2024

Nitrogen Application Timing and Cereal Rye (*Secale Cereale* L.) Cover Crop Influence Greenhouse Gas Emissions

Graig Reicks
South Dakota State University, graig.reicks@sdstate.edu

NITROGEN APPLICATION TIMING AND CEREAL RYE (*SECALE CEREALE L.*) COVER CROP INFLUENCE GREENHOUSE GAS EMISSIONS

BY

GRAIG REICKS

A dissertation submitted in partial fulfillment of the requirements for the

Doctor of Philosophy

Major in Plant Science

South Dakota State University

2024

DISSERTATION ACCEPTANCE PAGE
Graig Reicks

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Sharon Clay
Advisor Date

David Wright
Department Head Date

Nicole Lounsbery, PhD
Director, Graduate School Date

ACKNOWLEDGMENTS

I want to thank The Natural Resources Conservation Service (NRCS), South Dakota Corn Growers Association, and South Dakota State University for funding this project. I would also like to thank my advisor, Dr. Sharon Clay; my committee members, Dr. David Clay, Dr. Thandi Nleya, and Dr. Gary Hatfield; and my graduate faculty representative, Dr. Cynthia Elverson for all their time and guidance. I'd like to thank my coworkers and former coworkers, Dr. Janet Miller, Dr. Deepak Joshi, Dr. Stephanie Bruggeman, Dr. Shaina Westhoff, Sam Thies, Doug Fiedler, and Andrew Engel for all their assistance with field and lab work. And last, but definitely not least, I'd like to thank my wife, Stephanie, and my sons, Camden and Cayler for their love, patience, and support during this process.

TABLE OF CONTENTS

ABBREVIATIONS

AMO .... Ammonia monooxygenase

CO_2e .... CO_2 equivalent

DOC .... Dissolved organic carbon

FFT .... Fast Fourier Transform

GDD .... Growing degree day

GWP .... Global warming potential

GWP_{100} .... 100-year global warming potential

GHG .... Greenhouse gas

IPCC .... Intergovernmental Panel on Climate Change

MMO .... Methane monooxygenase enzyme

PLFA .... Phospholipid fatty acid

PVC .... Polyvinyl chloride

SARE .... Sustainable Agriculture Research and Education

SOC .... Soil organic carbon

UAN .... Urea ammonium nitrate

VE .... Seed emergence corn growth stage

V2, V3, V4, V5, V6 .... 2, 3, 4, 5, & 6- leaf corn growth stages, respectively

WFPS .... Water-filled pore space (%)

LIST OF FIGURES

Figure

Page

LIST OF TABLES

Table **Page**

Supplementary Tables

ABSTRACT

NITROGEN APPLICATION TIMING AND CEREAL RYE (*SECALE CEREALE L.*) COVER CROP INFLUENCE GREENHOUSE GAS EMISSIONS

GRAIG REICKS

2024

Dryland contributions to greenhouse gas (GHG) emissions are increased by N fertilizer applications and high soil water contents. Fertilizer timing and spring growth of cover crops prior to cash crop planting were investigated in separate studies to examine impacts on overall GHG emissions using a near continuous measurement system. There was a significant interaction between N fertilizer rate (0 vs. 224 kg N ha^{-1} surface-applied as urea) and application date ($p= 0.01$) for CO_2 emissions. This interaction occurred because N fertilizer increased CO_2 emissions by 35% for the 21 d interval following early spring application. When application was delayed until mid-spring, the opposite response occurred, and N fertilizer application reduced CO_2 emissions by 19% ($p=0.06$). CO_2 and non-CO_2 emissions (N_2O plus CH_4) were analyzed separately in this study because previous research demonstrated that C addition from crop residues can offset CO_2 emissions at this location. The soil was a CH_4 sink for all six application dates, but atmospheric CH_4 consumption was 85% greater during spring and early summer [averaging -85.8 g CO_2e $(ha\times h)^{-1}$] than during fall/early winter [averaging -46.3 g CO_2e $(ha\times h)^{-1}$]. The soil consumed CH_4 at a rate to offset N_2O emissions by 9.6% during mid-spring and early summer when N fertilizer wasn't applied and by 3.1% when N fertilizer was applied. Dormant-seeded cereal rye (*Secale cereale L.*) produced an average of 445 kg biomass ha^{-1} during the first three weeks of spring growth, which is normally prior to corn (*Zea mays L.)* emergence. The cover crop reduced N_2O emissions by 53% and did

not increase CO_2 emissions. However, since N_2O emissions only made up 4% of total GHG emissions, the cover crop only reduced total GHG emissions by 2%. These results indicate that early spring in eastern South Dakota climates may be a key time to target mitigation strategies for soil GHG emissions in a corn and soybean production system. Delaying N fertilizer application from early spring to mid-spring has more potential to reduce these emissions (26% reduction) than growing a cereal rye cover crop that is terminated just prior to corn planting (2% reduction).

CHAPTER 1 INTRODUCTION

Types of Greenhouse Gases (GHG)

In the 21st century, reducing the impact of annual cropping systems on global warming should involve the wide scale adoption of science-based climate smart practices. Goals of climate smart practices include increasing crop productivity and resilience, while concomitantly reducing emissions of the three major greenhouse gases (GHG), which are CO_2, N_2O, and CH_4. Of these gases, CO_2 typically receives the most attention since it makes up 79.4% of all U.S. GHG emissions. When expressed as CO_2 equivalents (CO_2e), CH_4 and N_2O represent 11.5% and 6.2% of the GHG emissions, respectively (EPA, 2023). CH_4 and N_2O cause 27.9 and 273 times as much global warming as CO_2 over a 100-year period, respectively. Therefore, their CO_2e is calculated by multiplying their emission values by 27.9 and 273, respectively.

According to the U.S. Environmental Protection Agency (EPA, 2023), agriculture contributes 9.4% of the total annual U.S. GHG emissions. Practices that improve crop productivity, such as N fertilization to increase yields, are generally conceptualized as increasing, rather than decreasing, GHG emissions. For example, N_2O emissions from soil management (mostly from N fertilizer addition) comprise 49.2% of the total GHG emissions from U.S. agriculture (4.6% of U.S. total GHG) and are the largest source of U.S. N_2O emissions. In their report, they cited rice cultivation as the only U.S. cropping practice that contributes CH_4 emissions, which contributed 2.8% of the total U.S. agricultural GHG emissions. In their global inventory of the soil CH_4 sink, Dutaur and Verchot (2007) reported forests and grasslands as both sinks and sources of CH_4. They reported cultivated lands only as sources of CH_4, although their dataset for cultivated lands was only 47 of their 318 datapoints.

Sources of GHG in Agriculture and Uptake by Soils

CH_4 is part of a cycle with CO_2, where CH_4 is the most reduced form of C, whereas CO_2 is the most oxidized. This cycle begins with decomposition of soil organic matter by methanogenic bacteria under anaerobic conditions (>60% soil moisture), which produces CH_4. Under drier aerobic conditions (<60% soil moisture), CH_4 is oxidized (i.e. consumed) by methanotrophs, and therefore sequestered from the atmosphere as one of several intermediates, eventually returning to the atmosphere as CO_2. CH_4 is a relatively short-lived gas with an average residence time of only 11.8 yrs. in the atmosphere before it is oxidized in the atmosphere with excess OH^{-1} and returns to CO_2 (Canadell et al., 2021). The most recent version of IPCC (Smith et al. 2021) was the first version to assign different GWP_{100} values to CH_4 depending on its source. For example, oxidation of CH_4 from fossil sources is viewed as adding new CO_2 to the atmosphere and receives a GWP_{100} value of 29.8. When CH_4 originates from non-fossil sources, it's viewed as part of the existing cycle between atmospheric CO_2 and CH_4 and receives a lower a GWP_{100} of 27.0 CO_2e. A weighted average of these two sources was used to estimate the overall GWP_{100} of 27.9 CO_2e for CH_4.

N_2O emissions are typically associated with denitrification, which is the microbial reduction of soil NO_3^- to N_2O under anaerobic soil conditions. N_2O emissions may also occur under aerobic conditions during nitrification, which is the oxidation of NH_4^+ to NO_3^-. N_2O has a much longer residence time of 116 years in the atmosphere (Canadell et al., 2021) compared to CH_4. Application of N fertilizer from 0 to an optimum level increases crop productivity as well as N_2O emissions. Thies et al. (2020) measured N_2O emissions from soil for 21 d following a urea application that provided 200 kg N ha^{-1} and reported that N_2O emissions in response to fertilizer varied by season. N fertilizer did not increase N_2O emissions for most application timings in their study (early fall, mid-fall, early winter, early spring, and mid-spring). The application timing where N fertilizer did increase N_2O emissions was early summer, which was by 293% over the other season's averages, and accounted for 0.06% of the N applied lost as N_2O within 21 of application. It's important to note that the Thies et al. (2020) study wasn't designed to separate N_2O emissions from their N source (i.e. fertilizer vs. soil residual). In addition to directly contributing fertilizer N to the inorganic N pool, the N fertilizer may have also contributed indirectly by stimulating soil microbes, which mineralized N from soil organic matter. Similar responses can occur even when N is applied at two lower rate applications (i.e. split applied) rather than as one high rate (Venterea and Coulter, 2015; Venterea et al., 2016).

Methods to Reduce GHGs

There are many ways to reduce GHG emissions from a corn and soybean production system. Two commonly proposed methods are through better N fertilizer timing and the inclusion of a cover crop.

N Fertilizer Timing

Phillips et al. (2009) conducted a study near Mandan, ND where soils were fertilized with 70 kg N ha^{-1} as urea in early spring (1 April) or late-spring (13 May) to corn that was planted on May 15. They concluded that CO_2 fluxes were greater and that

CH_4 fluxes were lower when soils were fertilized in late spring. They observed no significant difference between N_2O emissions between the application dates. Their study did not have an unfertilized control, so the effects of application timing and not of fertilizer were compared. Their N fertilizer rate was also about half of what farmers would apply to corn in eastern SD.

Cover Crops

Growing cover crops prior to planting cash crops is on the rise, but still relatively uncommon. The United States Department of Agriculture's National Agricultural Statistics Service (USDA-NASS) releases a Census of Agriculture every five years. The 2022 Census of Agriculture reported that 18.0 million acres of cover crops were planted in the U.S. This was up 17% from 15.4 million acres in 2017. However, the number of acres with a cover crop in 2022 only covered 6.0% of U.S. harvested cropland.

The U.S. Census of Agriculture did not provide specifics of cover crop species or the cropping systems utilizing cover crops. Instead, the Sustainable Agriculture Research and Education's (SARE) National Cover Crop Survey Report for 2022-2023 provided more insight. This report surveyed 795 growers from 49 states and reported that 48.8% of the respondents grew a cover crop in rotation with corn. Of those growers, 59.5% grew the cover crop prior to planting corn. Cereal rye was the most popular species, grown on 50.9% of the acres either in a mixture or monoculture. Radish was the next closest but was only grown on 16.4% of the acres.

Cover crop services can include increased soil organic C stocks, reduced soil erosion, reduced soil compaction, improved soil aggregation, reduced soil temperature, improved cycling of N, weed control, water conservation, increased cash crop yields, and biomass for livestock or biofuel production (Blanco-Canqui et al., 2015). Given the short timeframe for growth, and biomass production, its benefits may be limited. In a four-year Nebraska study, Ruis et al. (2020) grew either cereal rye or a mix of cereal rye, winter pea (*Pisum sativum L.*), hairy vetch (*Vicia villosa L.*), and radish (*Raphanus sativus L.*). They concluded that the low biomass (<1,000 kg ha^{-1}) from these cover crops in a corn-soybean rotation did not impact soil properties, such as soil organic C and wet aggregate stability.

Cereal rye is a popular species because it can quickly produce biomass during the short timeframe of early spring prior between thaw and planting a cash crop, which occurs when soil temperatures have reached 10.0 to 15.6°C. In eastern SD, cover crops are either interseeded during the growing season of the previous cash crop or are seeded following harvest of the previous cash crop. The two methods are outlined below.

Interseeding into standing crops

Interseeding into the previous cash crop usually consists of an aircraft (airplane or helicopter) dropping seed over the field, which mostly lands on the soil surface beneath the crop canopy. This method allows for an earlier seeding date and can be a very efficient process due to the high speed of aircraft. The biggest disadvantage of this method is that sufficient rain or irrigation is required to achieve germination of seeds broadcast on the soil surface. If this doesn't occur, the operation could be a failure. For this reason, broadcast interseeding is conducted as soon as possible in late-summer to increase the chances for rain exposure. In addition, a higher seeding rate is often used to compensate for the lower amount of seed-to-soil contact in a surface broadcast compared to drilling seed into the soil. Mohammed et al. (2020) broadcast seeded cereal rye onto the soil surface in standing corn or soybeans on three different dates ranging from mid-August to late-September at three different sites. Biomass was measured the following spring on May 1, where 1864, 786, and 724 kg ha^{-1} at Ames, IA; Fargo, ND; and Morris, MN, respectively. The biomass production was lower as the seeding date was later in the fall late seeding date, but the difference typically evened out by May 1. The location of the study reported in this dissertation is near Brookings, SD and is geographically between the Fargo/Morris and Ames study locations, but more similar to the Fargo/Morris locations in terms of heat units and precipitation for growing rye biomass. Based on these results, we could estimate 1000 kg rye biomass ha^{-1} if the cover crop were broadcast into standing corn or soybeans from mid-August through early-September and termination were to occur on May 1 the following growing season. Again, this would be prior to corn emergence, but not necessarily prior to corn planting, as corn is sometimes planted in late-April if soil conditions are favorable.

Drill Seeding after Corn or Soybean Harvest

Seeding with a drill is far less efficient, since a tractor pulling a drill only travels at small fraction of an airplane's speed. However, the extra time needed to drill the cover crop seed into the soil might be the difference between having a cover crop and not having one. In addition, significantly lower seeding rates are often possible with a drill since there's better seed-to-soil contact. Kantar and Porter (2014) drilled a cereal rye cover crop at two week increments during the fall and then measured biomass the following spring at two dates in May near Lamberton, MN (Table 1.1), which is only 125 km east of Brookings, SD and has a similar climate. Soybean harvest typically occurs between first and second seeding dates (mid-Sept. and early Oct.) used in their study, and if seeded simultaneously with soybean harvest, the cover crop may be planted by the early October seeding date. Since corn typically follows soybeans in the crop rotation, cover crop termination in early May around the time of corn planting produced 1014 kg biomass ha^{-1} in 2007, but only 19 kg ha^{-1} in 2008. These extremes show the challenges of incorporating a cereal rye cover crop into a corn and soybean rotation, especially during the corn growing season.

The mid-October date was dormant seeded (Table 1.1), as Kantar and Porter (2014) reported no biomass was produced during the fall from this seeding date. Since corn is typically harvested following soybeans and often lasts into late-October or early-November, dormant seeding a cover crop is more likely following corn harvest than following soybean harvest. During the following spring, soybeans normally follow corn in the crop rotation. Soybean planting typically begins after corn planting has finished when soils have reached 15.6°C. This can extend the cover crop growing season by a few weeks into mid-May. In Kantar and Porter (2014), these additional few weeks of cereal rye growth increased biomass production by about 200% (or by three-fold), which was especially beneficial to a dormant seeded cover crop, as the biomass production went from 502 to 1644 kg ha^{-1} when terminated on 5/9/07 and 5/21/07, respectively. They did not measure GHG emissions, only rye biomass production.

Table 1.1. Cover crop biomass production at different seeding and termination dates near Lamberton, MN. Adopted from Kantar and Porter (2014).

Seeding Date	Sampling Date	Biomass	Sampling Date	Biomass	% biomass increase[2]
		kg ha^{-1}		kg ha^{-1}	
9/19/06	5/9/07	1231 a[1]	5/21/07	3419 a	178
10/4/06		1014 a		2735 b	170
10/17/06		502 b		1644 c	227
9/20/07	5/4/08	475 a	5/18/08	1331 a	180
10/5/07		~0 b		302 b	
10/19/07		0		~0	

[1]Values followed by a different letter within the same sampling date are significantly different at $p<0.05$.
[2]Percent biomass increase over early sampling date

GHG Reductions from cover crops

Basche et al. (2014) conducted a meta-analysis of GHG emissions from cover crops that included a broad spectrum of cover and cash crop species, tillage systems, N fertilization rates, and geographies. In their analysis, they reported that 40% of cover crop trials decreased N_2O emissions, while 60% increased N_2O emissions. Net emissions were close to zero for studies that reported on an entire year of data, generally with net reductions during the growing phase of the cover crop and net emissions during the decomposition phase. One of the cereal rye cover crop trials in their meta-analysis was Jarecki et al. (2009), who measured N_2O emissions over a year from a mollisol in central IA that had a winter rye cover crop broadcast onto the soil surface just prior to soybean leaf drop. Corn was the following cash crop, which received 175 kg N ha^{-1} as a UAN sidedress. The cover crop appeared to reduce cumulative N_2O emissions by about 25% (visual estimation from a bar graph). However, this difference was not reported as statistically significant. Measurements were only taken once per week during the growing season and less frequently for the rest of the year. Lack of data may explain this non-significant difference.

In a 10-yr corn-soybean rotation in central IA, where the treatments were either a post-harvest drill-seeded rye cover crop (following both corn and soybeans) vs. no cover crop, Parkin et al. (2016) reported that the rye reduced cumulative NO_3 leaching losses by 53.5% (359 vs. 167 kg ha^{-1}) over the course of the study. Despite the large NO_3 leaching reduction and a significant decrease in cumulative annual N_2O emissions in 5 of the 10 years, rye did not reduce cumulative N_2O emissions over the entire study. Even though it wasn't mentioned in the context of their paper, the three years with the lowest rye biomass production (500, 610, and 1,080 kg ha^{-1}) occurred during spring of the soybean growing season, and were three of the five seasons where rye reduced N_2O emissions. This shows that smaller amounts (<1,000 kg ha^{-1}) of cereal rye cover crop biomass may have a larger impact on reducing N_2O emissions than higher quantities of biomass. Due to the cold spring seasons in the Upper Midwestern U.S., it will likely be a challenge to produce large amounts of biomass prior to planting corn or soybeans.

