1 Nov. 2017) and early spring (1 May 2018) the fertilizer application increased $\text{CO}_2\text{-C}$ emissions.

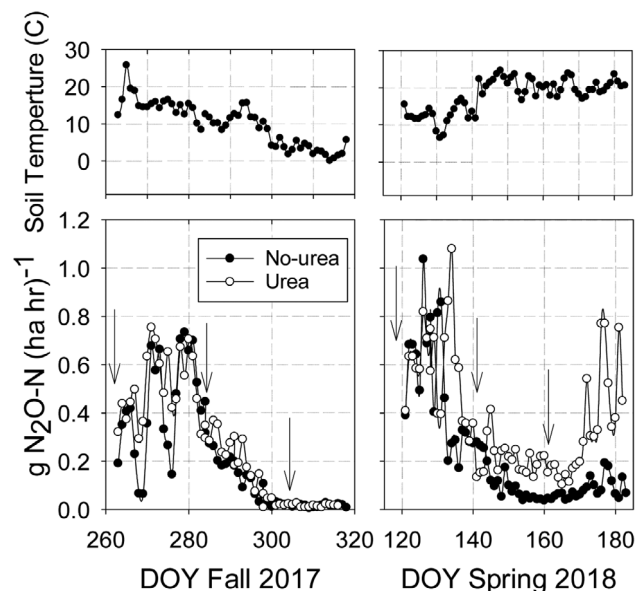


FIGURE 1 Average daily nitrous oxide emission rates across a 24-h period and soil temperatures (0-5 cm) from the six fertilizer application dates as influenced by day of the year (DOY). The figure on the left shows data from the early fall, mid-fall, early winter treatment and the figure on the right shows early spring, mid-spring, and early summer. The data shown represents the average daily emissions. The arrows represent when the fertilizer was applied. Soil temperature in the chart and soil moisture are in Figure 2

TABLE 3 Total cumulative N₂O-N emissions per hectare for each time interval from the six fertilizer application dates. In the urea treatment 224 kg urea-N ha⁻¹ was applied

Treatments	Days after application				Treatments	Days after application			
	0-7	8-14	15-21	0-21		0-7	8-14	14-21	0-21
	kg N ₂ O-N ha ⁻¹					kg N ₂ O-N ha ⁻¹			
Early fall					Early spring				
Urea	0.062	0.102	0.097	0.261	Urea	0.0983	0.1072	0.0876	0.293
None	0.039	0.072	0.108	0.219	None	0.1056	0.09	0.0466	0.242
<i>p</i> -value	0.201	0.055	0.176	0.085	<i>p</i> -value	0.882	0.756	0.168	0.681
Mid-fall					Mid-spring				
Urea	0.0442	0.0327	0.0087	0.086	Urea	0.0358	0.0358	0.0312	0.103
None	0.0364	0.0189	0.0059	0.061	None	0.0223	0.0146	0.0074	0.044
<i>p</i> -value	0.666	0.413	0.411	0.511	<i>p</i> -value	0.342	0.216	0.103	0.194
Early winter					Early summer				
Urea	0.0027	0.0027	ND	0.005	Urea	0.0219	0.0514	0.0958	0.169
None	0.0022	0.0031	ND	0.005	None	0.0087	0.0148	0.0194	0.0429
<i>p</i> -value	0.007	0.11		>0.9	<i>p</i> -value	0.101	0.0043	0.0001	0.0001

For fertilizer applied in early fall (20 Sept. 2017), 0.43% of the applied N was lost through NH₃ volatilization (Table 5; Figure 3). However, delaying the application until mid-fall (Oct. 11), increased volatilization losses 33%. This increase was attributed to slowed nitrification resulting from low soil temperatures. In the following spring (1 May 2018), NH₃ losses were higher than fertilizer applied in the fall. High losses in early spring maybe related to a combination of factors including the impact of temperature on nitrification, NH₃ solubility and the pKa value for the equation, NH₄⁺ → NH₃ + H⁺ (Bates & Pinching, 1949; Clay et al., 1990b; Young, 1981).

3.3 | Seasonal differences in N₂O-N, CO₂-C and NH₃-N emissions

Correlations between GHG emissions and soil temperature and moisture were not consistent across fertilizer application dates (Table 7). In the fall, decreasing soil temperatures slowed nitrification (Table 6), which reduced N₂O-N emissions and increased NH₃-N volatilization. The positive relationship between the average daily temperature and cumulative N₂O-N emissions in the unfertilized plots in the fall supports this hypothesis (Figure 4). Other studies have reported similar differences (Adair, Barbieri, Schiavone, &

TABLE 4 CO₂-C cumulative emissions for each time interval for the fall and spring fertilizer application dates

Treatment	Days after fertilizer application				Treatment	Days after fertilizer application			
	0-7	8-21	15-21	0-21		0-7	8-14	15-21	0-21
	kg CO ₂ -C ha ⁻¹					kg CO ₂ -C ha ⁻¹			
Early fall					Early spring				
Urea	167.4	86.6	100.3	354	Urea	185	180	262	627
None	128.8	108.9	143.9	382	None	120	153	191	464
<i>p</i> value	0.212	0.184	0.001	0.435	<i>p</i> value	0.001	0.372	0.116	0.044
Mid-fall					Mid-spring				
Urea	122	97	65	294	Urea	195	152	168	515
None	96	83	65	243	None	197	193	247	637
<i>p</i> value	0.165	0.467	0.985	0.266	<i>p</i> value	0.953	0.052	0.038	0.126
Early winter					Early summer				
Urea	75	43		120	Urea	197	151	148	496
None	34	31		43	None	190	201	253	644
<i>p</i> value	0.0099	0.0141		0.01	<i>p</i> value	0.798	0.225	0.059	0.219