In a Japanese study, Gong et al. (2021) seeded either a hairy vetch or cereal rye cover crop between crops of continuous soybeans. They reported that cereal rye and fallow had similar CH_4 consumption and N_2O emission levels. Hairy vetch, a legume, had net CH_4 emissions (instead of consumption) but similar N_2O emissions as rye and fallow. When factoring in CO_2, fallow, rye, and hairy vetch all had similar net global warming potential (GWP). This study produced relatively large amounts of biomass compared to the cereal rye studies previously mentioned, at 8.1, 3.2, and 3.1 Mg ha^{-1} for the rye, hairy vetch, and fallow (weeds) treatments, respectively. In addition, they did not have a true vegetation-free control and only compared rye to weeds.

In an irrigated New Mexico study, Acharya et al. (2022) had different results than those previously discussed and reported that various winter cover crop mixtures (annual ryegrass and triticale for grasses, daikon radish and turnip for brassicas, and clover and winter pea for legumes) increased both CO_2 and N_2O emissions during the cover crop growth phase compared to a no cover crop control. After the cover crop was terminated and corn was planted (i.e. the cash crop phase) emissions were similar between cover crop and no cover crop treatments. Archarya et al. (2022) did not report cover crop biomass amounts.

Impact of Termination Timing on Corn Yield

Even though information on whether a spring-grown cover crop can reduce GHG emissions is lacking, there is information on the quantity of cereal rye biomass that can reduce corn yields. A two-year study in central IA by Acharya et al. (2017) found that approximately 1,000 kg rye biomass ha^{-1} reduced corn yields by 11.5% in 2014 when rye was terminated the day after corn planting. A much earlier growing season in 2015 produced 1,000 kg biomass ha^{-1} when they terminated 25 days before planting, which still reduced corn yields by 6.6%. Corn yields were not impacted when approximately 500 kg biomass ha^{-1} were produced and terminated at 10 days before planting in 2014. Unfortunately, their study was unable to generate 500 kg biomass ha^{-1} at or shortly after planting to observe the impact on yields.

Moriles-Miller et al. (2024) terminated cover crops at 2 weeks prior to planting (BP), at corn planting (V0), and then at the V2 and V4 growth stages of corn. In the wetter year of 2019, rye terminated at the V2 growth stage of corn had 722 kg biomass ha^{-1} and did not reduce silage yield. However, if rye was terminated at the V4 growth stage, rye biomass was 1120 kg ha^{-1} and silage yield were reduced by 36.5% compared to a treatment without rye. In a drier year (2020), the negative effects occurred when the rye was terminated at V0 and had 62 kg biomass ha^{-1}, which still reduced grain yield by 19.3%. When terminated at V2, rye had 634 kg biomass ha^{-1} and resulted in a grain yield loss of 28.9%.

Except for the Moriles-Miller et al. (2024) trial, most of the trials discussed produced at least 500 kg aboveground biomass ha^{-1} by corn planting. This was the only trial that featured a dormant seeded the cover crop in late-October, which resulted in essentially no fall growth. The other trials were seeded in late-summer or early-fall and began growing in the fall. When dormant seeding, spring growth will likely be much slower because the cover crop still needs to go through the germination process. Therefore, the fall dormant seeded cover crop may require a later termination date, possibly after the corn has emerged, to experience the ecosystem services that the cover crop can provide. There's a heightened risk for yield loss by having a living (or even dying) cover crop in proximity to corn seedlings, even if the cover crop is small and not competitive for light, nutrients, and water. Moriles-Miller et al. (2012) showed that altered transcriptome signaling by weeds can reduce corn yields even when they're small and not taking resources from the corn plants.

Statement of the Problem

As GHG emissions continue to rise, agriculture is at the crossroads of whether the industry wants to be perceived as a net emitter, net reducer, or GHG neutral. CO_2 is the most discussed GHG, and agriculture can certainly play a role in reducing its atmospheric concentration through reduced fossil fuel inputs and sequestration of carbon into soil. N_2O and CH_4 are less frequently discussed, but agriculture also plays an important role in both producing and reducing these gases. N fertilizer is needed to produce most crops profitability. However, its application typically results in higher N_2O emissions than unfertilized soil. Farmers probably cannot eliminate N fertilizer from their operations, but perhaps they may consider methods to manage N fertilizer differently if it improves their income. We've reached the point where farmers can be paid for reductions in GHG emissions to produce their crops. Since limited information exists on whether N fertilizer and its application timing impacts CO_2 and CH_4 emissions, the first null hypothesis for this research is that N fertilizer and its application timing will not affect total GHG emissions from soils in a dryland cropping system. In the second part of our research, we investigated whether a cereal rye cover crop grown during the spring, and terminated at cash crop planting, could reduce total GHG emissions. Potential GHG reductions would likely be through reduced N_2O emissions stemming from uptake of soil moisture and/or N by the cover crop. The null hypothesis for the second part of our research is that a cereal rye cover crop will not significantly affect overall GHG emissions.

CHAPTER 2: GREENHOUSE GAS EMISSIONS WERE IMPACTED BY UREA APPLICATION TIMING

Abstract

Three important greenhouse gases connected to annual crop management are CO_2, N_2O, and CH_4. It's widely accepted that N fertilizer application increases N_2O emissions from soils. However, little is known about N fertilizer impacts on CO_2 and CH_4 emissions, especially at different application timings. Therefore, the objective was to determine the impact of urea application timing on all three of these gases. In this study, urea was surface applied at two rates (0 and 224 kg urea-N/ha) in the early fall (Sept. 21 - Oct. 11, 2017), mid-fall (Oct. 11 - Nov. 1), early winter (Nov. 1 – Nov. 15), early spring (May 1-22, 2018), mid-spring (May 22 – Jun. 12), and early summer (Jun. 12 – Jul. 4). Emissions were measured near-continuously with an automated system. There was a significant N fertilizer x application date (p=0.01) interaction for CO_2 emissions. This interaction occurred because in early spring, the fertilized treatment emitted 35% more CO_2 [109,433 g CO_2 $(ha\times d)^{-1}$] than the unfertilized treatment [80,976 g CO_2 $(ha\times d)^{-1}$] during the 21 d following application. During mid-spring, the opposite occurred, and N fertilizer reduced CO_2 emissions by 19% (p=0.06) compared to unfertilized soil. The soil was a CH_4 sink for all six application dates, consuming CH_4 at a rate to offset N_2O emissions by 9.6% during mid-spring and early summer when N fertilizer wasn't applied and by 3.1% when N fertilizer was applied. It was a stronger CH_4 sink during spring and early summer [averaging -85.8 g CO_2e $(ha\times d)^{-1}$] than during fall and early winter [averaging -46.3 g CO_2e $(ha\times d)^{-1}$]. N fertilizer had a negative impact on CH_4 consumption, reducing it by 20%, from -74.5 to -59.9 g CO_2e $(ha\times d)^{-1}$. These findings suggest that by delaying N fertilizer application 21 d in the spring, which

in eastern South Dakota would be from around the time of corn planting until shortly after corn emergence, may have large impacts on reducing CO_2 emissions. In addition, CH_4 consumption by soils can offset a portion of soil N_2O emissions that may be worth considering in analyses.

INTRODUCTION

In the 21st century, reducing the impact of agriculture on global warming will involve the wide scale adoption of science-based climate smart practices. These climate smart practices have the goal of increasing productivity and resilience, while reducing greenhouse gas (GHG) emissions. However, practices that improve productivity may have opposite impacts on GHG emissions. For example, increasing the N rate from 0 to an optimum level generally increases productivity and N_2O emissions. Similar responses can occur when N fertilizer is split applied (Venterea and Coulter, 2015, 2016). Therefore, practices designed to improve productivity need to be tested for their impact on CO_2, N_2O, and CH_4 emissions. Of these gases, CH_4 is the least understood.

Methane is an important GHG that is produced during crop production, and its concentration in the atmosphere has increased 142% since the pre-industrial era. It is estimated that approximately 60% of the global methane emissions are linked to ruminant livestock and rice production (Karakurt et al., 2012; Saunois et al., 2016). Methane is also emitted by natural sources, such as wetland soils, where anaerobic methanogens thrive. Methane has a global warming potential (GWP) value of 25, meaning that 1 kg of CH_4 causes 25 times as much warming over a 100-year period as 1 kg of CO_2.

Prior research is inconclusive on whether dryland cropping systems are a source (increase atmospheric CH_4) or sink (reduce atmospheric CH_4) of methane. Ussiri et al. (2009) reported that in an Ohio dryland continuous corn system, no-till soils were a net methane sink, whereas tilled soils were a methane source. Xiangyin and Groffman (2018) reported that Northeastern U.S. forests can be important sinks, however due to increasing rainfall, the strength of sink has been decreasing. In the United Kingdom and Ireland, Cowan et al. (2020) reported that agricultural soils are a source of CH_4. However, this interpretation may be based on the experiment containing cattle slurry, food waste digestate, and feces and urine treatments. They reported that each treatment type had a different emission rate where some treatments were sources and others were sinks. For example, at their Boghall site that used cattle slurry, CH_4 emissions were 0.05 nmol $(m^2 \times s)^{-1}$, whereas at Lincolnshire where fertilizer was not applied, the soil had a consumption rate of 0.04 nmol $(m^2 \times s)^{-1}$.

Differences between the study sites may be related to impact of treatment on soil inorganic N. Aronson and Helliker (2010) concluded that because the oxidation reactions of CH_4 to CO_2 and NH_4 to NO_3^- compete for the same active sites on the methane monooxygenase (MMO) and ammonia monooxygenase (AMO) enzymes, treatments that add NH_4 can reduce the strength of the sink (Figure 2.1). To the best of our knowledge, research has not investigated the impact of N fertilizer application timing on CH_4 flux. This is an important question because if the primary CH_4 removal mechanism is microbial oxidation, then changes in microbial respiration or NH_4 concentrations can affect the flux rate. Therefore, the objective of this research was to determine the impact of N fertilizer application timing on CH_4 emissions.

MATERIALS AND METHODS

Details on the field experiment are reported in Thies et al. (2019, 2020) and are summarized below. The study site was near Aurora, South Dakota (44º 18’ 20.57” N, 96º 40’ 14.04” W), and was located on the border between the Bsh (semi-arid) and DFa (continental wet all seasons) Köppen climate groups. The soil was a well-drained Brandt silty clay loam (Fine-silty, mixed, superactive, frigid Calcic Hapludolls) (Soil Survey Staff, 2018) on a 0 to 2% slope with parent materials of loess (0-60 cm) over glacial outwash. It 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) in the surface 15 cm. 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).

Site Preparation and Sampling Intervals

Soybean (*Glycine max*) were planted at the site immediately prior to this study and N 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 September 14, 2017. The experiment was conducted during six different seasons (Table 2.1), with each season lasting 21 days, except for early winter 2017, which ended after 14 days due to soil freezing. To start each season, eight experimental units, which were 314 cm^2 PVC rings, were inserted 5 cm into the soil. All of the rings were within 2 m of each other during a given season and the experimental units were kept weed free. The experiment contained two N rates (0 and 224 kg N ha^{-1}), where the fertilizer was urea (46-0-0) dissolved in 10 mL of water and sprinkled onto the soil surface within each ring. The no urea treatment received the same amount of water, but without dissolved urea. This application was made on the first date of each season. For each subsequent season, the rings were moved to another location within the trial site.

GHG measurements

At the start of each season, a LI-COR LI-8100-104 long-term opaque chamber (8100-104 LI-COR, Lincoln, NE) was placed over each of the eight rings. Each chamber was programmed to pivot over the top and enclose its respective ring for 15 min. once every 4 hours. All eight chambers were connected to a central Picarro® Cavity Ringdown Spectrometer (model G2508; Picarro Inc., Santa Clara, CA) that measured CH_4, N_2O, CO_2, and NH_3 concentrations inside the chamber headspace during each 15 min. sampling period, where one measurement per second was recorded for a total of 900 measurements. Sampling periods were from: 0000 to 0230 h, 0400 to 0630 h, 0800 to 1030 h, 1200 to 1430 h, 1600 to 1830 h, and 2000 to 2230 h. This allowed for sampling of the average (0930 to 1030 h), minimum (0530 to 0630 h), and maximum air temperatures (1330 to 1430 h) during each day (Thies et al., 2019). The original chamber sampling order was selected at random but remained constant for each application date. The gas within the chamber was mixed and a vent equalized the chamber and atmospheric pressures. Corrections were then made to each individual chamber to account for air volume differences. Flux values were calculated using version 4.01 LI-COR SoilFluxPro™ software (v. 4.01; LICOR, Lincoln, NE). For example, the CH_4 flux was a balance between production and consumption by the soil. Positive flux values indicate the soil was net CH_4 source, whereas negative flux values show the soil was a net CH_4 sink.

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. These were taken from two different areas adjacent to the chambers. During the experiments, power outages or machine failures resulted in two short gaps in two datasets (Sept 21 to 22 and May 1 to May 2). Missing information was replaced with time-appropriate information collected from each chamber.

Soil Sampling

To avoid soil sampling inside the treatment rings during each measurement period, an adjacent one m^2 area was fertilized with 200 kg N ha^{-1} to serve this purpose. Urea was applied to this square on the same date that was applied inside the rings. Each soil sample consisted of eight cores from the surface 15 cm were collected on the first day of each season from both inside (treated) and outside (untreated) the fertilized squares. Samples also were collected during the middle of each season and at the end of the season. Samples were dried, ground, sieved and analyzed for NH_4^+ -N and NO_3^{-1}–N (Clay et al., 2005; Kim et al., 2008). The NH_4^+ and NO_3^- values from these three sampling dates were averaged for each season.

The soil bulk density of the surface 15 cm was 1.29 and 1.34 g cm^3 for fall/early winter 2017 and spring/early summer 2018, respectively. Bulk densities and volumetric water contents were used to calculate the water filled pore space, using the assumption that soil particle density was 2.65 g cm^{-3}. The bulk densities were then used to convert the soil gravimetric values to volumetric values.

Soil samples (0- to 15-cm) for microbial community assessment were collected on 11 Oct. 2017 and 12 June 2018. Sampling and storage methods were outlined in Veum et al. (2019). Phospholipid Fatty Acid (PFLA) analysis was conducted by WARD Laboratories, Inc. (WARD Labs Inc, Kearney, NE) that used a modification of Hamel et al. (2006). Details of this modification are provided in Thies et al. (2019, 2020).

Data Analysis

CH_4 was converted to CO_{2e} by multiplying g CH_4 m^{-2} by 27.9 and N_2O emissions were converted to CO_2e by multiplying g N_2O m^{-2} by 273. Total daily GHG emissions were the sum of the CO_2, CH_4, and N_2O values (all expressed in CO_2e) for each day from each chamber. Statistical analysis was conducted using the Agricolae package in R Studio (R Core Team, 2023). For Analysis of Variance, the average daily emission value from each chamber for a given application timing was analyzed using the aov function in R Studio (Posit team, 2023). Post hoc analysis was conducted was conducted using the Duncan test in the Agricolae Package of R Studio (de Mendiburu, 2023). When p-values were <0.05, means were considered significantly different.

For modeling, separate analyses were conducted for fall 2017 and for spring/early summer 2018. Each data point was the mean daily from the four measurement chambers for an N fertilizer treatment (i.e. September 21, 2017 fertilized with urea). For 2017, there were 84 datapoints (21 days for early fall + 21 days for mid-fall x 2 fertilizer treatments per day). Early winter was not included because there wasn't soil sampling data to accompany the GHG data. In addition, early winter only lasted 14 days. For 2018, there were 126 datapoints (21 days for early spring + 21 days for mid-spring + 21 days for early summer x 2 fertilizer treatments per day). Since soil wasn't sampled every day, the missing values between sampling dates were estimated by filling in values in a linear fashion. Correlation analysis was used to determine the relationship among the measured values. Forward stepwise multiple regression was used to create a model using the OLSRR package in R Studio (Hebbali, 2020).

RESULTS AND DISCUSSION

In the fall, soil and air temperatures decreased from 21 Sept 2017 to 15 November 2017 and increased the following spring from 1 May 2018 to 4 July 2018 (Table 2.1). These changes are expected in the Northern U.S. climates of this study site. Soil moisture was dependent on rainfall that varied during the study. Weather was relatively normal, except for the Early Fall 2017 fertilizer application, which was exceptionally wet, with 17.7 cm of precipitation that resulted in soil WFPS >0.85 cm^{-1} cm^{-1} for all three weeks following fertilizer application.

Application Date x N Fertilizer Interaction for CO_2 Emissions

An application date x N fertilizer interaction was observed for CO_2 emissions (Table 2.2). This interaction occurred because N fertilizer application increased CO_2 emissions by 35% compared to unfertilized soil during early spring, which is normally around the time of corn planting. N fertilizer, however, decreased CO_2 emissions by 19% (p=0.06) during mid-spring, which is normally between VE and V4 growth stages. This CO_2 emission decrease (23%) continued into early summer, which is normally during sidedress N application between the V4 and V10 growth stages. At the other application dates, N fertilizer did not significantly affect CO_2 emissions.

Table 2.1 Average 7-d soil temperatures, soil moisture, water filled pore space (WFPS) contents for the surface 5 cm and amounts of rainfall by week and totals over the 21 d. The time intervals shown are 0 to 7, 8 to 14, and 15 to 21 d after fertilizer application. The values in parenthesis for volumetric soil moisture represent the range in values over each study period. This is a modified table from Theis et al. (2020).