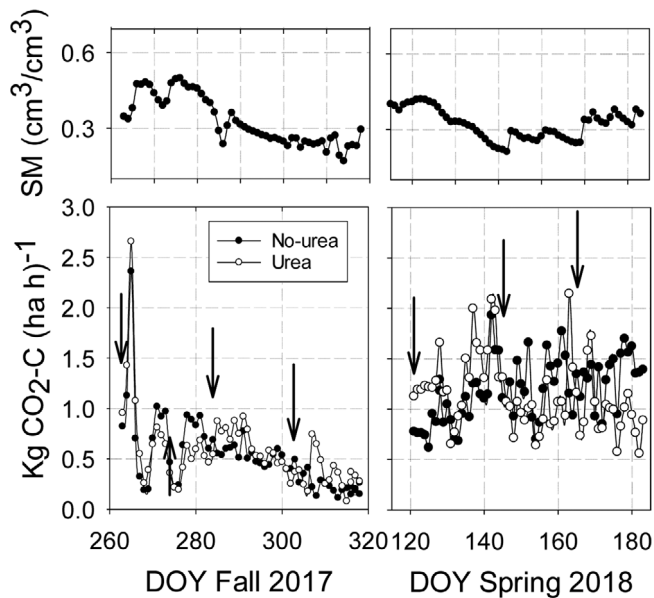


FIGURE 2 Average daily carbon dioxide emissions rates across a 24-h period for the three fall and three spring fertilizer application dates as influenced by day of the year (DOY). Data from the early fall, mid-fall, early winter treatments are on the left and data from the early spring, mid-spring, and early summer are on the right. Arrows represent when the single rate of fertilizer was applied to each plot. Soil moisture (SM) is provided in the chart and soil temperature is in Figure 1

Darby, 2019; Drake, Giasson, Spiller, & Finze, 2013; Dusenbury, Engel, Miller, Lemke, & Wallander, 2008; Liu et al., 2019; McGowan et al., 2018; Phillips, Tanaka, Archer, & Hanson, 2009).

Across all fertilizer application dates, soil moisture was positively correlated to N_2O (Figure 5; Table 7). However, a consistent relationship between soil moisture and N_2O

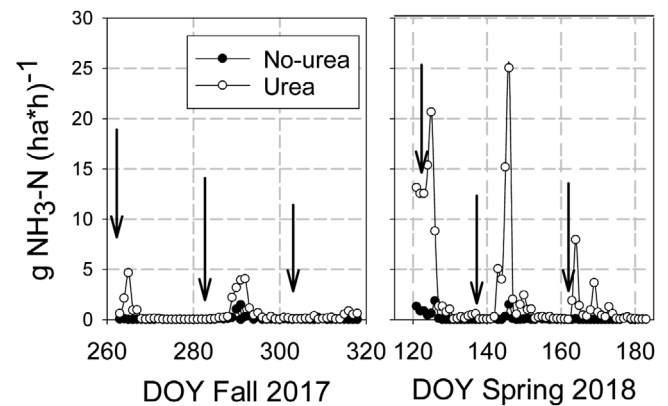


FIGURE 3 Average daily ammonia volatilization rates for fertilizer applied in the fall of 2017 and spring of 2018. Each value represents the sum of 6 measurement collected during the 24-h period. Data from the early fall, mid-fall, early winter treatment are on the left and data from the early spring, mid-spring, and early summer are on the right. The average soil temperature and moisture values are shown in Figures 1 and 2. The arrows represent when the fertilizer was applied

emissions between fertilizer application dates was not apparent. For example, N_2O and soil moisture were correlated to soil moisture for early fall and early winter but were not correlated for mid-fall.

3.3.1 | Relationship among temperature, moisture, and N_2O and CO_2-C emissions

The correlation analysis between the individual components shows that N_2O-N emissions were influenced by both soil temperature and soil moisture (Table 7). Others have reported similar findings (Abagandura et al., 2019). However, our

TABLE 5 Total cumulative NH_3-N volatilization for each time interval for fertilizer applied in early fall (20 Sept. 2017), mid-fall (11 Oct. 2017), early winter (1 Nov. 2017), early spring (1 May 2018), mid-spring (22 May 2018), and early summer (12 June 2018)

Treatment	Days after fertilizer application				Treatment	Days after fertilizer application			
	0-7	8-14	15-21	0-21		0-7	8-14	15-21	0-21
	kg NH_3-N ha ⁻¹					kg NH_3-N ha ⁻¹			
Early fall					Early spring				
Urea	0.24	0.007	0.001	0.25	Urea	1.812	0.0866	0.0368	1.9354
None	0.003	0.003	0.001	0.01	None	0.121	0.0003	0.0022	0.1235
<i>p</i> value	0.009	0.262	0.992	0.01	<i>p</i> value	0.159	0.175	0.152	0.0062
Mid-fall					Mid-spring				
Urea	0.1534	0.25	0.02	0.42	Urea	1.243	0.156	0.018	1.417
None	0.0337	0.06	0.005	0.1	None	0.045	0.0082	0.0045	0.0577
<i>p</i> value	0.306	0.281	0.055	0.04	<i>p</i> value	0.0121	0.0152	0.00152	0.12
Early winter					Early summer				
Urea	0.02	0.06		0.08	Urea	0.3656	0.0796	0.0126	0.4578
None	0.006	0.002		0.01	None	<0.002	<0.002	<0.002	0
<i>p</i> value	0.161	0.025		0.03	<i>p</i> value	0.036	0.022	0.759	0.0304

TABLE 6 Correlation coefficients between daily average N₂O, CO₂, and NH₃ emission rates as impacted by N treatment, season, and the soil temperature and soil moisture contents. Bolded correlation coefficients were significant at $p \leq .05$

Season	Date	N rate kg N ha ⁻¹	Temperature			Moisture		
			N ₂ O r	CO ₂ r	NH ₃ r	N ₂ O r	CO ₂ r	NH ₃ r
Early fall	20 Sept. 2017	all	-0.05	0.59	0.52	-0.14	-0.62	-0.48
Mid-fall	11 Oct. 2017	all	0.66	0.56	0.32	0.58	0.67	0.35
Early winter	1 Nov. 2017	all	-0.09	0.48	0.06	-0.16	0.31	0.23
Early spring	1 May 2018	all	-0.25	0.14	-0.18	0.43	-0.5	0.11
Mid spring	22 May 2018	all	0.54	-0.15	0.25	0.09	0.37	0.12
Early summer	12 June 2018	all	-0.28	0.25	-0.33	0.48	-0.26	-0.3
Sig. r values	P _{0.05} = 0.3, P _{0.01} = 0.393							
Early fall	20 Sept. 2017	0	-0.13	0.49	0.03	-0.52	-0.59	-0.34
Mid-fall	11 Oct. 2017	0	0.61	0.55	0.33	0.61	0.6	0.31
Early winter	1 Nov. 2017	0	-0.38	0.73	0.02	-0.49	0.45	-0.11
Early spring	1 May 2018	0	-0.45	0.47	0.05	0.35	-0.55	0.07
Mid spring	22 May 2018	0	0.18	0.18	0.12	0.38	0.19	0.09
Early summer	12 June 2018	0	-0.23	0.4	-0.14	0.65	0.12	0.33
Sig. r value	P _{0.05} = 0.423, P _{0.01} = 0.608							
Early fall	20 Sept. 2017	224	0.05	0.67	0.76	0.17	-0.65	-0.55
Mid-fall	11 Oct. 2017	224	0.74	0.61	0.43	0.59	0.76	0.49
Early winter	1 Nov. 2017	224	0.27	0.63	0.4	0.23	0.4	0.43
Early spring	1 May 2018	224	0.15	0.7	0.05	0.54	-0.64	0.15
Mid spring	22 May 2018	224	0.28	-0.007	0.11	-0.14	0.58	0.16
Early summer	12 June 2018	224	-0.09	0.02	-0.3	0.77	-0.54	-0.45
Sig. r values	P _{0.05} = 0.423, P _{0.01} = 0.608							
Fall		all	0.72	0.64	0.23	0.75	0.27	-0.07
Spring		all	-0.6	0.13	-0.13	0.69	-0.16	0.1
Fall		0	0.64	0.68	0.07	0.68	0.44	-0.12
Spring		0	-0.72	0.6	-0.21	0.68	-0.42	0.22
Fall		224	0.8	0.59	0.33	0.82	0.11	-0.08
Spring		224	-0.58	-0.12	-0.13	0.78	0.05	0.13
Sig r values	P _{0.05} = 0.174, P _{0.01} = 0.226							

research also showed that while temperature is correlated to N₂O emissions, this relationship may be positive in the fall and negative in the spring (Figure 4). In our experiment, the highest N₂O emissions (0.542 ± 0.079 g N₂O-N ha⁻¹ h⁻¹) occurred in the early spring, when temperatures were low and soil moisture (0.3387 ± 0.011 cm³ cm⁻³) and CO₂-C (1111 ± 107 g CO₂-C ha⁻¹ h⁻¹) emissions were high. The high CO₂-C emissions in early spring was attributed to soil freezing and thawing.

Soil moisture was positively correlated to N₂O for all treatments. These results are similar to those reported by Abagan-dura et al. (2019) and Linn and Doran (1984). The lowest N₂O

emissions (0.165 ± 0.002 g N₂O-N ha⁻¹ h⁻¹) occurred in the early winter which corresponded to low soil moisture contents (0.234 ± 0.014 cm³ cm⁻³) and low CO₂-C (285 ± 67 g CO₂-C ha⁻¹ h⁻¹), whereas the highest emissions occurred in the early spring when soil moisture and CO₂ emissions were high.

3.4 | Seasonal differences in PLFA microbial community structure

Seasonal differences in GHG emissions may be attributed to seasonal microbial community structure changes. However,

TABLE 7 Average daily rate of N₂O-N, CO₂-C and NH₃-N emissions for the six fertilizer application dates. Values with same letter within a fertilizer treatments and gas species were not different ($p = .05$). To convert these values to loss per day, multiply each value by 24 h, and to convert these values to average loss over the study period, multiply the daily values by the number of days in the study period

Application date	Urea			No-urea			
	N ₂ O-N	CO ₂ -C	NH ₃ -N	N ₂ O-N	CO ₂ -C	NH ₃ -N	
	g ha ⁻¹ h ⁻¹	kg ha ⁻¹ h ⁻¹	g ha ⁻¹ h ⁻¹	g ha ⁻¹ h ⁻¹	kg ha ⁻¹ h ⁻¹	g ha ⁻¹ h ⁻¹	
Early fall	20 Sept. 2017	0.514b	0.68c	0.4b	0.43b	0.75c	0.013c
Mid-fall	11 Oct. 2017	0.170d	0.56d	0.8b	0.12c	0.48d	0.198ab
Early winter	1 Nov. 2017	0.017e	0.36e	0.3b	0.02d	0.20e	0.026bc
Early spring	1 May 2018	0.597a	1.28a	4.0a	0.50a	1.95b	0.254a
Mid-spring	12 May 2018	0.211d	1.05b	2.9a	0.09c	1.30a	0.118abc
Early summer	12 June 2018	0.346c	1.016b	0.9b	0.09c	1.32a	0.023bc
<i>p</i> value		<.001	<.001	<.001	<.001	<.001	0.0077

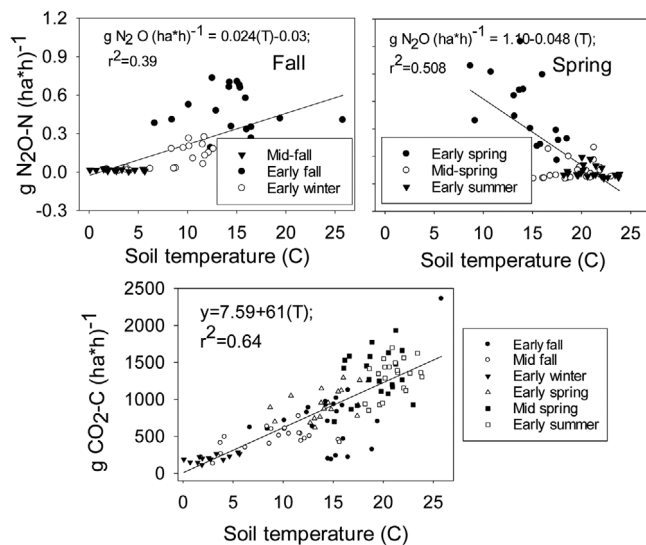


FIGURE 4 Influence of temperature on the average daily N₂O-N (left) and CO₂-C (right) emissions over 7 d in the fall of 2017 and spring of 2018

because the relationship between emission and the microbial community structure are not understood, the purpose of this section was to continue this dialog on how the microbial community influence greenhouse gas emissions.