Season	**Date Range**	**Soil Temp.**	**Volumetric soil moisture**	**WFPS**	**Precip.**
		˚C	$cm^3\ cm^{-3}$	$cm^3\ cm^{-3}$	cm
Early fall 2017	Sept. 21 - 27	17.5	0.44 (0.12-0.52)	0.857	11
	Sept. 28 – Oct. 4	15.2	0.45 (0.36-0.51)	0.877	6.5
	Oct. 5 – 11	11.8	0.45 (0.34-0.49)	0.877	0.2
	Average	**14.8**	**0.45**	**0.870**	**17.7**
Mid-fall 2017	Oct. 11 - 18	10.9	0.32 (0.28-0.39)	0.624	0.3
	Oct. 19 - 25	12.2	0.32 (0.25-0.45)	0.643	0
	Oct. 26 – Nov. 1	4.0	0.28 (0.18-0.45)	0.643	0.4
	Average	**9.0**	**0.31**	**0.637**	**0.7**
Early winter 2017	Nov. 1 - 8	3.3	0.24 (0.23-0.26)	0.468	0.7
	Nov. 9 - 15	2.2	0.24 (0.17-0.32)	0.468	0
	Average	**2.8**	**0.24**	**0.468**	**0.7**
Early spring 2018	May 1 - 8	12.6	0.37 (0.36-0.41)	0.749	1.3
	May 9 - 15	10.4	0.45 (0.28-0.41)	0.91	2.3
	May 16 - 22	14.4	0.33 (0.30-0.42)	0.668	0
	Average	**12.5**	**0.38**	**0.776**	**3.6**
Mid-spring 2018	May 22 - 29	21.9	0.27 (0.20-0.33)	0.546	0.3
	May 30 – Jun. 5	18.9	0.27 (0.19-0.32)	0.546	1.2
	Jun. 6 - 12	18.5	0.26 (0.19-0.34)	0.526	0.9
	Average	**19.8**	**0.27**	**0.539**	**2.4**
Early summer 2018	Jun. 12 - 19	21.1	0.29 (0.22-0.37)	0.546	2.3
	Jun. 20 - 27	18.9	0.34 (0.12-0.56)	0.465	4.2
	Jun. 28 – Jul. 4	21.0	0.36 (0.26-0.68)	0.607	3.8
	Average	**20.3**	**0.33**	**0.539**	**10.3**

One possible explanation for the reduced CO_2 emissions during mid-spring and early summer was microbial inhibition by acidity generated through nitrification. Chen et al. (2016) also reported a reduction in CO_2 emissions in response to N fertilization. In their study, they applied either H_2SO_4 or NH_4NO_3 and saw a downward trend in soil respiration and pH from each of these products that almost paralleled one another. By applying a product that did not contain N, such as H_2SO_4, they were able to isolate whether the reduction in CO_2 emissions from the NH_4NO_3 addition was due to acidification or some other factor related to N. In our study, it might seem appropriate to attribute the N-induced reductions in CO_2 emissions during mid-spring and early summer to acidity generated from nitrification. However, N application during early spring also stimulated similar amounts of nitrification as mid-spring and early summer (Figure 2.1), which should have generated acidity to lower CO_2 emissions. However, CO_2 emissions increased during early spring. One possible explanation for this increase was that soil NH_4^+ levels were usually less than half that of soil NO_3^- levels (Figure 2.1) during early spring. This was a very different trend than what was observed in mid-spring and early summer, where soil NH_4^+ was usually greater than or similar to NO_3^-. These levels suggest that a substantial amount of N from the urea applied in early spring became rapidly consumed by CO_2-producing microbes that were N-limited at the time. Choi et al. (2011) reported a 127% increase in CO_2 emissions from urea over ammonium nitrate and concluded that applying N fertilizers with higher NO_3^- concentration could reduce CO_2 emissions from soils. The fact that N fertilizer application may actually reduce CO_2 emissions sounds appealing. However, it's important to note that the 224 kg N ha^{-1} applied in our study was an experimental rate and was about 33% higher than what a

South Dakota farmer would normally apply to corn.

During spring and early summer, CO_2 emissions were statistically similar and were 53% higher than early fall (Table 2.3). This was also reflected in soil microbial biomass measurements, which were 3510 mg/kg on June 10, 2018, but only 2360 mg/kg on 11 October 2017 (Thies et al., 2019). Early spring and early fall are an interesting comparison because despite having very different net GHG emissions, they both had similar soil temperatures (Table 2.1). Soil freezing and thawing prior to early spring may have been a contributing factor, as these cycles have been shown to lyse microbial cells that subsequently release dissolved organic carbon (DOC) into the soil (Christensen and Christensen, 1991) for microorganisms to readily consume and subsequently respire CO_2.

Application Date x N Fertilizer Interaction for N_20 Emission Offset by CH_4 Consumption

For all application dates, there was a reduction in the CH_4 concentration in the measurement chambers, as indicated by all negative values (Table 2.2). This suggests that methanotrophic bacteria in the soil were consuming more CH_4 than methanogenic bacteria in the soil were emitting. There was a significant interaction between N fertilizer and its application date on the percentage of N_2O emissions that were offset by CH_4 consumption (Table 2.2). Since early winter had very little N_2O emissions compared to the other application dates, the 40% offset by CH_4 consumption (not shown in Table 2.2) was misleading and negated this interaction, giving it a p-value of 0.55 (not shown in Table 2.2) instead of 0.01. Therefore, early winter was omitted from the analysis of this interaction. The interaction was mostly due to the amount of denitrification that occurred following the various application dates. For example, early fall and early spring both had the highest amounts of denitrification whether the soil was fertilized or not. Therefore, CH_4 consumption only offset N_2O emissions by 0.9% and 2.3% during early fall and early spring, respectively (Table 2.3). During mid-spring and early summer, N_2O emissions averaged only 39% of those of during early fall and early spring. These lower N_2O emissions during mid-spring and early summer allowed CH_4 consumption to offset N_2O emissions by 9.6% when N fertilizer wasn't applied and by 3.1% when fertilizer was applied. This average offset of 6.4% for mid-spring and early summer simulates a common cropping system in eastern SD where farmers grow approximately half of their acres as corn and approximately half as soybeans, and the corn receives N fertilizer, but the soybeans do not.

N Fertilizer Effect on CH_4 Consumption

The reduction in CH_4 consumption for the N fertilized treatment during mid-spring and early summer may have been due to increased soil NH_4^+ levels during this time. Methanotrophic bacteria need the methane monooxygenase (MMO) enzyme to catalyze the oxidation of CH_4 to CO_2 (Figure 2.2). If soil NH_4^+ levels are high, nitrifiers, which primarily use the ammonium monooxygenase (AMO) enzyme for nitrification, can also use the MMO enzyme to oxidize NH_4^+ to NO_3^-. This makes MMO and AMO less-available to methanotrophic bacteria to oxidize CH_4 to CO_2. Aronson and Helliker (2010) published a meta-analysis of 33 different studies involving N fertilizer applied to aerated soils and concluded that at 100 kg N ha^{-1} yr^{-1}, nitrifiers become the dominant user of these enzymes over methanotrophs. Their results generally agree with ours, as we observed reductions in CH_4 consumption during mid-spring and early summer when soil

NH_4^+ levels were close to 100 kg ha^{-1} (Figure 2.1). In early spring, when N fertilizer did not reduce CH_4 consumption, soil NH_4^+ levels were <50 kg ha^{-1}.

N Application Timing Effect on CH_4 Consumption

CH_4 consumption was 84% greater (more negative) during spring and early summer than during fall and early winter, averaging -85.8 and -46.6 g CO_2e $(ha \times h)^{-1}$, respectively. Increased dissolved organic carbon (DOC) levels during spring and early summer may have caused this increase in CH_4 consumption. Although we did not measure DOC, Sullivan et al. (2013) reported a strong relationship (r=0.76) between CH_4 consumption and DOC. They also found isotopically labeled glucose in methanotroph cells, indicating that the methanotrophs utilized DOC in addition to CH_4. Prior to their work, it was widely accepted that methanotrophs only consumed CH_4. The consistently high soil moisture levels of early fall, which were >0.85 WFPS for all three weeks (Table 2.1), may have also contributed to reduced CH_4 consumption relative to early spring. In incubation experiments under a series of soil moisture conditions, van den Pol-van Dasselaar et al. (1998) reported that when soil moisture contents exceeded greater than 50% (w/w), CH_4 consumption rapidly declined. The optimum range for CH_4 consumption was 20-35% (w/w) soil moisture.

Table 2.2. Interaction between fertilizer application timing and N rate on greenhouse gas emissions.

Application date	N rate	†CO_2	†N_2O	CH_4	‡N_20 Offset
	kg N/ha	------------g CO_{2e}/(ha d)--------			%
Early fall	0	66,578	4,901	-48.5	0.9
Early fall	224	61,816	5,810	-35.0	0.6
p-value		0.67	0.50	0.40	0.96
Mid fall	0	42,554	1,366	-45.1	4.2
Mid fall	224	49,482	1,908	-54.3	3.0
p-value		0.53	0.67	0.57	0.13
Early winter	0	16,963	120	-51.3	
Early winter	224	30,966	120	-45.5	
p-value		0.31	0.99	0.80	
Early spring	0	80,998	5,403	-93.6	2.3
Early spring	224	109,463	6,539	-94.9	1.4
p-value		**0.01**	0.40	0.94	0.53
Mid spring	0	111,059	987	-107.0	9.9
Mid spring	224	89,777	2,294	-64.6	4.5
p-value		**0.06**	0.35	**0.01**	**0.06**
Early summer	0	112,303	958	-90.1	9.2
Early summer	224	86,536	3773	-64.9	1.7
p-value		**0.03**	**0.05**	0.12	**0.01**
Interaction p-value		**0.01**	0.75	0.25	**0.01**

†Reported in Thies et al. (2019).
‡Percent reduction in N_2O emissions offset by CH_4 consumption.
§Values within the same column followed by a different letter are significantly different at $p<0.05$.

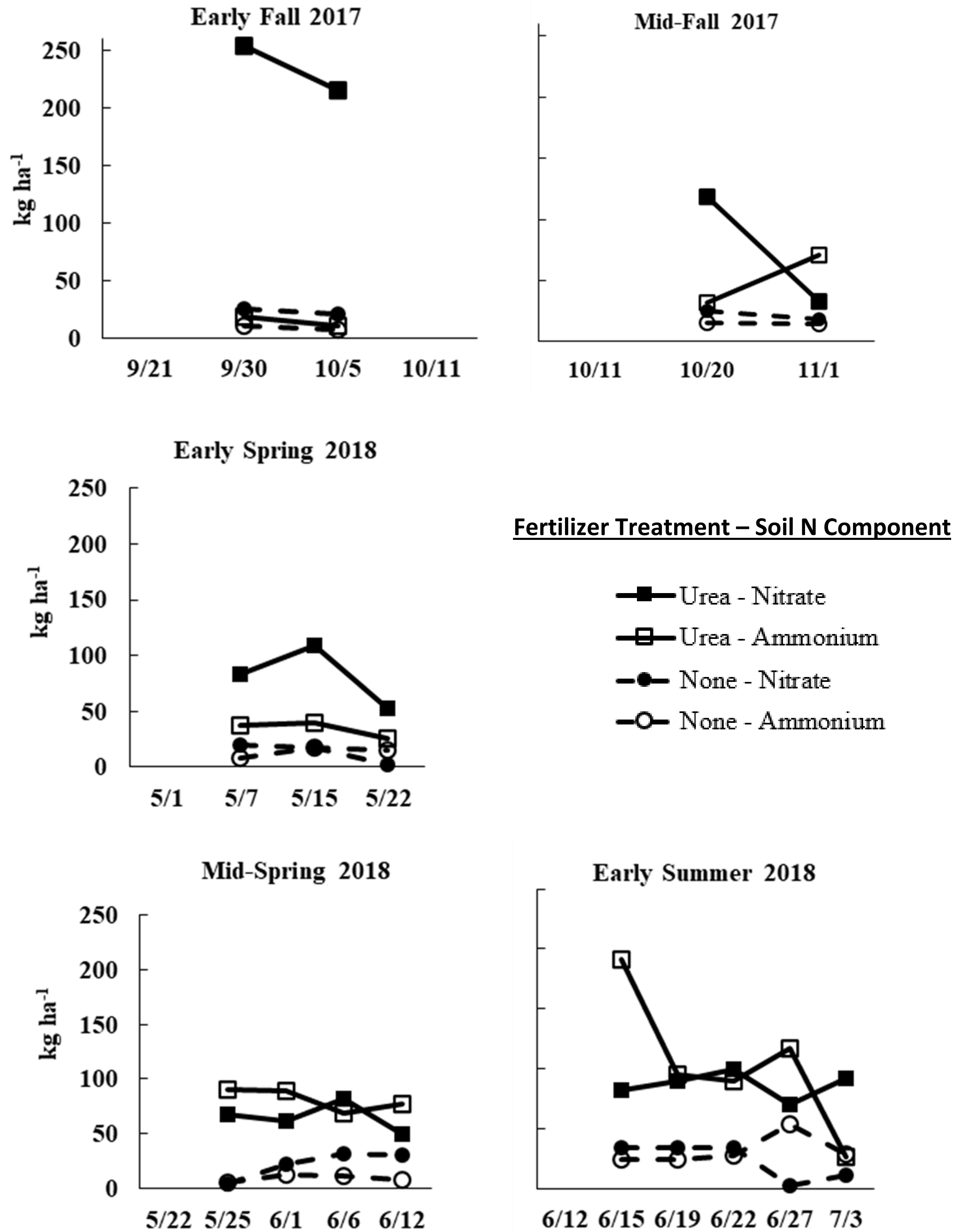


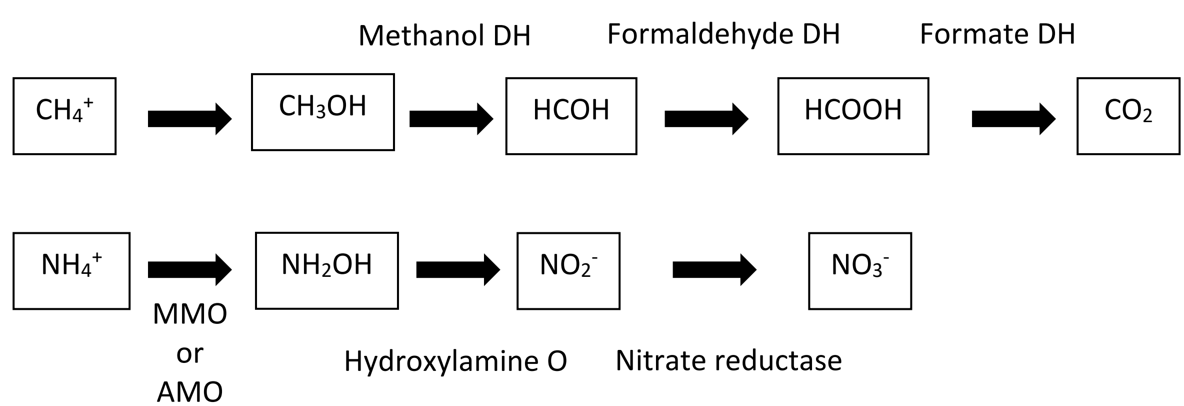
Figure 2.1. Soil inorganic N levels (15 cm depth) for all N fertilization application timings. The application date is the first date listed on the x-axis.

Table 2.3. Main effects of fertilizer application timing and N rate on greenhouse gas emissions.

Application date	N rate	CO_2	N_2O	CH_4	‡N_20 Reduction
	kg N/ha	---------g CO_{2e}/(ha d)---------			%
Early Fall		64,197 b§	4,902 a	-41.8 b	0.9 c
Mid-fall		46,018 c	1,498 b	-49.7 b	4.4 bc
Early winter		26,299 d	164 b	-47.5 b	
Early spring		95,230 a	5,465 a	-94.2 a	2.3 c
Mid-spring		100,418 a	1,501 b	-85.8 a	8.8 a
Early summer		99,419 a	2,165 b	-77.5 a	6.6 ab
p value		**<0.01**	**<0.01**	**<0.01**	**<0.01**
	0	76,188	2486	-74.5	6.5
	224	69,601	3407	-59.9	2.7
p-value		†0.16	†0.08	**0.03**	0.01

†Reported in Thies et al. (2019).
‡Percent reduction in N_2O emissions offset by CH_4 consumption.
§Values within the same column followed by a different letter are significantly different at $p<0.05$.

Figure 2.2. A simplified version of the CH_4 to CO_2 and the NH_4^+ to NO_3^- oxidation reactions and the enzymes involved. Both compounds compete for the methane monooxygenase (MMO) or the ammonium monooxygenase (AMO) enzymes, meaning that high NH_4^+ concentrations can reduce the enzymes availability for methane oxidation. (modified from Topp and Pattey, 1997)



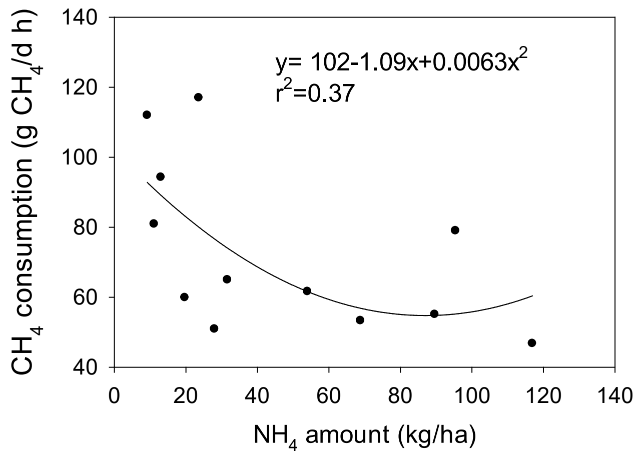


Figure 2.3. The relationship between CH_4 consumption and soil NH_4 amounts during the mid spring and early summer.

Modeling

Since increased CH_4 consumption was indicated by increasingly negative CH_4 flux values, a positive relationship between CH_4 consumption and other variables were defined by a negative correlation coefficient. For example, CH_4 consumption had a negative relationship (i.e. positive correlation coefficient) with soil NO_3^- and NH_4^+ during spring/early summer, but not during fall (Table 2.4). CH_4 consumption also had a negative relationship with WFPS, but during both seasons. The one variable that CH_4 consumption had a positive relationship with was CO_2 emissions, which occurred during both seasons. This can be explained by the fact that aerated soils consume CH_4 and respire CO_2. In addition, it might seem appropriate to attribute some of this relationship to the CO_2 produced from the CH_4 consumption (Figure 2.2). However, the amount of CH_4 oxidized was typically <0.1% of the total CO_2 emitted. Therefore, the contribution of CH_4 oxidation to CO_2 emissions was relatively small. For multiple regression, CO_2 emissions and WFPS explained 73.6% of the variation in CH_4 consumption during fall (Table 2.5). Multiple regression wasn't as powerful during the spring / early summer, as

CO_2 emissions, soil NH_4^+, WFPS, and soil temperature explained 57% of the variation in CH_4 consumption. Adding N_2O emissions slightly improved the model to explaining 59% of the variation.