The total microbial biomass was not different in the fall of 2017 and spring of 2018 ($p = 0.149$) (Table 8). However, seasonal differences in percentage of bacteria, actinomycetes,

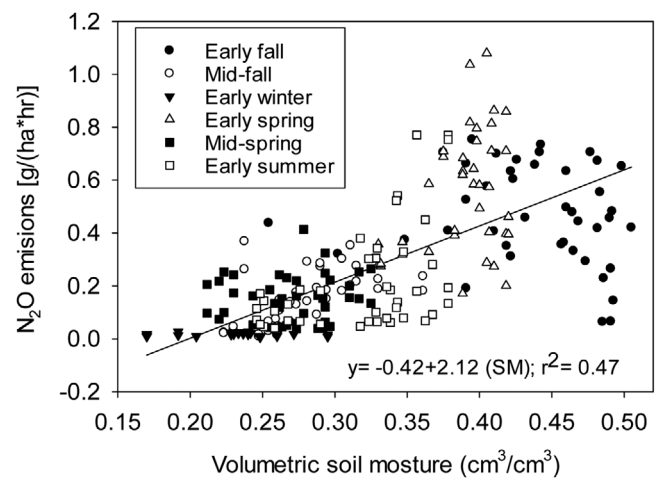


FIGURE 5 Influence of fertilizer application date and volumetric soil moisture (SM) on the average daily N₂O-N emission rates

arbuscular mycorrhizae, and undifferentiated soil organisms were observed. Others have reported seasonal changes in the microbial community structure (Andresen et al., 2014). Lipson, Schadt, and Schmidt (2002) reported that fungal/bacteria ratios were higher in winter than in spring. Buckeridge, Banerjee, Siciliano, and Grogan (2013) reported that from the winter to spring, fungal populations decreased and bacterial populations increased. Reese et al. (2014) reported that in South Dakota, the fungi to bacteria ratio was higher in the spring than the fall.

TABLE 8 Seasonal differences on the total microbial biomass, bacteria, fungi, actinomycetes, arbuscular mycorrhizae, undifferentiated microbial biomass, and the fungi to bacteria ratio as determined using the PLFA technique

Sample date	Total biomass	Bacteria	Fungi	Actino mycetes	Arbuscular mycorrhizae	Undifferentiated	Fungi/ bacteria ratio
	ng g ⁻¹	g kg biomass ⁻¹					
11 Oct. 2017	2360	546	101	118	34	345	0.18
12 June 2018	3510	408	125	73	12	461	0.32
<i>p</i> -value	0.149	0.003	0.481	0.006	0.016	0.025	0.217

Fungi are generally more efficient, have slower growth rates, higher C to N ratios, are more tolerant to dry soil and pH conditions, and have greater potential to immobilize N than bacteria (de Vries, 2009; Rousk & Bååth, 2007; Rousk, Brookes, & Bååth, 2009). In addition, fungi do not fix N, however they do increase nutrient uptake through their hyphal network, produce glomalin that builds soil structure, have the ability to produce N_2O through denitrification, and are favored at lower temperatures (Clay et al., 1990b; de Vries, 2009; Pietikäinen, Pettersson, & Bååth, 2005; Seo & Delaune, 2010). Selected bacteria have the capacity to fix N_2 , release organic acids to solubilize nutrients, produce siderophores that chelate iron, convert ammonium to nitrate, and use nitrate as the final electron acceptor. These findings suggest that climatic changes may impact the microbial community structure and nutrient cycling (Andresen et al., 2014; Maeda et al., 2015).

3.5 | Findings relative to IPCC N_2O default value of 1% of applied N

The findings reported in this paper differ from the current IPCC protocol that uses a 1% default value for N_2O emissions (DeKlein et al., 2006). Low fertilizer-derived N_2O -N losses were attributed to several factors including that:

1. Fertilizer induced emissions were calculated as opposed to total emissions,
2. Approximately, 50% of the applied N was immobilized/fixed into non-exchangeable forms that were not nitrified, and
3. Emissions were measured with a near continuous system and the same experiment was repeated in the early fall, mid-fall, early winter, early spring, mid-spring, and early summer.

Others have also reported that <1% of the applied N is emitted as nitrous oxide (Fujinuma, Venterea, & Rosen, 2011; Halvorson & Del Grosso, 2013; Macdonald, Rochester, & Nadelko, 2015; Niraula et al., 2019). However, the literature contains N_2O emission values that are highly variable. This variability could be attributed to: 1) research conducted at targeted locations that do not represent the entire watershed; 2) experiments that apply N at rates exceeding the plant requirement; 3) estimating cumulative emissions from experiments containing highly variable point measurements; and 4) using protocols with low accuracy and precision (Lehman et al., 2017; Parkin, 2008; Johnson & Barbour, 2019; Maharjan & Venterea, 2013; Steusloff, Nelson, Motovalli, & Singh, 2019; Chiesa, Pineiro, & Yahdjian, 2019; Davis, Groh, Jaynes, Parkin, & Isenhardt, 2019; Hoben, Gehl, Millar, Grace, & Robertson, 2010; Macdonald et al., 2015; Omonode, Kovács, & Vyn, 2015; Parkin & Hatfield, 2013; Thies

et al., 2019). In spite of these difficulties, obtaining accurate information is critical for estimating the carbon footprint for the entire watershed. One approach to overcome these barriers to conduct research at targeted locations and then using mathematics or a simulation model to calculate area corrected emission estimates (Chang, Clay, Clay, Smart, & Ohrtman, 2017).

4 | SUMMARY

This paper represents the second paper in series of papers utilizing this dataset. In the first paper, Thies et al. (2019) showed that the diurnal temperature cycle and soil moisture interacted to influence emissions across a 24-h period. This paper expands on this discussion and investigates the influence of fertilizer application date on N cycling. The current study showed that, when urea was added to soil it was hydrolyzed resulting in the production of NH_3 and NH_4^+ . A portion of the N was immobilized/fixed into non-exchangeable forms, emitted from the soil as either N_2O or NH_3 , or reduced to NO_3^- that could be denitrified or leached with percolating water. Mass-balance calculations suggest that the applied N was split between extractable and non-extractable forms, and that 11.63 ± 11.9 kg N ha^{-1} of the applied N was transported below the surface 30 cm. The relatively low NO_3^- losses were attributed to a large percentage of the applied N that was immobilized or fixed, that water percolation occurred prior to nitrification, and/or that nitrate was weakly sorbed to the soil matrix (Clay, Zhen, Liu, Clay, & Troien, 2004).

The relationships between GHG emissions and soil temperature moisture contents were dependent on the fertilizer application date. In the fall, the relationship between the N_2O -N emission rate and temperature was N_2O -N ($g\ ha^{-1}\ h^{-1}$) = $-0.03 + 0.024 \times \text{temperature } (^{\circ}C)$, $r^2 = 0.39$, $p < .01$, whereas in the spring the relationship was N_2O -N ($g\ ha^{-1}\ h^{-1}$) = $1.10 - 0.048 \times \text{temperature } (^{\circ}C)$, $r^2 = 0.508$, $p < .01$. Higher emissions in the spring than the fall were attributed to the spring freeze-thaw cycle that released labile organic substrates. The highest N_2O emissions occurred in the early spring when temperatures were low, and soil moisture and CO_2 emissions were high.

In the fall when the average 7-day temperature was less than $12^{\circ}C$, nitrification was slow, which resulted in increased ammonia volatilization. For fertilizer applied in the spring, ammonia volatilization losses were consistently greater in the fertilized than non-fertilized plots. These findings suggest that lower NH_3 -N volatilization losses for fertilizer applied in mid-fall (11 Oct. 2017) than early spring (1 May 2018) or mid-spring (22 May 2018) were attributed to low temperatures. Ammonia volatilization peaks generally occurred within 14 days of applying fertilizer. Based on the measured


values, the combined emissions of N_2O -N and NH_3 -N were generally less for the fall than the spring applied N. These findings show that management can be used to reduce N_2O -N and NH_3 -N emissions, and they confirm that delaying fall applied N until after the soil temperature decrease below $10^\circ C$ slows nitrification and potential losses.


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REFERENCES

- Abagandura, G. O., Sentürklü, S., Singh, N., Kumar, S., Landblom, D. G., & Ringwall, K. (2019). Impacts of crop rotational diversity and grazing under integrated crop-livestock system on soil surface greenhouse gas flux. *Plos One*, *14*, e0217069. <https://doi.org/10.1371/journal.pone.0217069>
- Adair, E. C., Barbieri, L., Schiavone, K., & Darby, H. M. (2019). Manure application decisions impact nitrous oxide emissions during non-growing season thaws. *Soil Science Society of America Journal*, *83*, 163–172. <https://doi.org/10.2136/sssaj2018.07.0248>
- Allison, F. E. (1973). Chapter 11: Non-biological immobilization of nitrogen. In F. E. Allison (Ed.), *Soil organic matter and its role in crop production* (pp. 206–229). New York: Elsevier Scientific Press.
- Andresen, L. C., Dungait, J. A. J., Bol, R., Selsted, M. B., Ambus, P., & Michelsen, A. (2014). Bacteria and fungi respond differently to multifactorial climate change in temperate heathland, traced with ^{13}C -glycine and FACE CO_2 . *PLOS*, *9*, e85070.
- Azum, F., Simmons, F. W., & Mulvaney, R. L. (1993). Immobilization of ammonium and nitrate and their interaction with native N in three Illinois mollisols. *Biology & Fertility of Soils*, *15*, 50–54. <https://doi.org/10.1007/BF00336288>
- Baer, S. E., Connelly, T. L., Sipler, R. E., Yager, P. L., & Bronk, D. A. (2014). Effect of temperature on rates of ammonium uptake and nitrification in the western coastal Arctic during winter, spring, and summer. *Global Biogeochemical Cycles*, *28*, 1455–1466. <https://doi.org/10.1002/2013GB004765>
- Barnes, R. T., Smith, R. L., & Aiken, G. R. (2012). Linkages between nitrification and dissolved organic matter quality, Boulder Creek watershed, Colorado. *Journal of Geophysical Research*, *117*, G01014. <https://doi.org/10.1029/2011JG001749>
- Bates, R. G., & Pinching, G. D. (1949). Acidic dissociation constants of ammonia ion at 0° to $50^\circ C$, and the base strength of ammonia. *J Res Nat Bureau Stan*, *42*, 419–430.
- Blackmer, A. M., & Bremner, J. M. (1978). Inhibitory effect of nitrate on reduction of N_2O to N_2 by soil microorganisms. *Soil Biology & Biochemistry*, *10*, 187–191.
- Blackmer, A. M., & Bremner, J. M. (1979). Stimulatory effect of nitrate on the reduction of N_2O to N_2 by soil microorganisms. *Soil Biology & Biochemistry*, *11*, 313–315. [https://doi.org/10.1016/0038-0717\(79\)90078-6](https://doi.org/10.1016/0038-0717(79)90078-6)
- Broadbent, F. E. (1965). Effect of fertilizer nitrogen on the release of soil nitrogen. *Soil Science Society of America Journal*, *29*, 692–696.
- Buckeridge, K. M., Banerjee, S., Siciliano, S. D., & Grogan, P. (2013). The seasonal pattern of soil microbial community structure in a mesic low arctic tundra. *Soil Biology & Biochemistry*, *65*, 338–347. <https://doi.org/10.1016/j.soilbio.2013.06.012>
- Burzaco, J. P., Smith, D. R., & Vyn, T. J. (2013). Nitrous oxide emissions in Midwest US maize production vary widely with band-injected N rates, timing, and nitrinaprin presence. *Environmental Research Letters*, *8*, 035031.
- Chang, J., Clay, D. E., Clay, S. A., Smart, A., & Ohrtman, M. (2017). A rapid method for measuring feces ammonia-nitrogen and carbon dioxide-carbon emissions and decomposition rate constants. *Agronomy Journal*, *109*, 1–9.
- Chiesa, T. D., Pineiro, G., & Yahdjian, L. (2019). Gross, background, and net anthropogenic soil nitrous oxide emissions from soybean, corn, and wheat cropland. *Journal of Environmental Quality*, *48*, 16–23.
- Clay, D. E., Clay, S. A., Lyon, D. J., & Blumenthal, J. M. (2005). ^{13}C discrimination in corn grain can be used to separate and quantify yield losses due to water and nitrogen stresses. *Weed Science*, *53*, 23–29.
- Clay, D. E., Clay, S. A., Brix-Davis, K., & Scholes, K. A. (1994). Nitrate movement after anhydrous ammonia application in a ridge tillage system. *Journal of Environmental Quality*, *23*, 9–13.
- Clay, D. E., Clay, S. A., Moorman, T. B., Brix-Davis, K., Scholes, K. A., & Bender, A. R. (1996). Temporal variability of organic C and nitrate in a shallow aquifer. *Water Research*, *30*, 559–568.
- Clay, D. E., Malzer, G. L., & Anderson, J. L. (1990a). Tillage and dicyandiamide influence on nitrogen fertilizer immobilization, remineralization, and utilization by maize. *Biology and Fertility of Soil*, *9*, 220–225.
- Clay, D. E., Malzer, G. L., & Anderson, J. L. (1990b). Ammonia volatilization from urea as influenced by soil temperature, soil water content, and nitrification and hydrolysis inhibitors. *Soil Science Society of America Journal*, *54*, 263–266.
- Clay, D. E., Reicks, G., Carlson, C. G., Moriles-Miller, J., Stone, J. J., & Clay, S. A. (2015). Residue harvesting and yield zone impacts C storage in a continuous corn rotation. *Journal of Environmental Quality*, *44*, 803–809.
- Clay, D. E., Zhen, Z., Liu, Z., Clay, S. A., & Trooien, T. P. (2004). Bromide and nitrate movement through undisturbed soil columns. *Journal of Environmental Quality*, *33*, 338–342.
- Davis, M. P., Groh, T. A., Jaynes, D. B., Parkin, T. B., & Isenhardt, T. M. (2019). Nitrous oxide emissions from saturated riparian buffers: Are we trading a water quality problem for an air quality problem. *Journal of Environmental Quality*, *48*, 261–269.
- de Vries, F. T. (2009). Soil fungi and nitrogen cycling: Causes and consequence of changing fungal biomass in grassland. PhD thesis. Wageningen University. ISBN 978-90-8585-325-1. Retrieved from <http://library.wur.nl/WebQuery/wurpubs/fulltext/1757>
- DeKlein, C., Novoa, R. S. A., Ogle, S., Smith, K. A., Rochette, P., Wirth, T. C., McConkey, B. G., Mosier, A., & Rypdal, K. (2006). Chapter 11: N_2O emissions from managed soils, and CO_2 emissions