CO_2 emissions were more straightforward than CH_4 consumption, where a positive relationship between CO_2 and another variable was indicated by a positive correlation coefficient. CO_2 had a positive relationship with soil temperature during both fall and spring / early summer (Table 2.4). CO_2 had a negative relationship with soil NH_4^+ during both fall and spring/early summer, but only with soil NO_3^- and during spring/early summer. For multiple regression, the model included CH_4 flux, WFPS, soil temperature, and NH_3 flux for both the fall and spring/early summer seasons (Table 2.5). Similar to the CH_4 model, the CO_2 model explained the variation fall data far more effectively than spring/early summer data, at 77 and 34%, respectively.

Table 2.4. Correlation coefficients between soil temperature, CH_4 consumption, CO_2 emissions, N_2O emissions, NH_3 emissions, soil NO_3^-, and soil NH_4^+. This includes all data except for early winter 2017. Values in bold font are significant at $p<0.05$.

	WFPS	Soil Temp.	CH_4	CO_2	N_2O	NH_3	Soil NO_3^-
Fall							
Soil Temp.	**0.49**						
CH_4	**0.31**	-0.10					
CO_2	0.13	**0.48**	**-0.76**				
N_2O	**0.69**	**0.35**	-0.01	**0.31**			
NH_3	-0.13	0.00	-0.21	**0.30**	-0.09		
Soil NO_3^-	**0.30**	0.16	0.19	0.00	**0.44**	0.18	
Soil NH_4^+	**-0.59**	**-0.33**	-0.03	**-0.25**	**-0.51**	0.12	0.02
Spring / E. Sum.							
Soil Temp.	**-0.76**						
CH_4	**0.25**	0.05					
CO_2	**-0.20**	**0.21**	**-0.50**				
N_2O	**0.63**	**-0.59**	0.09	-0.14			
NH_3	0.01	0.02	0.13	0.09	0.12		
Soil NO_3^-	0.05	-0.09	**0.28**	**-0.25**	**0.44**	0.14	
Soil NH_4^+	**-0.38**	**0.31**	**0.43**	**-0.26**	0.05	**0.22**	**0.63**

Table 2.5. Forward stepwise regression model between CH_4 removal and CO_2 emissions, water-filled pore space (WFPS), soil NH_4^+ (15 cm depth), soil temperature (5 cm depth), and N_2O emissions.

	Factors(s)	**Intercept**	**Slope**	**Adj. R^2**
Fall				
		-12.4		
	CO_2		$-6.23e^{-4}$	0.568
	CO_2 + [a]WFPS		14.6	0.736
Spring / E. Sum.				
		-45.7		
	CO_2		$-4.47e^{-4}$	0.24
	$CO_2 + NH_4^+$		0.0737	0.36
	$CO_2 + NH_4^+$ + WFPS		42.4	0.42
	$CO_2 + NH_4^+$ + WFPS + [b]ST		0.659	0.57
	$CO_2 + NH_4^+$ + WFPS + ST + N_2O		$-2.68e^{-3}$	0.59

Abbreviations: [a]percent water-filled pore space, [b]soil temperature.

Table 2.6. Forward stepwise regression model between CO_2 emissions and water-filled pore space (WFPS), soil temperature (5 cm depth), and NH_3 emissions.

	Factors(s)	**Intercept**	**Slope**	**Adj. R^2**
Fall				
		-6031		
	CH_4		-982	0.57
	CH_4 + [a]WFPS		6890	0.71
	CH_4 + WFPS + [b]ST		355	0.75
	CH_4 + WFPS + ST + NH_3		330	0.77
Spring / E. Sum.				
	Intercept	-6434		
	CH_4		-513	0.24
	CH_4 + [a]WFPS		11401	0.29
	CH_4 + WFPS + [b]ST		510	0.32
	CH_4 + WFPS + ST + NH_3		24.6	0.34

Abbreviations: [a]percent water-filled pore space, [b]soil temperature.

PRACTICAL IMPLICATIONS

Farmers are not likely to change their N application timing based on reduced GHG emissions, but the results of this trial help us better understand a system. In our study, we found that applying N fertilizer during early spring around the time of corn planting increased total CO_2 emissions by 35%. CO_2 emissions were decreased by 19% when N application was delayed by three weeks until around the time of corn emergence (i.e. mid-spring). Brugler et al. (2024) also reported that N fertilizer application timing impacted CO_2 emissions. In their study, they addressed many of this study's limitations by: 1) applying a more-normal N rate for SD corn production (157 versus 200 kg N ha^{-1}), 2) including a split application (78.5 + 78.5 kg N ha^{-1}), and 3) monitoring emissions for an entire growing season as opposed to just 21 days following application. Their work was conducted on the same research farm on the same soil type. In their findings, they reported no response in CO_2 emissions to N fertilizer during 2021. However, in 2022, CO_2 emissions were increased by 548 and 348% in the single and split application treatments (over no N), respectively. Results of our study and that of Brugler et al. (2024) show that additional studies are needed to confirm whether N application can lower CO_2 emissions at certain times of year. It's also likely that poorly drained soils and long-term no-till soils may have different results.

In this study, we also found that the soil had net CH_4 consumption during all times measured, but spring and early summer had 84% more CH_4 consumption than fall and early winter. However, the amount of CH_4 consumed by the soil during spring/early summer only reduced total GHG emissions by 0.08% because CO_2 made up 96.8% of the total GHG emissions. It's important to note that our study was conducted on bare soil without the addition of decomposing crop residues or growing plants. On this same soil type on this same research farm, Clay et al. (2015) reported that decomposing corn residues can offset all CO_2 emissions from a corn field during a growing season and add additional C to the soil. In situations such as these, where all the annual CO_2 emissions are negated by C sequestration, the remaining GHGs to account for are N_2O and CH_4. When this is the case, CH_4 consumption becomes more relevant. In North America, many farms have a corn and soybean annual crop rotation. N fertilizer is rarely applied during the soybean year. Our analysis suggests that in the soybean year, 9-10% of the N_2O emissions could be offset by CH_4 consumption during mid-spring and early summer (Table 2.3). Therefore, CH_4 consumption may be more important to GHG fluxes from croplands than others have recognized. N fertilizer is normally applied during the corn growing year. In this study, we found N fertilizer to reduce CH_4 consumption by 20% when averaged across all application dates.

One cropping system where CH_4 flux is not ignored is rice production. In this system, the soil can be flooded for a portion of the growing season. Weiler et al. (2018) reported that the DayCent model underestimated rice yield and overestimated CH_4 flux by 0.43 kg/(ha day)$^{-1}$. Gou et al. (2023) had slightly different results and explained between 58 to 63% of the daily CH_4 fluxes and reported that models could be improved by accounting for tillage changes.

To date, few mitigation strategies are available to reduce CH_4 emissions by promoting CH_4 consumption. For example, table 8.3 in Smith et al. (2007) identified that in cropland mitigation, strategies and calculation approaches exist for rice production and that minimal strategies exist for integrating methane consumption into the CO_{2e} values for agricultural products. As shown by this research, when and how much N fertilizer is applied impacts the calculated values. In animal agriculture, USDA is focused on reducing CH_4 emissions by installing lagoon covers and/or anaerobic methane digesters that collect methane for use or destruction; installing solid separators that reduce methane-producing slurries; providing conservation assistance for transitions to alternative manure management systems, such as deep pits, composting, transitions to pasture, or other practices that have a lower greenhouse gas profile; and supporting rice management that reduces methane emissions, such as alternate wetting and drying. What is missing from IPCC and USDA is the acceptance that soil can help mitigate atmospheric CH_4 (Smith et al., 2007; White House, 2021).

CHAPTER 3: WINTER CEREAL RYE COVER CROP DECREASED NITROUS OXIDE EMISSIONS DURING EARLY SPRING

Abstract

Despite differences between the cover crop growth and decomposition phases, few greenhouse gas (GHG) studies have separated these phases from each other. This study's hypothesis was that a living cover crop reduces soil inorganic N concentrations and soil water, thereby reducing N_2O emissions. We quantified the effects of a fall-planted living cereal rye (*Secale cereale*) cover crop (2017, 2018, 2019) on the following spring's soil temperature, soil water, water filled porosity (WFP), inorganic N, and GHG (N_2O-N and CO_2-C) emissions and compared these measurements to bare soil. The experimental design was a randomized complete block, where years were treated as blocks. Rye was fall planted in 2017, 2018, and 2019, but mostly emerged in the following spring. GHG emissions were near-continuously measured from early spring through June. Rye biomass was 1049, 428, and 2647 kg ha^{-1} in 2018, 2019, and 2020, respectively. Rye reduced WFP in the surface 5 cm by 29, 15, and 26% in 2018, 2019, and 2020 and reduced soil NO_3-N in surface 30 cm by 53% in 2019 ($p = 0.04$) and 65% in 2020 ($p=0.07$), respectively. Rye changed the N_2O and CO_2 frequency emission signatures. It also reduced N_2O emissions by 66% but did not influence CO_2-C emissions during the period prior to corn seed emergence (VE). After VE, rye and bare soils N_2O emissions were similar. These results suggest that to assess the influence of cover crops more precisely on seasonal N_2O-N emissions, sampling protocols must account for early season impacts of the living cover.

Abbreviations: greenhouse gas, GHG; nitrous oxide, N_2O-N; water-filled porosity, WFP; carbon dioxide, CO_2;

INTRODUCTION

Cover crops can have many positive effects on soil health (Smeltekop et al., 2002; SARE, 2007), and mixed impacts on GHG emissions (Basche et al., 2016; Çerçioğlu et al., 2019; Antosh et al., 2020) and soil productivity (Bich et al., 2014; Reese et al., 2014). The mixed effect of cover crops on GHG emissions is difficult to assess because early season emissions are often under sampled and many experiments do not provide critical information, such as bulk density, NO_3-N and NH_4-N concentrations, and soil water contents (Mitchell et al., 2013; Ruis et al., 2018; Wegner et al., 2018; Sanz-Cobena, et al., 2014; Abdalla et al., 2014).

Interpreting conflicting results for land managers can result in mixed messages that slows conservation practice adoption (Wang et al., 2020). We can start improving our understanding on how, when, and why cover crops affect N_2O emissions by separating the growing season into two distinct phases, growth and decomposition. During cover crop growth, nutrients are scavenged from soil and water are transpired, whereas during decomposition, nutrients are returned, and the cover crop mulch can reduce evaporation. The stark differences between growth and decomposition may partially explain the mixed impacts of cover crops on GHG emissions (Shan & Yan, 2013; Seiz et al., 2019; Johnson & Barbour, 2019; Nielsen et al., 2015). However, this explanation cannot be confirmed because little research has been conducted exclusively during the cover crop growth phase (Basche et al., 2017). Therefore, this study quantified the influence of an unfertilized growing rye cover crop on soil temperatures, soil moisture, inorganic N, the N_2O frequency emission signatures, and total N_2O-N and

CO_2-C emissions in a well-drained frigid soil from the start of growth in April/May through termination in late June.

MATERIALS AND METHODS

Experimental Design and Treatments

Rye was planted in the fall of 2017, 2018, and 2019 in field studies conducted near Aurora, South Dakota (44º 18’ 20.57” N, 96º 40’ 14.04” W). The site was located on the border between the Bsh (semi-arid) and DFa (continental wet all seasons) Köppen climate groups and the soil had a frigid temperature regime. The soil at the site was a Brandt silty clay loam (a fine-silty, mixed, superactive frigid Calcic Hapludoll), and 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%) of soil organic carbon (SOC). The no-tillage first-order rate constant and half-life of SOC for this soil were 0.00675 kg (kg-C × year)$^{-1}$ and 103 years, respectively (Clay et al., 2015). The soil $pH_{water\ 1:1}$ was 5.8, and the soil parent materials were loess (0- to 60-cm) over glacial outwash. The surface soil hydraulic conductivity was 0.72 m d^{-1} and the slope was between 0 and 2%. Additional information about the study site is available in Thies et al. (2020). Rainfall was determined based on data collected at the site. Our study was not irrigated and following cover crop seeding it was not cultivated. Prior to the study, the long-term rotation was corn followed by soybeans (*Glycine max*).

Four experimental units, each consisting of a PVC pipe covering 317 cm^2, were driven into the soil to a depth of 14 cm with about 6.3 cm of the pipe extending above the soil surface. The pipes were spaced about 1.5 m apart. The surface 2.5 cm was cultivated in all four pipes and rye was hand-planted inside two of them chosen at random at 56 kg ha^{-1} (39,500 seeds kg^{-1} or 220 seeds m^{-2}) on October 20, 2017, October 16, 2018, and October 23, 2019. Planting depth was 2.5 cm. Fertilizer was not applied, residue cover was minimal, and all soils were exposed to the prevailing climatic conditions. Seed emergence was monitored in late November each year and 17, 15, and 36% of the planted seeds emerged in 2017, 2018, and 2019, respectively.

The following discussion is intended to provide a reference for the system that simulated GHG emissions prior to the emergence of the cash crop. Because a cash crop was not seeded into the study area, the changes in GHG emissions and soil properties were attributed to rye. In the region, corn is generally planted between the last week of April and the third week of May. However, the date varies, and it is based on the frost-free period, which is between May 13 and 14, soil temperatures, and moisture content. Cover crops and other weeds are generally killed prior to the critical weed free period of corn (from VE to V5). However, in some situations, cover crop control may be delayed or not conducted if conditions are not conducive for planting the cash crop. Under these conditions, the cover crop biomass can be harvested for other purposes.

The cover crop growth period was separated into three sampling intervals (Table 3.1). At the end of each interval, the rye was clipped near the soil surface to allow the chamber lid to close and to simulate grazing. During the first interval, rye emerged, and the interval ended prior to corn emergence (VE). For most of the farmers in the region, the cover crop growth would be terminated at the completion of this interval. Emergence dates were calculated by assuming the seed would not be planted until the risk of frost damage for corn was reduced (soil temperature > 10 C) and the soil moisture was less than 0.33 cm^3 cm^{-3}. A formula from Nleya, Chungu, & Kleinjan (2016) was used to calculate growing degree days (GDD) for corn (lower limit 10 and upper limit 30 °C). These authors also reported that approximately 51.7 GDD were needed for corn seeds to germinate and emerge.

During the 2nd interval, the cover crops continued to grow and based on accumulated growing degree days, corn plants at the end of the 2nd interval would have been between the V2 to V3 growth stages. In our region, cover crop growth through the 2nd interval would be considered delayed control and may be suitable for crops that are seeded later than corn, such as soybeans. The third interval ranged from V2 or V3 to V5 or V6 and probably would not be part of a corn or soybean production system.

Table 3.1. The relationship between the experiment events and corn growth periods. Clip 1 provides a reference for early season GHG emissions prior to corn emergence, whereas clips 2 and 3 provide a reference for delayed control, grazing or seeding.

Year	Reference period	Events	2018	days of sampling
2018	Start measurement		7-May	
	Prior to VE	clip 1	25-May	18
	VE to corn at V2	clip 2	15-Jun	21
	V2 to V5	clip 3	3-Jul	18
2019	Start measurement		26-Apr	
	Prior to VE	clip 1	13-May	17
	VE to corn at V2	clip 2	29-May	16
	V2 to V5	clip 3	24-Jun	26
2020	Start measurement		8-Apr	
	Prior to VE	clip 1	4-May	30
	VE to corn at V2	clip 2	31-May	27
	V2 to V5	clip 3	26-Jun	26

Carbon Dioxide and Nitrous Oxide Emissions

Greenhouse gas emissions measurements were initiated in the spring as soon as it was physically possible to set up measuring equipment in the field. LI-COR LI-8100-104 long-term opaque chambers (8100-104 LI-COR, Lincoln, NE) were used to measure emissions. Each of the four chambers covered an area of 317 cm^2. Prior to sampling, the cover pivots over the PVC pipe, creating an enclosed volume. Gas samples were collected for 15-minutes six times daily (between 0000 to 0230 h, 0400 to 0630 h, 0800 to 1030 h, 1200 to 1430 h, 1600 to 1830 h, and 2000 to 2230 h). At each gas sampling event, the chambers were sampled in a designated sequence, and corrections were applied to each individual chamber to account for air volume differences. During the individual sampling event, the gas within the chamber was mixed with a pump, a vent was used to equalize the chamber and atmospheric pressures, and thermistor measured the air temperature.

Gas drawn from the chamber was analyzed for N_2O-N and CO_2–C concentrations every second, for a total of 900 measurements, using a Picarro® Cavity Ringdown Spectrometer (model G2508; Picarro Inc., Santa Clara, CA). Based on each chamber's volume, N_2O-N emissions were calculated with data obtained between 45 to 900 seconds, whereas CO_2-C emissions were determined with data obtained between 45 and 165 seconds, both using 4.01 LI-COR SoilFluxPro™ software (v. 4.01; LICOR, Lincoln, NE). To assess accuracy, standard gases were used prior to and at the completion of all experiments. Adjacent to the chambers in an identically treated area, soil moisture and temperatures for the surface 5 cm were measured using LI-COR LI-8150-205 Soil

Moisture Probes (LI-COR, Lincoln, NE) and LI-COR LI-8150-203 Soil Temperature Probes (LI-COR, Lincoln, NE), respectively.

Emissions were measured from May 7 to July 3, 2018, April 25 to June 24, 2019, and from April 8, 2020 to June 26, 2020 (Table 3.1). When rye reached a height of 15 cm, plants were clipped to 3-cm height, which occurred three times each year. At each clipping date, rye biomass was dried, weighed, ground, and analyzed for total N and C using a stable isotope C and N analyzer (Clay et al., 2015).

Soil Sampling

In 2018, 2019, and 2020 soil samples from the 0- to 15- and 15- to 30-cm soil depths were collected with a 2-cm diameter soil probe. For each experimental unit, an area outside of the GHG chambers was sampled when GHG sampling was initiated (Table 3.1). When the study was completed, soil samples from within the chambers at the same depths were collected. 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- and 15- to 30- cm depths in 2018 were 1.33 and 1.32 g cm^{-3}, respectively. In 2019, the bulk densities for the 0- to 15- and 15- to 30- cm depths were 1.31 and 1.28 g cm^{-3}. In 2020, the bulk densities for those same depths were 1.33 and 1.29 g cm^{-3}. Based on the measured bulk densities and volumetric moisture contents, the percent water filled porosities were determined. This calculation assumed that the soil particle density was 2.65 g cm^{-3}. Soil samples were dried at 40°C, ground (<2mm) and analyzed for NH_4^+-N and NO_3^-–N (Clay et al., 2015).

Statistical Analysis

Based on N_2O-N and CO_2-C, 5400 measurements from each chamber over 24-hour period daily emissions were determined. Due the large number of measurements, we conducted an analysis to determine the replication requirements. This analysis is available in Thies et al. (2019). To demonstrate differences between the sampling systems we compared average daily emissions from samples collected between 9:30 and 10:30 with near continuous measurement. The variances, which were different at $p < 0.001$ for near continuous measurement and point sampling between 9:30 and 10:30 AM were 0.00768 and 0.0227, respectively. This analysis showed that the daily N_2O-N variances were reduced 300% by converting from point to near continuous measurements. If the replication requirement (n) was calculated with the equation, $n=(4s^2/B^2)$, where s^2 is the variance and B is the bound of the estimation error, then the measured variances decreases would have produced a corresponding decrease in the replication (n) requirement. Based on this analysis, the experimental protocol used in this experiment was designed and tested (Thies et al., 2020).

The experimental model was a randomized block design, where the 3 years were treated as blocks. Each treatment within a year was replicated twice. Years (i.e. blocks) and cover crop treatments were fixed effects. The model was years, treatments, and year by treatment interaction (R Core Team, 2023). Our hypothesis was that the growing rye plant reduced soil moisture and N_2O-N emissions and increased CO_2-C emissions.

A fast Fourier transform (FFT) was conducted on soil temperatures, N_2O-N, and CO_2-C emission to determine the FFT frequency signatures (Klingenberg, 2005). The FFT frequency signature is composed of frequencies each with a magnitude and is often used to assist in interpreting repeating complex data sets (Brummell et al., 2014; Krijnen et al., 2013). Each frequency represents a repeating function, and the magnitude provides information on the relative importance of that frequency. Frequencies with larger magnitudes explain more of the variability. To determine the relative importance of different frequency regions, the FFT were separated into two regions, 0.75 to 0.85 and 0.98 to 1.01 cycles d^{-1}. The average value of the magnitudes for the 0.75 to 0.85 cycles d^{-1} was arbitrary and provided a benchmark for nondiurnal cycles and the average value of the magnitudes for the 0.99 to 1.01 cycles d^{-1} provided a reference for diurnal cycles. The averages and confidence intervals of the magnitudes within these frequencies were determined.

RESULTS AND DISCUSSION

Rye Biomass Production, Inorganic N, Precipitation, Moisture, and Temperature

Rye biomass production was highest in 2020 and lowest in 2019 (Table 3.2). The low 2019 yields were attributed to cool and wet conditions (25 GDD from April 26 to May 13) which hampered rye growth and development. Because rye does not have ability to fix atmospheric N_2, the N contained in the biomass was derived from N provided by the soil.

In 2018, the initial NO_3-N and NH_4-N amounts in the surface 30 cm were 3.7 and 6.68 ±0.57 mg ka^{-1}, respectively, and when rye was terminated on July 3, 2018 the NO_3-N concentrations in the soil and rye treatments were similar but numerically lower in the rye (7.11 ±0.91 mg kg^{-1}) than soil (9.03 ±2.94 mg kg^{-1}) treatments. At termination, the NH_4-N concentrations in the soil and rye treatments were similar and the average concentration was 5.41 ±0.83 mg kg^{-1}. In 2019 when the experiment was initiated the initial NO_3-N concentration (April 26) was 14.3 ±7.3 and the initial NH_4-N concentration was 20.3±4.75 mg kg^{-1}. When rye was terminated on June 24, 2019, the NO_3-N concentration in the soil was 8.66 ±1.84 and it was 4.12 ± 0.26 mg kg^{-1} in the rye. However, rye did not influence the NH_4-N concentrations and was 10.3 ±3.99 mg kg^{-1} in both treatments. In 2020, the NO_3-N and NH_4-N concentrations in the surface 30 cm prior to the study were 6.25 ±1.22 and 43.6 ±21 mg kg^{-1}, respectively. When the experiment was terminated on June 26, 2020, NO_3-N in the surface 30 cm was 7.11 ± 1.95 in the soil and 2.5 ±1.56 mg kg^{-1} in the rye. However, at termination rye did not influence NH_4-N concentration and was 2.8 ±1.77 mg kg^{-1} in both treatments.

Table 3.2. The total precipitation, rye biomass produced, and growing degree days (GDD) for each sampling interval, and average water filled porosity (WFP) of the bare soils and the rye cover crop during the sampling intervals in 2018, 2019, and 2020.

Sampling intervals	Prec.	GDD	Dry Rye	C in Rye	N in Rye	Avg. WFP Bare Soil	Avg. WFP Rye
2018	--cm--	C	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}	cm^3 cm^{-3}	
May 7- May 25	2.6	132	279	120	12.2	0.74	0.60
May 26-June 15	2.1	240	392	169	17.2	0.54	0.42
June 16-July 3	10.3	225	378	163	16.6	0.74	0.52
Total	15.0	597	1049	452	46		
2019							
April 26-May 13	7.1	25	106	47	4.4	0.524	0.583
May 14-May 29	8.1	64	69	31	2.9	0.613	0.665
May 30-June 24	5.4	232	253	112	10.5	0.511	0.435
Total	20.6	321	428	190	17.7		
2020							
April 8 -May 4	0.5	78	951	385	32.0	0.441	0.248
May 5 -May 29	8.5	112	883	358	29.7	0.595	0.439
May 30-June 26	7.8	291	843	342	28.3	0.423	0.306
Total	16.8	481	2677	1085	90		

Abbreviations: GDD – Growing Degree Days, BM – Biomass, Prec.- Precipitation, WFP – Water-filled porosity

These findings show that large temporal changes in inorganic N occurred during the study. In 2018, NH_4-N were similar at the beginning and end of the study, whereas in 2019 NH_4-N concentrations decreased from 20.3±4.75 to 10.3±3.99. The largest decrease occurred in 2020 when NH_4-N concentrations decreased from 43.6±21 to

2.82±1.75. Decreases in NH_4-N concentrations over the study were attributed to nitrification and plant uptake. Nitrified N should have increased NO_3-N concentrations during the study. However, these increases would have been reduced by fixation, leaching, and plant uptake. Lower NO_3-N concentrations in the rye than soil treatments in 2019 and 2020 were attributed to plant uptake.

Temporal changes in inorganic N concentration are important because N_2O is emitted from nitrification and denitrification and the relationship between N additions and N_2O-N emissions may follow an 'S" shaped curve which can be mathematically described using a logistic model (Kim, Herandez-Ramirez, & Gilstrap, 2011). Because rye utilized inorganic N, the effect of rye on N_2O emissions may have partially resulted from changes in enzyme efficiencies. The logistic model predicts that at low and high nitrate-N levels, small changes in nitrate can have a minimal impact on N_2O-N emissions. The predication for low N levels, is attributed to increased efficiency of nitrous oxide reductase (more of the N_2O is further reduced to N_2). Thomas et al. (2017) suggested that N_2O-N emissions are reduced when NO_3-N level decrease below 6 ppm, and Millar et al. (2010) reported that a nonlinear relationship exists between N_2O-N emissions and N rate. The predication for high N levels is attributed to respiration being carbon limited as opposed to N limited. This hypothesis is supported by Weier et al. (1993), who showed that in carbon limited systems, adding additional N will not increase denitrification. Blackmer & Bremner, (1978, 1979) also showed that denitrification efficiency is influenced by NO_3-N. Findings from Senbayram et al. (2011) also showed denitrification can be limited by carbon availability. However, not all experiments follow the logistic model (Eagle et al., 2017). Regardless of the model, logistic, exponential, or linear all models predict that decreasing the N rate reduces N_2O-N emissions.

Soil moisture and precipitation also should be considered when evaluating GHG emissions because as soil pores fill with water, oxygen flux into the soil decreases. Decreases in the oxygen flux can result in soil microbial communities that switch from aerobic to anaerobic respiration (Linn & Doran, 1984).

During the experiment, soil moisture was not constant and generally decreased between precipitation events. This decrease was attributed to drainage and evapotranspiration. For example, across years changes in soil moisture [*d*(soil moisture)] during the experiments could be explained by the equation, *d*(soil moisture) = -0.0116+0.0004×rye biomass (kg/ha), $r=0.79$, $p<0.01$). Following precipitation soil moisture increased rapidly. In all three years there were intervals where the water filled porosity was greater than 60%. This value is the tipping point where Lynn & Doran (1984) reported respiration switch from aerobic to anaerobic. In 2018, between May 7 and 25 and between June 16 and 26 the WFP in the bare soil generally exceeded the 60% water-filled porosity (Table 3.2, Supplementary Table 1). However, rye reduced the WFP for these sampling intervals. In 2019, due to high rainfall, rye had a minimal effect on WFP between April 26 and May 29 (Supplementary Table 1 and 2). However, as the season progressed and cover crop growth increased, soil moisture contents decreased at a rate 2.8 times faster than bare soil (Figure 3.1). In 2020, the cover crop had lower WFP for all periods when compared with bare soil. These results were attributed to high biomass production and transpiration, especially from May 30 to June 26.

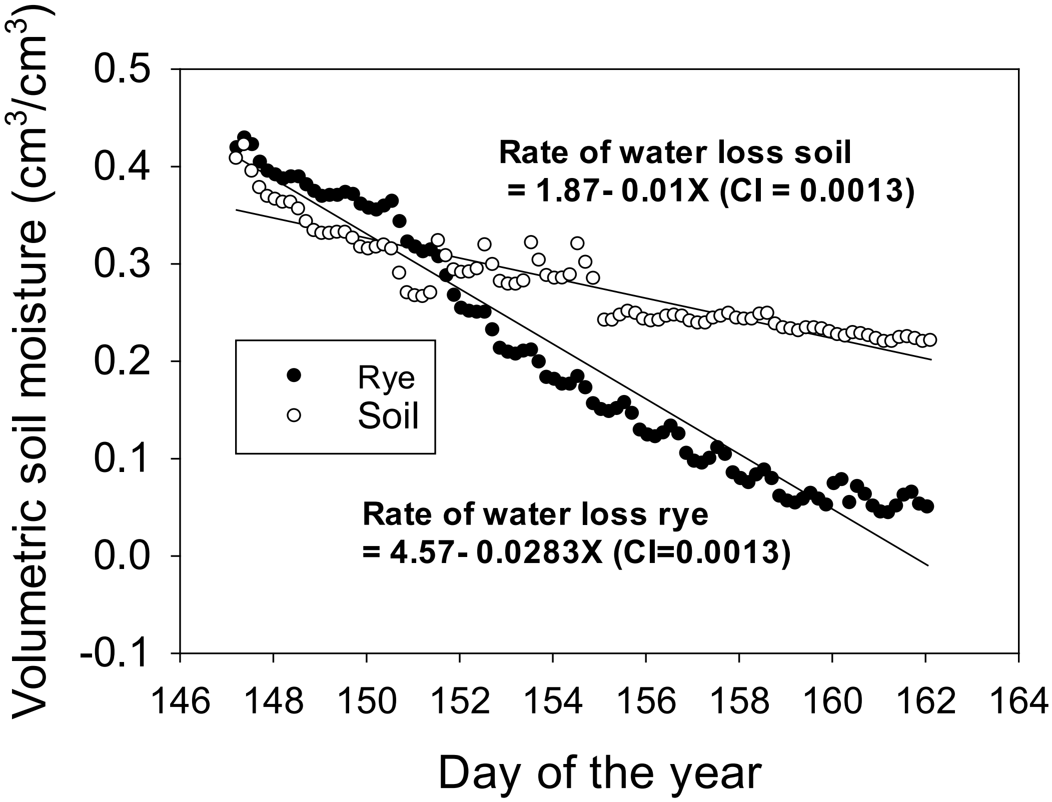


Figure 3.1. Soil moisture depletion in the surface 5 cm of soil between May 26 (146 day of the year) and June 11 (162 day of the year) in 2019. The rate of water loss [(cm^3 (cm^3 x d)$^{-1}$ are shown from the bare soil and rye cover crop treatments. CI represents 95% confidence interval.

The soil temperature in the surface 5 cm differed among years, and it was generally lower in 2019 than 2018 or 2020 (Supplementary Table 2). Across years, the rye and bare soil treatments had similar soil temperatures. However, differences were observed at selected times. For example, between April 26 and May 13 in 2019 at 1000 and 1400 h, the soil temperatures in the rye treatment were generally higher than the bare soil, whereas in 2020 between May 30 and June 26 soil temperatures were cooler in the rye the bare soil. Temperature changes are important when evaluating GHG emissions because it influences gas solubility, equilibrium relationships, microbial activity, and plant growth.

Nitrous oxide and carbon dioxide frequency emissions signatures

To determine if N_2O-N fluxes followed a predictable pattern, we conducted an FFT, which converts time domain data into the frequency domain. The transformation results in a series of frequencies and associated magnitudes (Figures 3.2, 3.3, 3.4).

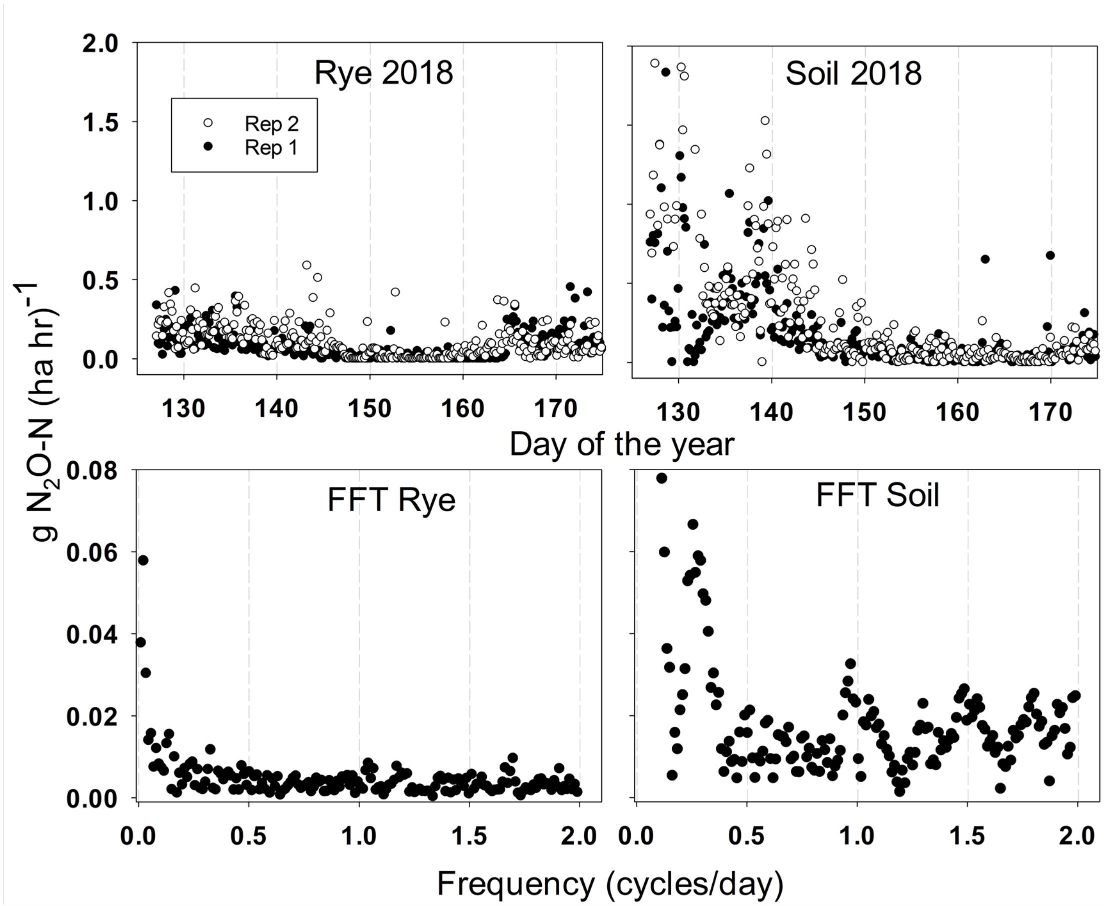


Figure 3.2. The 2018 N_2O emissions (top) and frequency emission signatures (bottom) for the rye and bare soil treatments. For the frequency data, the magnitude is on the y-axis and the frequency is on the x-axis.