- from line and urea application. In *2006 IPCC guidelines for national greenhouse gas inventories*, 11.3–11.563. Retrieved from https://www.ipcc-nggip.iges.or.jp/public/2006gl/pdf/4_Volume4/V4_11_Ch11_N2O&CO2.pdf
- Del Grosso, S. J., Parton, W. J., Mosier, A. R., Ojima, D. S., Kulmala, A. E., & Phongpan, S. (2000). General model for N₂O and N₂ emissions from soil due to denitrification. *Global Biogeochemical Cycles*, *14*, 1045–1060.
- Drake, J. E., Giasson, M. A., Spiller, K. J., & Finze, A. C. (2013). Seasonal plasticity in the temperature sensitivity of microbial activity in three temperate zone forest soils. *Ecosphere*, *4*, 1–21.
- Dusenbury, M. P., Engel, R. E., Miller, P. R., Lemke, R. L., & Wallander, R. (2008). Nitrous oxide emissions from a Northern Great Plains soil as influenced by nitrogen management and cropping systems. *Journal of Environmental Quality*, *37*, 542–550.
- Eagle, A. J., Olander, L. P., Locklier, K. L., Heffernan, J. B., & Bernhardt, E. S. (2017). Fertilizer management and environmental factors drive N₂O and NO₃ losses in corn: A meta-analysis. *Soil Science Society of America Journal*, *81*, 1191–1202. <https://doi.org/10.2136/sssaj2016.09.0281>
- Farquharson, R. (2016). Nitrification rates and associated nitrous oxide emissions from agriculture soils- a synopsis. *Soil Research*, *54*, 469–480. <https://doi.org/10.1071/SR15304>
- Fixen, P. E. (2007). Can we define a global framework within which fertilizer best management practices can be adapted to local conditions? IFA International Workshop on Fertilizer Best Management Practices (FBMPs), 7-9 March 2007, Brussels, Belgium, International Fertilizer Industry Association (IFA), Paris, France.
- Foster, N. W., Beauchamp, E. G., & Corke, C. T. (1985). Immobilization of nitrogen-15-labeled urea in a jack pine forest floor. *Soil Science Society of American Journal*, *49*, 448–452. <https://doi.org/10.2136/sssaj1985.03615995004900020035x>
- Fujinuma, R., Venterea, R. T., & Rosen, C. (2011). Broadcast urea reduces N₂O but increases NO emissions compared with conventional and shallow-applied anhydrous ammonia in a coarse-textured soil. *Journal of Environmental Quality*, *40*, 1806–1815.
- Halvorson, A. D., & Del Grosso, S. J. (2013). Nitrogen placement and source effects on nitrous oxide emissions and yields of irrigated corn. *Journal of Environmental Quality*, *42*, 312–322.
- Hamel, C., Hanson, K., Selles, F., Cruz, A. F., Lemke, R., McConkey, B., & Zentner, R. (2006). Seasonal and long-term resource-related variation in soil microbial community in wheat-based rotations of the Canadian prairie. *Soil Biology & Biochemistry*, *38*, 2104–2116. <https://doi.org/10.1016/j.soilbio.2006.01.011>
- Han, Z., Walter, M. T., & Drinkwater, L. E. (2017). N₂O emissions from grain cropping systems: A meta-analysis of the impact of fertilizer-based and ecologically-based nutrient management strategies. *Nutrient Cycling Agroecosystems*, *107*, 335–355. <https://doi.org/10.1007/s10705-017-9836-z>
- Hoben, J. P., Gehl, R. J., Millar, N., Grace, P. R., & Robertson, G. P. (2010). Nonlinear nitrous oxide (N₂O) response to nitrogen fertilizer in on-farm corn crops of the Midwest. *Global Change Biology*, *17*, 1140–1152.
- Holtan-Hartwig, L., Dörsch, P., & Bakken, L. R. (2002). Low temperature control of soil denitrifying communities: Kinetics of N₂O production and reduction. *Soil Biology & Biochemistry*, *34*, 1797–1806. [https://doi.org/10.1016/S0038-0717\(02\)00169-4](https://doi.org/10.1016/S0038-0717(02)00169-4)
- IPCC. (2007). Summary for policymakers. In *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge, UK and New York, NY: Cambridge Univ. Press. Retrieved from http://www.ipcc.ch/publications_and_data/ar4/wg3/en/spm.html
- Johnson, J. M. F., & Barbour, N. W. (2019). Stover harvest did not change nitrous oxide emissions in two Minnesota fields. *Agronomy Journal*, *111*, 143–155. <https://doi.org/10.2134/agronj2018.09.0591>
- Kelly, K. R., & Stevenson, F. J. (1987). Effect of carbon source on immobilization and chemical distribution of fertilizer nitrogen in soil. *Soil Science Society of America Journal*, *51*, 946–951. <https://doi.org/10.2136/sssaj1987.03615995005100040023x>
- Kim, K. I., Clay, D. E., Carlson, C. G., Clay, S. A., & Trooien, T. (2008). Do synergistic relationships between nitrogen and water influence the ability of corn to use nitrogen derived from fertilizer and soil? *Agronomy Journal*, *100*, 551–556. <https://doi.org/10.2134/agronj2007.0064>
- Lehman, R. M., Osborne, S. L., & Duke, S. E. (2017). Diversified no-till crop rotation reduces nitrous oxide emissions, increases soybean yields and promotes soil carbon accrual. *Soil Science Society of America Journal*, *81*, 76–83. <https://doi.org/10.2136/sssaj2016.01.0021>
- Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and non-tilled soils. *Soil Science Society of America Journal*, *48*, 1267–1272. <https://doi.org/10.2136/sssaj1984.03615995004800060013x>
- Lipson, D. A., Schadt, C. W., & Schmidt, S. K. (2002). Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. *Microbial Ecology*, *43*, 307–314. <https://doi.org/10.1007/s00248-001-1057-x>
- Liu, R., Hu, H., Suter, H., Hayden, H. L., He, J., Mele, P., & Chen, D. (2016). Nitrification is the primary driver of nitrous oxide production in laboratory microcosms from different land-use soils. *Frontiers in Microbiology*, *9*, 1373. <https://doi.org/10.3389/fmicb.2016.01373>
- Liu, Y., Tang, H., Muhammad, A., Zhong, C., Li, C., Yang, B., & Huang, G. F. (2019). Rice yields and greenhouse gas emissions affect by Chinese milk vetch and rice straw retention with reduced N fertilization. *Agronomy Journal*, *111*, 1–11. <https://doi.org/10.2134/agronj2019.03.0145>
- Ma, B. L., Wu, T. Y., Tremblay, N., Deen, W., Morrison, M. J., McLaughlin, N. B., Gregorich, E. G., & Stewart, G. (2010). Nitrous oxide flux from corn fields: On-farm assessment and the amount and timing of nitrogen fertilizer. *Global Change Biology*, *16*, 156–170.
- Macdonald, B. C. T., Rochester, I. J., & Nadelko, A. (2015). High yielding cotton produced without excessive oxide emissions. *Agronomy Journal*, *107*, 1673–1681. <https://doi.org/10.2134/agronj14.0369>
- MacLean, A. A., & McRae, K. B. (1987). Rate of hydrolysis and nitrification of urea and implications of its use in potato production. *Canadian Journal of Soil Science*, *67*, 679–686. <https://doi.org/10.1038/srep09697>
- Maeda, K., Spor, A., Edel-Hermann, V., Heraud, C., Breuil, M.-C., Bizouard, F., Toyoda, S., Yoshida, N., Steinberg, C., & Philippot, L. (2015). N₂O production, a widespread trait in fungi. *Scientific Reports*, *5*, 9697.
- Mahal, N. K., Osterholz, W. R., Miguez, F. E., Poffenbarger, H. J., Sawyer, J. E., Olk, D. C., Archontoulis, S. V., & Castellano, M. J. (2019). Nitrogen fertilizer suppresses mineralization of soil organic matter in maize agroecosystems. *Frontiers Ecology Evolution*, *7*, 59. <https://doi.org/10.3389/fevo.2019.00059>