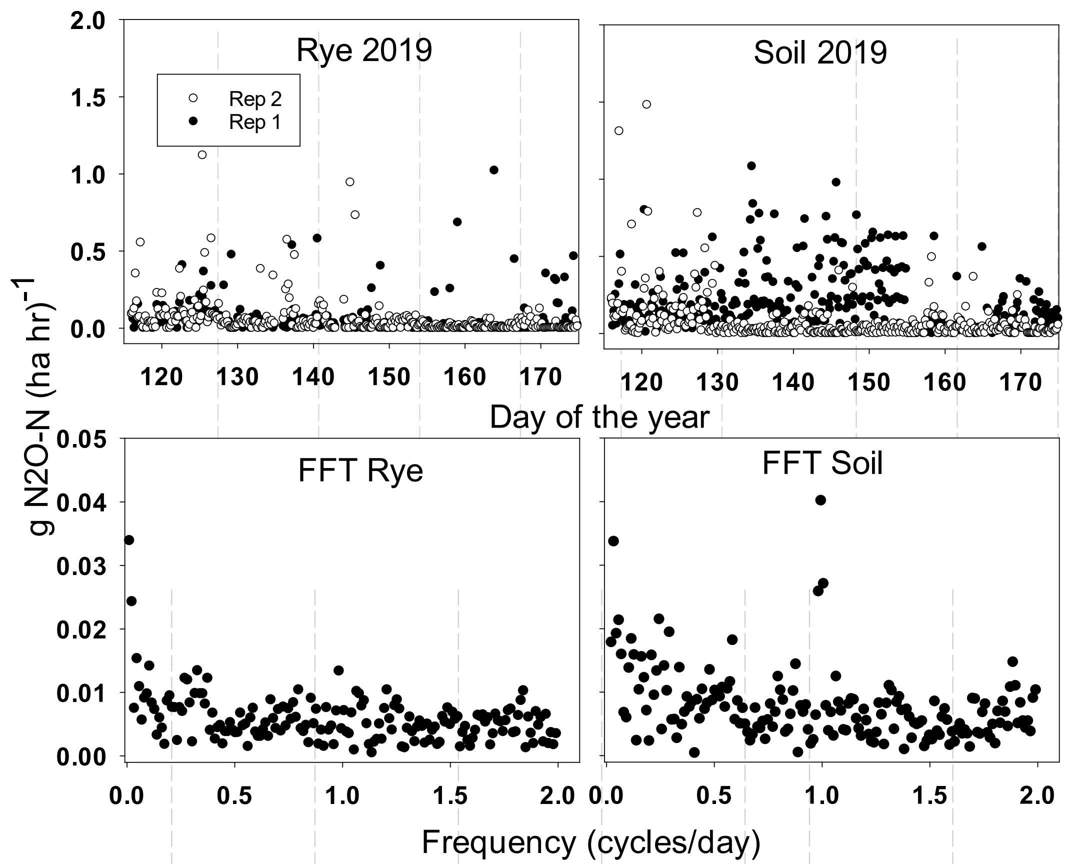


Figure 3.3. The 2019 N2O emissions (top) and frequency emission signatures (bottom) for the rye and bare soil treatments. For the frequency data, the magnitude is on the y-axis and the frequency is on the x-axis.

The size of the magnitude provides an assessment of the importance of each frequency. Across all three years, rye reduced the magnitudes 80% for the frequencies between 0.98 to 1.01 cycle's day^{-1} and 42% for the frequencies between 0.75 to 0.85 cycle's day^{-1} (Table 3.3). In addition, across years, rye reduced the ratio 66% between the non-diurnal period (0.75 and 0.85) and the diurnal (0.98 and 1.01 cycles d^{-1}) period.

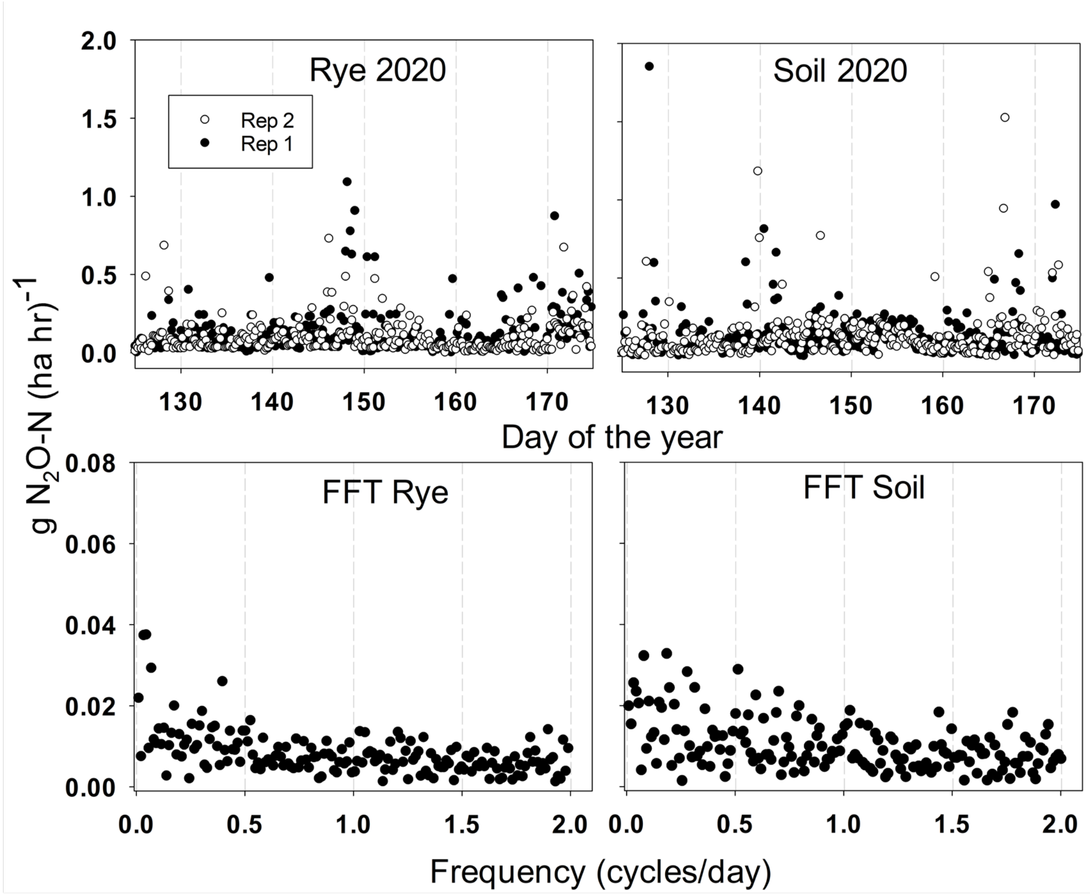


Figure 3.4. The 2020 N_2O emissions (top) and frequency emission signature (bottom) for the rye and bare soil treatments. For the frequency data, the magnitude is on the y-axis and the frequency is on the x-axis.

The larger ratio for bare soil (2.9) than rye (0.95) indicates that bare soil had a stronger diurnal cycle for emissions than rye. We attributed these results to cover crop-induced differences in soil physical, chemical, and biological properties that were previously discussed. Others have seen similar responses. For example, Shurpali et al. (2016) reported that when N_2O flux was low and the plant was N limited, the N_2O emission pattern switched, with emissions being higher during the night than day. This change in

FFT signature has implications on the sampling requirement and suggests that near-continue sampling may be required for precise and accurate measurement.

Rye had a mixed effect on the FFT CO_2-C emission signatures. For the non-diurnal benchmark (frequencies between 0.75 and 0.85 cycle d^{-1}) rye increased the magnitudes in 2020, reduced the magnitudes in 2019 and did not influence the magnitudes in 2018 when compared with the soil treatment. However, for the diurnal frequencies (between 0.98 to 1.01 cycles d^{-1}) rye either increased or did not influence the

Table 3.3. Analysis of the N_2O-N and CO_2-C frequency signatures. The average magnitudes for two frequency ranges (0.75 to 0.85 and 0.99 to 1.01 cycles day^{-1}) and the ratio between these magnitudes for the bare soil and rye treatments in 2018, 2019, and 2020. Confidence intervals for the 90% level are shown.

		N_2O-N Frequency			CO_2-C Frequency		
Year	Trt.	0.75 -0.85	0.98 -1.01	Ratio	0.75-0.85	0.98 - 1.01	Ratio
		g N_2O-N/ (ha×h)			g CO_2-C/ (ha×h)		
2018	Soil	0.01	0.031	2.82	29.8	99.4	3.34
2018	Rye	0.0031	0.0025	0.81	27.9	159.0	5.7
2019	Soil	0.0071	0.0310	4.36	20.3	70.4	3.47
2019	Rye	0.0057	0.0081	1.39	38.5	132.2	3.43
2020	Soil	0.00935	0.0143	1.53	82.5	85.3	1.03
2020	Rye	0.0065	0.00440	0.67	67.2	118	1.76

magnitudes. Across the three years, the ratio between two frequency periods was 2.61 for soil and 3.63 for rye. These values suggest that rye increased the importance of the CO_2-C diurnal cycle.

Vegetative rye impact on early season N_2O-N flux and total emissions

Across the three years, rye reduced N_2O-N emissions ($p= 0.05$) by 66% during the first sampling interval (Table 3.4). These results were attributed to rye scavenging the soil for inorganic N and water (Linn & Doran, 1984; Del Grosso et al., 2000; Kallenback et al., 2010; Thies et al., 2020). However, contrary to the first sampling interval, rye did not affect emissions during the 2nd and 3rd sampling intervals. The temporal effect of rye on N_2O-N emissions could be attributed to treatment differences in the amount of NH_4-N that was nitrification and NO_3-N that was denitrified and that relationship between N_2O-N emissions and NO_3-N concentration most likely followed a logistic model (Kim et al., 2011).

Across the sampling intervals, the highest emissions were observed during the first period. Higher emissions in the early spring could be the results of soil freezing–that lyses microbial cells releasing labile organic compounds into the soil solution. These compounds when mineralized result in CO_2–C emissions and higher soil NH_4–N concentration in the soil solution, which is subsequently reduced to NO_3–N and susceptible to denitrification. Increasing soil temperatures during the spring may have also released N_2O during soil thawing (Wegner-Riddle et al., 2017). Our findings differ from Ruis et al. (2018), where rye had a minimal impact on N_2O-N emissions. Differences between Ruis, et al. (2018) and our study were attributed to four factors. First, Ruis, et al. (2018) sampled their system 14 times from late April 2018 to June 2019 and collected point samples from the treatments biweekly between 1000 and 1400 h. In comparison, we measured emissions over 1100 times over three years. Second, Ruis et al. (2018) applied N fertilizer, whereas in our study N was not applied. As discussed earlier, the application of N fertilizer may have placed Ruis et al. (2018) in the high emissions portion of S-Curve where the amount of N uptake up by the cover crop wasn't enough to affect N_2O-N emissions. Third, Ruis et al. (2018) reported that in a dryland system, the cover crop had a minimal impact on soil moisture, whereas in our rye reduced soil moisture. Fourth, Ruis et al. (2018) reported that between March 6 and April 25 an N_2O-N flush was not observed and changes in soil inorganic N were not reported. Whereas, in our study, rye reduced N_2O-N emissions during the first sampling period in all three years.

Vegetative rye impact on early season CO_2-C flux and total emission

For CO_2-C emissions, the soil and rye treatments had diurnal cycles in 2018, 2019, and 2020 (Table 3.3). The diurnal CO_2-C cycles were attributed to diurnal temperature cycles which influenced CO_2 water solubility and microbial activity. In 2018, CO_2-C emission rates were not constant during the study and increased at a rate 14.6 g CO_2-C ±3.1 $(ha \times h \times d)^{-1}$) in the bare soil and 26.6 ±3.5 g CO_2-C $(ha \times h \times d)^{-1}$) in the cover crop. Across years, rye only increased CO_2-C emissions in 2019. The higher rate in rye was attributed to the increased importance of non-heterotrophic respiration.

SUMMARY

In this experiment, the impact of an unfertilized growing cover crop on soil moisture, inorganic N, and GHG emissions and frequency signatures were investigated. Our research showed that when compared to bare soil, rye reduced the surface soil WFP 29, 15, and 26% in 2018, 2019, and 2020, respectively. Rye also reduced the NO_3–N concentration in surface 30 cm of soil by 52 and 64% in 2019 and 2020, respectively. Associated with these reductions was a 66% decrease in N_2O-N emissions for the first sampling period across years. The study also showed that the cover crop changed the N_2O-N and CO_2–C FFT emission signatures which could complicate the interpretation of a single sample collected at a prescribed time every 2 wk. In addition, during the cover crop first sampling period, N_2O emissions were consistently reduced, whereas during the second and third sampling interval the cover crop did not influence emissions. Temporal changes on cover crop induced differences in N_2O may be related to changes in the inorganic N during the study. Rye induced changes in soil nitrate are important because N additions (NO_3–N) and N_2O-N emissions may follow a logistic model. This model predicts that at low N and high N levels, changes in the NO_3–N concentration may result in minimal changes in N_2O-N emissions. However, at moderate N levels, N_2O emissions increase exponentially with increasing N. Nitrified N should have increased NO_3–N concentrations during the study. However, large increases in NO_3–N were not observed and generally NO_3–N concentrations were relatively low in this unfertilized soil. In 2018, NO_3–N increased from 3.7 to 9.03 mg kg^{-1} in the soil and 7.11 mg kg^{-1} in the rye treatment. In 2019, NO_3–N concentrations decreased from 14.3 mg kg^{-1} at the start of the experiment to 8.66 mg kg^{-1} in the soil treatment and 4.1 mg kg^{-1} in the rye treatment during the study. Slightly different results were observed in 2020 where NO_3–N at initiation was 6.25 mg kg^{-1} and at termination it was 7.11 in the soil and 2.5 mg kg^{-1} in the rye treatments. Our findings support the hypothesis that N_2O emissions would be reduced during cover crop growth. Additional research is needed to confirm these results over a range of environments and NO_3–N concentrations. For this experiment, additional information on the impact of cover crop on corn growth is available in Moriles-Miller et al. (2024) and the effect of the decomposing cover crop on GHG emissions are available in Joshi et al. (2022).

Table 3.4. Greenhouse gas emissions from bare soil and soil with a living rye cover crop during the first, second, and third runs of each year.

		CO_2	N_2O	†CH_4
		---------------g CO_{2e}/(ha d)---------------		
First Run				
Soil	May 7 – May 25, 2018	105458	4213	-44.2
	Apr. 26 – May 13, 2019	43040	1670	-72.2
	Apr. 8 – May 4, 2020	36850	1920	232.9
	mean	61783	2601	38.8
Rye	May 7 – May 25, 2018	91941	1430	-252.9
	Apr. 26 – May 13, 2019	35714	919	-177.4
	Apr. 8 – May 4, 2020	61099	1301	43.5
	mean	62918	1217	-128.9
	p-value	0.47	**0.09**	
Second Run				
Soil	May 26 – June 15, 2018	108315	713	-108.1
	May 14 – May 29, 2019	71905	1872	-58.7
	May 5 – May 29, 2020	50110	1314	158.3
	mean	76776	1300	-2.8
Rye	May 26 – June 15, 2018	140952	507	-133.7
	May 14 – May 29, 2019	77253	790	-62.0
	May 5 – May 29, 2020	68132	1194	49.5
	mean	95446	830	-48.7
	p-value	**0.07**	0.13	
Third Run				
Soil	Jun. 16 – Jul. 3, 2018	153337	919	-58.5
	May 30 – Jun. 24, 2019	51905	940	-29.2
	May 30 – Jun. 26, 2020	141538	1391	-176.3
	mean	115593	1084	-88.0
Rye	Jun. 16 – Jul. 3, 2018	153846	825	-150.2
	May 30 – Jun. 24, 2019	123406	404	-90.6
	May 30 – Jun. 26, 2020	146667	1190	-190.5
	mean	141306	806	-143.8
	p-value	0.19	**0.09**	

†CH_4 values originally reported in Pandit et al. (2024).

Table 3.5. Soil NH_4-N and NO_3-N in the surface 30 cm at the start and at the end of the experiment for each year.

	-------------bare soil--------		-------------rye-------------	
Sampling Date	NH_4-N	NO_3-N	NH_4-N	NO_3-N
	------------------------------------kg N ha^{-1}--------------------------------			
5/7/18	6.68 ±0.57	3.7		
7/3/18	5.41 ±0.83	9.03 ±2.94	5.41 ±0.83	7.11 ±0.91
4/26/19	20.3±4.75	14.3 ±7.3		
6/24/19	10.3 ±3.99	8.66 ±1.84	10.3 ±3.99	4.12 ± 0.26
4/8/20	43.6 ±21	6.25 ±1.22		
6/26/20	2.8 ±1.77	7.11 ± 1.95	2.8 ±1.77	2.5 ±1.56

CHAPTER 4: SUMMARY OF DISSERTATION

There are many ways for agriculture to reduce its GHG emissions and still maintain high levels of productivity. The two studies in this document showed that growing a cereal rye cover crop during the spring prior to growing cash crops and/or adjusting N fertilizer application timing can help achieve those goals. In the first study, urea was applied (0 or 224 kg N ha^{-1}) at three different timings starting in the fall of 2017 (early fall, mid-fall, and early winter) and at three different timings starting in the spring of 2018 (early spring, mid-spring, and early summer). GHG emissions were measured every 4 h for 21 d after each application. A significant interaction between N fertilizer and its application date occurred for CO_2 emissions (p=0.01). The early spring application (5/1/18), which would normally be around corn planting, increased CO_2 emissions by 35% during the 21 d interval following application. When application was delayed until mid-spring, the opposite occurred, and CO_2 emissions were reduced by 19% (p=0.06). This trend continued into early summer, where CO_2 emissions of N fertilized soil were 23% less than those of unfertilized soil.

A significant N fertilizer x application date interaction also occurred for the percentage of N_2O emissions offset by CH_4 consumption (p=0.01). During mid-spring and early summer, CH_4 consumption offset N_2O emissions by 9.6% when N fertilizer wasn't applied and by 3.1% when fertilizer was applied. This offset between the fertilized and unfertilized treatment wasn't significantly different for the other applications timings and was only 0.9 and 2.3% during early fall and early spring, respectively.

When averaged across application dates, N fertilization reduced CH_4 consumption by 20%. Despite this, the soil still had net CH_4 consumption (not emissions) regardless of N fertilization or application timing. CH_4 consumption was 85% greater during spring and early summer (5/1/18 – 7/4/18) than during fall through early winter (9/21/17 – 11/14/17). When averaged across N fertilized (corn) and non-fertilized (soybeans) soils, CH_4 consumption offset N_2O emissions by an average of 6.4%, which is an important finding considering that agriculture is the largest contributor to N_2O emissions.