- Maharjan, B., & Venterea, R. T. (2013). Nitrite intensity explains N management effects on N₂O emissions in maize. *Soil Biology & Biochemistry*, *66*, 229–238. <https://doi.org/10.1016/j.soilbio.2013.07.015>
- McGowan, A. R., Roozeboom, K. L., & Rice, C. W. (2018). Nitrous oxide emission from annual and perennial biofuel cropping systems. *Agronomy Journal*, *110*, 84–92. <https://doi.org/10.2134/agnonj2018.03.0187>
- Nieder, R., & Benbi, D. K. (2008). Carbon and nitrogen transformations in soils. In R. Nieder, & D.K. Benbi (Eds.), *Carbon and nitrogen in the terrestrial environment* (pp. 137–159). Germany: Springer.
- Nieder, R., Benbi, D. K., & Scherer, H. W. (2011). Fixation and defixation of ammonium in soil. A review. *Biology Fertility Soil*, *47*, 1–14.
- Niraula, S., Rahman, S., Chatterjee, A., Cortus, E. L., Mehata, M., & Spiehs, M. J. (2019). Beef manure and urea applied to corn show variable effects on nitrous oxide, methane, carbon dioxide, and ammonia. *Agronomy Journal*, *111*, 1448–1467.
- Okereke, G. U., & Meints, V. W. (1985). Immediate immobilization of labeled ammonium sulfate and urea. *Soil Science*, *140*, 105–109.
- Omonode, R. A., Kovács, P., & Vyn, T. J. (2015). Tillage and nitrogen rate effects on area- and yield-scales nitrous oxide emissions from pre-plant anhydrous ammonia. *Agronomy Journal*, *107*, 605–614.
- Parkin, T. B., & Hatfield, J. L. (2013). Enhanced efficiency fertilizers: Effect on nitrous oxide emissions in Iowa. *Agronomy Journal*, *105*, 1–9. <https://doi.org/10.2134/agnonj2013.0219>
- Parkin, T. B. (2008). Effect of sampling frequency on estimates of cumulative nitrous oxide emissions. *Journal of Environmental Quality*, *37*, 1390–1395.
- Phillips, R. L., Mcmillan, A. M. S., Palmada, T., Dando, J., & Giltrap, D. (2015). Temperature effects on N₂O and N₂ denitrification end-products for a New Zealand pasture soil. *New Zealand Journal of Agricultural Research*, *58*, 89–95. <https://doi.org/10.1080/00288233.2014.969380>
- Phillips, R. L., Tanaka, D. L., Archer, D. W., & Hanson, J. D. (2009). Fertilizer application timing influences greenhouse gas fluxes over a growing season. *Journal of Environmental Quality*, *38*, 1569–1579. <https://doi.org/10.2134/jeq2008.0483>
- Pietikäinen, J., Pettersson, M., & Bååth, E. (2005). Comparison of temperature effects on soil respiration and bacteria and fungal growth rates. *Fems Microbiology Ecology*, *52*, 49–58. <https://doi.org/10.1016/j.femsec.2004.10.002>
- Rankoth, L. M., Udawatta, R. P., Gantzer, C. J., Jose, S., Veum, K., & Dewanto, H. A. (2019). Cover crop on temporal and spatial variation in soil microbial communities by phospholipid fatty acid profiling. *Agronomy Journal*, *111*, 1693–1703. <https://doi.org/10.2134/agnonj2018.12.0789>
- Reddy, G. B., & Reddy, K. R. (1993). Fate of nitrogen-154 enriched ammonium nitrate applied to corn. *Soil Science Society of America Journal*, *57*, 111–115.
- Reese, C. L., Clay, D. E., Clay, S. A., Bich, A. D., Kennedy, A. C., Hansen, S. A., & Moriles, J. (2014). Winter cover crops impact on corn production in semi-arid regions. *Agronomy Journal*, *106*, 1479–1488. <https://doi.org/10.2134/agnonj13.0540>
- Rousk, J., & Bååth, E. (2007). Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. *Soil Biology & Biochemistry*, *39*, 2173–2177. <https://doi.org/10.1016/j.soilbio.2007.03.023>
- Rousk, J., Brookes, P., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacteria growth suggest functional redundancy in carbon mineralization. *Applied Environmental Microbiology*, *75*, 1589–1596. <https://doi.org/10.1128/AEM.02775-08>
- Sainju, U. M., Stevens, W. B., Caesar-Tonthat, T., & Liebig, M. A. (2012). Soil greenhouse gas emissions affected by irrigation, tillage, crop rotation, and nitrogen fertilization. *Journal of Environmental Quality*, *41*, 1774–1786. <https://doi.org/10.2134/jeq2012.0176>
- Sánchez-Martin, L., Vallejo, A., Dick, J., & Skiba, U. M. (2008). The influence of soluble carbon and fertilizer nitrogen on nitric oxide and nitrous oxide emissions from two contrasting agricultural soils. *Soil Biology & Biochemistry*, *40*, 142–151. <https://doi.org/10.1016/j.soilbio.2007.07.016>
- Sebilo, M., Mayer, B., Nicolardot, B., Pinay, G., & Mariotti, A. (2013). Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences of the USA*, *110*, 18185–18189. <https://doi.org/10.1073/pnas.1305372110>
- Senbayram, M., Chen, R., Budai, A., Bakken, L., & Dittert, K. (2012). N₂O emissions and the N₂O/(N₂O + N₂) product ratio of denitrification as controlled by carbon substrates and nitrate concentrations. *Agriculture Ecology Environment*, *147*, 4–12. <https://doi.org/10.1016/j.agee.2011.06.022>
- Seo, D. C., & DeLaune, R. D. (2010). Fungal and bacteria mediated denitrification in wetlands: Influence of sediment redox condition. *Water Research*, *44*, 2441–2450. <https://doi.org/10.1016/j.watres.2010.01.006>
- Sherlock, R. R., & Goh, K. M. (1984). Dynamics of ammonia volatilization from simulated urine patches and aqueous urea applied to pasture: 1. Field experiments. *Fertility Research*, *5*, 181–195.
- Shurpali, N. J., Rannik, Ü., Jokinen, S., Lind, S., Biasi, C., Mammarella, I., Peltola, O., Pihlatie, M., Hyvönen, N., Rätty, M., Haapanala, S., Zahniser, M., Virkajävi, P., Vesala, T., & Martikainen, P. J. (2016). Neglecting diurnal variation leads to uncertainty in terrestrial nitrous oxide emissions. *Science Report*, *6*, 25739.
- Snyder, C. S., Bruulsema, T. W., Jensen, T. L., & Fixen, P. E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture Ecosystem Environment*, *133*, 247–266. <https://doi.org/10.1016/j.agee.2009.04.021>
- Soil Survey Staff (2018). Natural Resources Conservation Service. United States Department of Agriculture. Web Soil Survey. Retrieved from <https://websoilsurvey.sc.egov.usda.gov/>
- Steusloff, T. W., Nelson, K. A., Motovali, P. P., & Singh, G. (2019). Urea nitrapyrin placement effects on soil nitrous oxide emissions in claypan soil. *Journal of Environmental Quality*, *44*, 1444–1453. <https://doi.org/10.2134/jeq2019.01.0031>
- Thies, S. (2018). Greenhouse gas emissions from soil applied nitrogen fertilizers. Theses and Dissertations. 2956. South Dakota State University. Retrieved from <https://openprairie.sdstate.edu/etd/2956>
- Thies, S., Bruggeman, S. A., Clay, S. A., Mishra, U., Hatfield, G., Kumar, S., & Clay, D. E. (2019). Midmorning point sampling may not accurately represent nitrous oxide emissions following fertilizer application. *Soil Science Society of America Journal*, *83*, 339–347. <https://doi.org/10.2136/sssaj2018.08.0313>
- Thomas, B. W., Hao, X., Larney, F. J., Goyer, C., Chatigny, M. H., & Charles, A. (2017). Non-legume cover crops can increase non-growing season nitrous oxide emissions. *Soil Science Society of America Journal*, *81*, 189–199. <https://doi.org/10.2136/sssaj2016.08.0269>
- Tran, T. S., & Giroux, M. (1998). Fate of 15N-labelled fertilizer applied to corn grown on different soil types. *Canadian Journal of Soil Science*, *78*, 597–605.