The results from the cereal rye cover crop study and the N fertilizer timing study had a common theme in that early spring was the best timeframe for potential GHG reductions. Over three years (2018-2020), a fall dormant seeded cereal rye cover crop produced an average biomass yield of 445 kg ha^{-1} during the first three weeks of spring growth. This amount of biomass reduced N_2O emissions by 53% compared to soil without a cover crop. Also important, the early spring cover crop did not increase CO_2 or total GHG emissions. Terminating the cover crop at this time would typically align with a period between corn planting and emergence. At the end of the early spring growth period, the cover crop was clipped near the soil surface and allowed to grow an additional three weeks, which was classified as the mid-spring growing period. Termination following mid-spring simulates a cover crop termination between soybean planting and emergence. This extended period of cover crop growth did not reduce N_20 emissions and actually increased CO_2 emissions by 20%. Our research showed that when compared to bare soil, rye reduced the surface soil WFPS by 29, 15, and 26% in 2018, 2019, and 2020, respectively. Rye also reduced the NO_3-N concentration in surface 30-cm of soil by 52 and 64% in 2019 and 2020, respectively. The study also showed that the cover crop changed the N_2O-N and CO_2-C FFT emission signatures which could complicate the interpretation of a single sample collected at a prescribed time every two weeks. Our findings support the hypothesis that N_2O emissions would be reduced during cover crop growth.

The results of both studies indicate that early spring management could have a large impact in reducing GHG emissions. Delaying N fertilization application by three weeks from early May to mid-May did not increase overall GHG emissions. This would be from delaying the application from around the time of corn planting to around the time of emergence. The cereal rye cover crop reduced total GHG emissions, but only by 2.2%. Perhaps more important are the substantial reductions in N_2O emissions and in soil moisture levels that a cereal rye cover crop can provide.

Future Recommendations

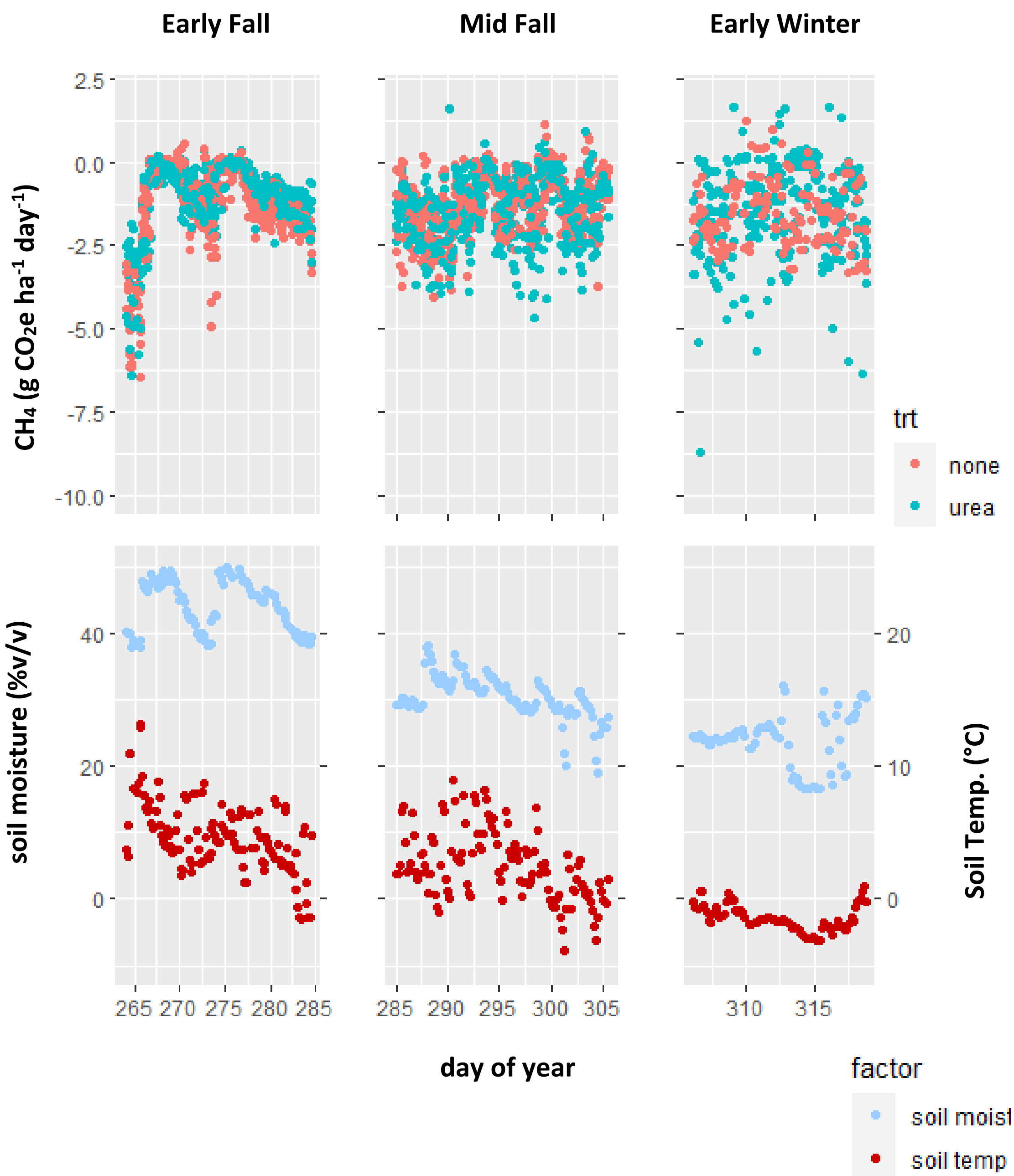
Future work should combine these two studies to evaluate the impact of growing a cereal rye cover crop during early spring followed by a mid-spring N fertilizer application to compare GHG emissions relative to the more-common practice of applying N fertilizer around the time of corn planting in early spring without growing a cover crop. Since a maximum of four treatments is attainable with the current GHG measuring system, the following treatments would be of greatest interest: 1) an untreated control, 2) N fertilizer applied during early spring, 3) N fertilizer applied during mid-spring, and 4) a cereal rye cover crop grown during early spring and with N applied during mid-spring following termination. Applying a lower rate of N fertilizer would also be beneficial, as the rate applied in the N fertilizer application trial was about 50% higher than a farmer would apply to corn in a corn/soybean rotation. The urea should also be treated with a urease inhibitor to minimize volatilization losses. In addition, conducting such a trial within a corn and/or soybean field would also better-simulate a production setting due to the soil moisture reductions and N fertilizer uptake by the cash crops.

Supplementary Table 1. The average volumetric soil moisture (cm3 cm^{-3}) during three sampling intervals and six sampling times over a 24-hour period in 2018, 2019, and 2020

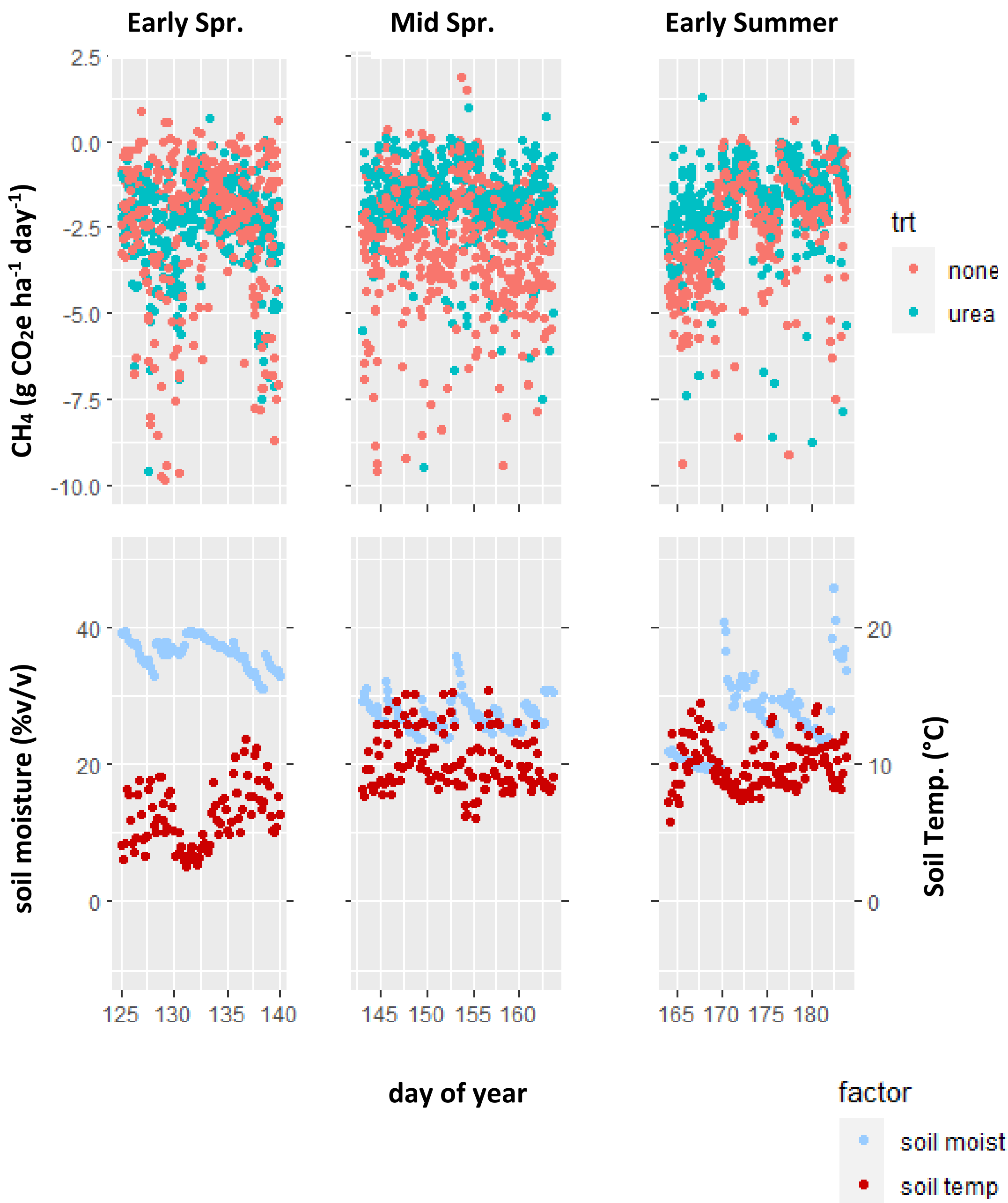
2018	May 7 - May 25			May 26 - June 15			June 16 – July 3		
Time	Soil	Rye	p-value	Soil	Rye	p-value	Soil	Rye	p-value
h	--cm^3/cm^3--			---cm^3/cm^3---			---cm^3/cm^3--		
200	0.3	0.31	<0.01	0.26	0.21	0.02	0.3	0.26	<0.01
600	0.3	0.31	<0.01	0.26	0.21	0.008	0.3	0.27	<0.01
1000	0.3	0.31	<0.01	0.26	0.21	0.007	0.3	0.26	<0.01
1400	0.3	0.31	<0.01	0.27	0.21	0.002	0.3	0.26	<0.01
1800	0.3	0.31	<0.01	0.27	0.2	<0.01	0.3	0.25	<0.01
2200	0.3	0.31	<0.01	0.27	0.2	0.001	0.3	0.25	<0.01
Avg.	0.3	0.31	<0.01	0.27	0.21	<0.001	0.3	0.26	<0.01
2019	April 26 - May 13			May 14 - May 29			May 30 - June 24		
	Soil	Rye	p-value	Soil	Rye	p-value	Soil	Rye	p-value
200	0.2	0.29	0.24	0.3	0.32	0.099	0.2	0.21	0.16
600	0.2	0.30	0.16	0.32	0.34	0.98	0.2	0.21	0.08
1000	0.2	0.3	0.16	0.31	0.34	0.89	0.2	0.22	0.15
1400	0.2	0.3	0.12	0.31	0.33	0.90	0.2	0.23	0.35
1800	0.2	0.28	0.16	0.29	0.32	0.90	0.2	0.22	0.17
2200	0.2	0.29	0.15	0.3	0.33	0.93	0.2	0.22	0.26
Avg.	0.2	0.29	<0.01	0.31	0.33	0.001	0.2	0.22	<0.00
2020	April 8 - May 4			May 5 - May 30			May 30 - June 26		
	Soil	Rye	p-val.	Soil	Rye	p-val.	Soil	Rye	p-val.
200	0.2	0.13	0.04	0.3	0.22	0.08	0.1	0.15	0.27
600	0.2	0.12	0.05	0.3	0.22	0.07	0.1	0.15	0.28
1000	0.2	0.11	0.04	0.31	0.23	0.08	0.2	0.16	0.26
1400	0.2	0.13	0.04	0.31	0.23	0.08	0.2	0.16	0.25
1800	0.2	0.13	0.03	0.3	0.22	0.08	0.1	0.14	0.03
2200	0.2	0.13	0.04	0.3	0.22	0.08	0.2	0.16	0.18
Avg.	0.2	0.13	<0.01	0.3	0.22	<0.01	0.1	0.15	<0.01

Supplementary Table 2: Average soil temperatures (ºC) for the surface 5 cm for the three sampling intervals in the bare soil and rye cover crop treatments in 2018, 2019, and 2020.

2018	May 7 – May 25			May 26 – June 15			June 16 – July 3		
Time	Soil	Rye	p-val.	Soil	Rye	p-val.	Soil	Rye	p-val.
h	ºC	ºC		ºC	ºC		ºC	ºC	
200	14.7	14.7	0.97	20.9	20.2	0.29	20.9	20.9	0.91
600	13.1	13.2	0.92	19.2	18.5	0.27	20.2	20	0.74
1000	12.5	15	0.04	18.7	21.4	<0.01	19.8	20.8	0.08
1400	16.0	19.4	0.04	22.2	26.5	<0.01	21	23.5	<0.01
1800	19.0	20.3	0.46	25.4	25.9	0.82	22.8	24.1	0.09
2200	17.5	17	0.76	23.7	22.8	0.25	22.1	22.2	0.87
Avg.	15.5	16.6	0.48	21.7	22.6	0.62	21.1	21.9	0.36
2019	April 26 – May 13			May 14 – May 29			May 30 – June 24		
	Soil	Rye	p-val.	Soil	Rye	p-val.	Soil	Rye	p-val.
200	6.5	6.5	0.93	11.5	11.3	0.83	14.3	17.7	<0.01
600	5.3	5.5	0.89	10.5	10.3	0.89	13.9	16.3	<0.01
1000	5.6	7.1	0.07	10.6	11.9	0.64	17.7	17.9	0.62
1400	10.8	11.1	0.82	14.4	15.7	0.41	21.1	22.3	0.22
1800	12.2	11.3	0.50	15.9	16	0.61	20.5	23.2	<0.01
2200	9.3	8.7	0.56	13.6	13.1	0.78	16.3	20	<0.01
Avg.	8.3	8.4	0.96	12.8	13.1	0.83	17.3	19.6	0.21
2020	April 8 - May 4			May 5 - May 29			May 30 - June 26		
	Soil	Rye	p-val.	Soil	Rye	p-val.	Soil	Rye	p-val.
200	6.6	6.6	0.98	10.1	10.6	0.27	18.9	18.7	0.60
600	5.2	5.2	0.95	10.7	9.7	0.57	18.0	17.6	0.53
1000	7.3	6.8	0.71	14.1	11.2	0.18	20.2	19.6	0.50
1400	14.6	13.0	0.49	16.7	15.1	0.58	18.0	17.6	0.53
1800	15.0	13.1	0.15	15.2	14.6	0.87	25.1	22.5	0.24
2200	9.7	9.0	0.26	11.9	12.6	0.54	21.0	20.8	0.82
Avg.	9.7	8.95	0.22	13.2	12.3	0.34	20.2	19.5	0.05



Supplementary Figure 1. A graphical depiction of the CH_4 emission data collected during fall and early winter of 2017 from the Urea Application Timing Study (Chapter 2). Urea was applied on the first date of each season, which were early fall (Sept. 21 – Oct. 11), mid-fall (Oct. 11 – Nov. 1), and early winter (Nov. 1 – Nov. 15). Each CH_4 datapoint is an average of the four LI-COR chambers from a given daily sampling period (i.e. from 0000 to 0230 h). There were six sampling periods per day.



Supplementary Figure 2. A graphical depiction of the CH_4 emission data collected during spring and early summer of 2018 from the Urea Application Timing Study (Chapter 2). Urea was applied on the first date of each season, which were early spring (May 1 – May 22), mid-spring (May 22 - Jun. 12), and early summer (Jun. 12 – Jul. 4). Each CH_4 datapoint is an average of the four LI-COR chambers from a given daily sampling period (i.e. from 0000 to 0230 h). There were six sampling periods per day.

Literature Cited

Abadalla, M., Hastings, A., Helmy, M., Prescher, A., Osborne, B., Lanigan, G., Forristal, D, Killi, D., Maratha, P., Williams, M., Rueangritsarakul. K., Smith, P., Nolan, P., & Jones M.B. (2014). Assessing the combined use of reduced tillage and cover crops for mitigating greenhouse gas emissions from arable ecosystems. *Geoderma*, **223-225**, 9-20.

Acharya, J., Bakker, M.G., Moorman, T.B., Kaspar, T.C., Lenssen, A.W., and Robertson, A.E. (2017). Time interval between cover crop termination and planting influences corn seedling disease, plant growth, and yield. *Plant Disease,* **101**:591-600.

Antosh, E, Idowa, J., Schutte, B., and Lehnhoft, E. (2020). Winter cover crops effect soil properties and sweet corn yield in semi-irrigated systems. *Agronomy Journal,* **112***,* 92-106.

Archarya, P., Ghimire, R., Paye, W.S., Ganguli, A.C., and DelGrosso, S.J. (2022). Net greenhouse gas balance with cover crops in semi-arid irrigated cropping systems. *Nature,* 12:12386.

Aronson, E.L. and Helliker, B.R. (2010). Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology*, **91(11),** 3242-3251.

Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., and Hergert, G.W. (2015). Cover crops and ecosystem services: insights from studies in temperate soils. *Agronomy Journal*, **107**, 2449-2474.

Basche, A.D., Kaspar, T.C., Archontoulis, S.V., Jaynes, D.B., Sauer, T.J., Parkin, T.B., and Miguez, F.E. (2016). Soil water improvements with the long-term use of winter rye cover crop. *Agricultural Water Management,* **172**, 40-50.