- Venterea, R., & Coulter, J. A. (2015). Split application of urea does not decrease and may increase nitrous oxide emissions in rainfed corn. *Agronomy Journal*, *107*, 337–348. <https://doi.org/10.2134/agronj14.0411>
- Veum, K. S., Lorenz, T., & Kremer, R. J. (2019). Phospholipid fatty acid profiles of soils under variable handling and storage conditions. *Agronomy Journal*, *111*, 1090–1096. <https://doi.org/10.2134/agronj2018.09.0628>
- Wagner-Riddle, C., & Thurtell, G. W. (1998). Nitrous oxide emissions from agricultural fields during winter and spring thaw as affected by management practices. *Nutrient Cycling Agroecosystems*, *52*, 151–163. <https://doi.org/10.1023/A:1009788411566>
- Weier, K. L., Doran, J. W., Powers, J. F., & Walters, D. T. (1993). Denitrification and dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society of America Journal*, *57*, 66–72. <https://doi.org/10.2136/sssaj1993.03615995005700010013x>
- Wlodarczyk, T., Stepniewska, Z., & Brzezinska, M. (2003). Denitrification, organic matter, and redox potential transformations in Cambisols. *International Agrophysics*, *17*, 219–227.
- Young, C. L. (1981). IUPAC solubility data series. *Oxides of nitrogen* (8). Oxford, England: International Union of Pure and Applied Chemistry, Analytical Chemistry Division, Commission on Solubility Data, Pergamon Press.

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