Basche, A.D, Miguez, F.E. Kaspar, T.C., and Castellano, M.J. (2014). Do cover crops increase or decrease nitrous oxide emission? A meta-analysis. *Journal of Soil and Water Conservation*, **69**, 471-482.

Bich, A.D., Reese, C.L., Kennedy, A.C, Clay, D.E., and Clay, S.A. (2014). Corn yield is not reduced by mid-season establishment of cover crops in northern Great Plains environment. *Crop Management*, **13**, 1-8.

Blackmer, A. M., and 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., and 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

Brugler, S., Clay, D.E., Joshi, D., Clay, S.A., Nleya, T., and Hatfield G. (2024). Does splitting the nitrogen rate reduce carbon intensity. *Agronomy Journal* (in review).

Brummell, M. E., Farrell, R. E., Hardy, S. P., and Siciliano, S. D. (2014). Greenhouse gas production and consumption in High Arctic deserts. *Soil Biology & Biochemistry*, **68**, 158–165.

Canadell, J.G., P.M.S. Monteiro, M.H. Costa, L. Cotrim da Cunha, P.M. Cox, A.V. Eliseev, S. Henson, M. Ishii, S. Jaccard, C. Koven, A. Lohila, P.K. Patra, S. Piao, J. Rogelj, S. Syampungani, S. Zaehle, and K. Zickfeld, 2021: Global Carbon and other Biogeochemical Cycles and Feedbacks. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 673–816, doi:10.1017/9781009157896.007.

Çerçioğlu, M., Anderson, S.H., Udawatta, R.P., and Algele, S. (2019). Effect of cover crop management on soil hydraulic properties. *Geoderma,* **343**, 247-253.

Chang, R. Y., Liu, X., Wang, T., Li, N., and Bing, H. (2021). Stimulated or inhibited response of methane flux to nitrogen addition depends on nitrogen levels. *Journal of Geophysical Research: Biogeosciences*, *126*, e2021JG006600. https://doi.org/10.1029/2021JG006600

Chen, D., Li, J., Lan, Z., Hu, S., and Bai, Y. (2016). Soil acidification exerts a greater control on soil respiration than soil nitrogen availability in grasslands subjected to long-term nitrogen enrichment. *Functional Ecology*, **30**, 658-669. doi: 10.1111/1365-2435.12525

Choi, Woo-Jung, Matushima, M., and Ro, H-M. (2011). Sensitivity of soil CO_2, emissions to fertilizer nitrogen species: urea, ammonium sulfate, potassium nitrate, and ammonium nitrate. *Journal of the Korean Society for Applied Biological Chemistry*, **56,** 1004-1007.

Christensen, S. and Christensen, B.T. (1991). Organic matter available for denitrification in different soil fractions: effect of freeze/thaw cycles and straw disposal. *European Journal of Soil Science, **42***, 637-647.

Clay D.E., Reicks G., Carlson C.G., Moriles-Miller J., Stone J.J., and Clay S.A. (2015). Tillage and corn residue harvesting impact surface and subsurface carbon sequestration. *Journal of Environmental Quality,* **44**, 803-809. https://doi.org/10.2134/jeq2014.07.0322

Clay, D. E., Clay, S. A., Moorman, T. B., Brix-Davis, K., Scholes, K.A., and Bender, A. R. (1996). Temporal variability of organic C and nitrate in a shallow aquifer. *Water Research*, ***30***, 559–568.

Clay, D. E., Clay, S. A., Lyon, D. J., and Blumenthal, J. M. (2005). 13C 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., and Scholes, K. A. (1994). Nitrate movement after anhydrous ammonia application in a ridge tillage system. *Journal of Environmental Quality*, ***23***, 9–13.

Cowan, N., Maire, J., Krol, D., Clory, J.M., Hargreaves, P., Murphy, R., Carswell, A., Jones, S.K., Hinton, N., Anderson, M., Famulari, D., Bell., M., Stack, O., Levy, P., Skibe, U., and Drewer, J. (2020). Agricultural soils: A sink or source for methane across the British Isles. *European Journal of Soil Science*, **77**, 1842-1862.

Del Grosso, S. J., Ojima, D. S., Parton, W. J., Stehfest, E., Heistemann, M., DeAngelo, B., and Rose, S. (2009). Global scale DAYCENT model analysis of greenhouse gas emissions and mitigation strategies for cropped soils. *Global and Planetary Change*, ***67*****(1-2)**, 44-50. https://doi.org/10.1016/j.gloplacha.2008.12.006

de Mendiburu, F. and Yaseen, M. (2023). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-7. https://cran.rproject.org/package=agricolae

Dietzel, R., Wolfe, D., and Thies, J.E. (2011). The influence of winter soil cover on spring nitrous oxide emissions from an agricultural soil. *Soil Biology and Biochemistry*, **43**, 1989-1991.

Dutaur, L. and L.V. Verchot. (2007). A global inventory of the soil CH_4 sink. *Global Biogeochemical Cycles,* **21**, 1-9.

Eagle, A.J., Olander, L.P., Locklier, K.L., Heffernan, J.B., and Bernhardt. (2017). Fertilizer management and environmental factors drive N_2O and NO_3 losses in corn: A meta analysis. *Soil Science Society of America Journal*, **81**, 1191-1202.

EPA 2023. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2021. U.S. Environmental Protection Agency, EPA 430-R-23-002. https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2021.

Fisher, W.B. Jr., and Parks, W.L. (1958). Influence of soil temperature on urea hydrolysis and subsequent nitrification. *Soil Science Society of America Journal*, **22**, 247-248.

Forster, P., T. Storelvmo, K. Armour, W. Collins, J.-L. Dufresne, D. Frame, D.J. Lunt, T. Mauritsen, M.D. Palmer, M. Watanabe, M. Wild, and H. Zhang, 2021: The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani,

S.L. Connors, C. Pean, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekci, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 923–1054, doi:10.1017/9781009157896.009.

Gong, Y., Li, P., Sakagami, N., and Komatsuzaki, M. (2021). No-tillage with rye cover crop can reduce net global warming potential and yield-scaled global warming potential in long-term organic soybean field. *Soil & Tillage Research*, **205**, 1-10.

Guo, Y., Zhang, G., Adballa. M., Kuhnert, M., Bao, H., Xu H., Ma, J., Bedgum K., and Smith, P (2023). Modelling methane emissions and grain yields for a double-rice system in Southern China with DayCent and DNDC models. *Geoderma,* **431**, 116364.

Hamel, C., Hanson, K., Selles, F., Cruz, A. F., Lemke, R., McConkey,B., and 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. and Drinkwater, L.E. (2017). N_2O emissions from grain cropping systems: A meta-analysis of the impact of fertilizer-based and ecologically-based nutrient management strategies. *Nutrient Cycles in Agroecosystems*, **107**, 335-355. doi: 10.1007/s10705-017-9836-z

Hebbali, A. (2020). olsrr: Tools for Building OLS Regression Models. R package version 0.5.3.

Jarecki, M.K., Parkin, T.B., Chan, A.S.K., Kaspar, T.C., Moorman, T.B., Singer, J.W., Kerr, B.J., Hatfield, J.L., and Jones, R. (2009). Cover crop effects on nitrous oxide emissions from a manure-treated mollisol. *Agriculture, Ecosystems, & Environment*, **134**, 29-35.

Joshi, D.R., Clay, D.E., Clay, S.A., Moriles-Miller, J., Daigh, A.L.M., Reicks, G. and Westhoff, S. (2022). Quantification and machine learning based on N_2O-N and CO_2-C emissions predictions from a decomposing rye cover crop. *Agronomy Journal,* 1-15. https://doi.org/10.1002/agj2.21185

Johnson, J.M.F. and Barbour, N.W. (2019). Stover harvest did not change nitrous oxide emissions in two Minnesota fields. *Agronomy Journal,* **111**, 143-155.

Kallenback, C.M., Rolston, D.E., and Horwath, W.R. (2010). Cover cropping affects soil N_2O and CO_2 emissions differently depending on type of irrigation. *Agriculture, Ecosystems & Environment,* **137**, 251-260. https://doi.org/10.1016/j.agee.2010.02.010

Kantar, M. and Porter, P. (2014). Relationship between planting date, growing degree days and the winter rye (*Secale cereale* L.) variety "Rymin" in Minnesota. *Crop Management*, **13**, 1-9. doi:10.2134/CM-2013-0096-RS

Karakurt, I., Aydin, G., and Aydiner, K. (2012). Sources and mitigation of methane emissions by sectors: A critical review. *Renewable Energy*, **39**, 40–48.

Kim, D-G., Hernandez-Ramirez, G., and Giltrap, D. (2011). Linear and nonlinear dependency of direct nitrous oxide emission on fertilizer nitrogen input. *Agriculture, Ecosystems, & Environment,* **168**, 53-65.

Kim, K. I., Clay, D. E., Carlson, C. G., Clay, S. A., and 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

Klingenberg, L. (2005). Frequency domain using Excel. San Francisco, CA: San Francisco State University, School of Engineering. Krijnen, F., Richman, D., Lemke, R., & Farrell, R. (2013). Evaluation of a closed-path Fourier transform infra-Red (FTIR) multi-component gas analyzer for the simultaneous measurement of nitrous oxide, carbon dioxide and methane. Saskatoon, SK, Canada: University of Saskatchewan. https://core.ac.uk/display/226146035

Krijnen, F., Richman, D., Lemke, R., and Farrell, R. (2013). Evaluation of a Closed-Path Fourier Transform Infra-Red (FTIR) Multi-Component Gas Analyzer for the Simultaneous Measurement of Nitrous Oxide, Carbon Dioxide and Methane, University of Saskatchewan, Canada, https://core.ac.uk/display/226146035

Linn, D. M., and 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(6),** 1267–1272. https://doi.org/10.2136/sssaj1984.03615995004800060013x

Millar, N., Robertson, G.P., Grace, P.R., Gehl, R.L., and Hoben, J.P. (2010). Nitrogen fertilizer management for nitrous oxide (N_2O) mitigation in intensive corn (Maize) production: an emission reduction protocol for US Midwest agriculture. *Mitigation & Adaptation Strategies for Global Change*, **15**, 185-204.

Mitchell, D.C., Castellano, M.J., Sawyer J.E., and Pantoja, J. (2013). Cover crop effects on nitrous oxide emissions: role of mineralizable carbon. *Soil Science Society of America Journal*, **77**, 1765-1773.

Mohammed, Y. A., Matthees, H. L., Gesch, R.W., Patel, S., Forcella, F., Aasand, K., Steffl, N., Johnson, B. L., Wells, M. S., and Lenssen, A. W. (2020). Establishing winter annual cover crops by interseeding into maize and soybean. *Agronomy Journal*, **112(2)**, 719– 732. https://doi.org/10.1002/agj2.20062

Moriles-Miller, J., Joshi, D.R., Reicks, G., Westhoff, S., Clay, D.E., and Clay, S.A. (2024). Delaying cover crop termination timing influenced vegetative corn growth and yield in a no-till system. *Agronomy Journal*. (in review).

Moriles-Miller, J., Hansen, S., Horvath, D.P., Reicks, G, Clay, D.E., and Clay, S.A. (2012). Microarray and Growth Analyses Identify Differences and Similarities of Early Corn Response to Weeds, Shade, and Nitrogen Stress. *Weed Science*, **60**, 158-166. https://doi.org/10.1614/WS-D-11-00090.1

Mosier, A.R., Halvorson, A.D., Reule, C.A., and Liu, X.J. (2006). Net global warming potential and greenhouse gas intensity in irrigated cropping systems in northeastern Colorado. *Journal of Environmental Quality*, **35**, 1584–1598.

Myers, R., Weber, A., and Tellatin, S. (2019). Cover crops economics: Opportunities to improve your bottom line in row crops. https://www.sare.org/resources/cover-crop-economics/

Nielsen, D.C., Lyon, D.J., Hergert, G.W., Higgins, R.K., and Holman, J.D. (2015). Cover crop biomass production and water use in the central Great Plains. *Agronomy Journal*, **107**, 2047-2058.

Nleya, T., Chungu, C., and Kleinjan. J. (2016). Chapter 5: Corn Growth and Development. In Clay, D.E., C.G. Carlson, S.A. Clay, and E. Byamukama (eds). iGrow Corn: Best Management Practices. South Dakota State University.

Pattey, E., Blackburn, L.G., Strachan, I.A., Desjardins, R. and Dow. D. (2008). Spring thaw and growing season N_2O emissions from a field planted with edible peas and a cover crop. *Canadian Journal of Soil Science*, **88**, 241-249

Parkin, T.B., Kaspar, T.C., Jaynes, D.B., and Moorman, T.B. (2008). Effect of sampling frequency estimates on cumulative nitrous oxide emissions. *Journal of Environmental Quality*, **37**, 1390-1395.

Parkin, T.B., Kaspar, T.C., Jaynes, D.B, and Moorman T.B. (2016). Rye cover crop effects on direct and indirect nitrous oxide emissions. *Soil Science Society of America Journal*, **80**, 1551–1559. doi:10.2136/sssaj2016.04.0120

Phillips, R. L., Tanaka, D. L., Archer, D. W., and Hanson, J. D. (2009). Fertilizer application timing influences greenhouse gas fluxes over a growing season. *Journal of Environmental Quality, 38***(4)**, 1569-1579. https://doi.org/10.2134/jeq2008.0483

Posit team 2023. RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL http://www.posit.co/.

R Core Team. (2023). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org.

Reese, C.L., Clay, D.E., Clay, S.A., Bich, A.D., Kennedy, A.C., Hansen, S.A., and Moriles, J. (2014). Winter cover crops impact on corn production in semi-arid regions. *Agronomy Journal,* **106**, 1479-1488.

Ruis, S.J., Blanco-Canqui, H., Jasa, P.J., Ferguson, R.B., and Slater, G. (2018). Impacts of early- and late- terminated cover crop on gas fluxes. *Journal of Environmental Quality,* **47**, 1426-1435.

Ruis, S.J., Blanco-Canqui, H., Elmore, R.W., Proctor, C., Koehler-Cole, K., Ferguson, R., Francis, C.A., and Shapiro, C.A. Impacts of cover crop planting dates on soils after four years. *Agronomy Journal*, **112**, 1649-1665.

Sanz-Cobena, A., Garcia-Marco, S., Quemada, M., Gabriel. J.L., Almendros, P., and Vallejo, A. (2014). Do cover crops enhance N_2O, CO_2, or CH_4 from soil in Mediterranean arable systems? *Science of the Total Environment*, **466-467**, 164-174.

SARE (2007). Managing Cover Crops Profitably, 3rd Ed. https://www.sare.org/resources/managing-cover-crops-profitably-3rd-edition (Accessed 10/16/2020)

SARE. *National Cover Crop Survey Report 2022-2023.* Sustainable Agriculture Research and Education; College Park, MD, USA: 2020. https://www.sare.org/wp-content/uploads/2022-2023-National-Cover-Crop-S-urvey-Report.pdf

Saunois, M., Bousquet, P., Poulter, B., Peregon, A., Ciais, P., Canadell, J., and Zhu, Q. 2016. The global methane budget 2000–2012. *Earth System Science Data*, **8**, 697-751.

Seiz, P., Guzman-Bustamante, I., Schulz, R., Müller, T., and Ruser, R. (2019). Effect of crop residue removal on nitrous oxide emissions from a horticulturally used soil in south Germany. *Soil Science Society of America Journal*, **83**, 1399-1409.

Senbayram, M., Chen, R., Budai, A., Bakken L., and Dittect, K. (2011). N_2O emissions and $N_2O/(N_2O+N_2)$ product ratio of denitrification as controlled by available carbon substrat and nitrate concentration. *Agriculture, Ecosystem, and Environment,* **147,** 4-12.

Shan, J., and Yan, X. (2013). Effects of crop residue returning on nitrous oxide emissions in agricultural soils. *Atmospheric Environment*, **71**, 170-175.

Shurpali, N.J., Rannik, U., Jokinen, S., Lind, S., Biasi, C.X., Mammarella, I., Peltola, O., Pihlatie, M., Hyvőnen, N., Raty, M., Haapanala, S., Zahniser, M., Virkajärvi, P., Vesala, T., and Martikainen, P.J. (2016). Neglecting diurnal variation leads to uncertainties in terrestrial nitrous oxide emissions. *Scientific Reports,* **6**, 25739.

Smeltekop, H., Clay, D.E., and Clay, S.A. (2002). The impact if intercropping annual ”sava” snail medic on corn production. *Agronomy Journal,* **94,** 917-924.

Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, O. Sirotenko, 2007: Agriculture. In Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [B. Metz, O.R. Davidson, P.R. Bosch, R. Dave, L.A. Meyer (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Smith, C., Z.R.J. Nicholls, K. Armour, W. Collins, P. Forster, M. Meinshausen, M.D. Palmer, and M. Watanabe, 2021: The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity Supplementary Material. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Available from https://www.ipcc.ch/.

Soil Survey Staff 2018. Natural Resources Conservation Service. United States Department of Agriculture. Web Soil Survey. Retrieved from https://websoilsurvey.sc.egov.usda.gov/

Stevens, R.J., Laughlin, R.J., Burns, L.C., Arah, J.R.M., and Hood, R.C. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology & Biochemistry,* **29**, 139–151. https://doi.org/10.1016/S0038-0717(96)00303-3

Sullivan, B.W., Selmants, P.C., and Hart, S.C. (2013). Does dissolved organic carbon regulate biological methane oxidation in semiarid soils? *Global Change Biology*, **19**, 2149-2157. doi:10.1111/gcb.12201

Thies, S., Bruggeman, S., Clay, S.A., Mishra, U., Hatfield, G., Kumar, S. and Clay, D.E. (2019). Midmorning point sampling may not accurately represent nitrous oxide emission following fertilizer application. *Soil Science Society of America Journal*, **83**, 339-347

Thies, S., Joshi, D.R., Bruggeman, S.A., Clay, S.A., Mishra, U., Moriles-Miller, J. and Clay, D.E. (2020). Fertilizer timing affects nitrous oxide, carbon dioxide, and ammonia emissions from soil. *Soil Science Society of America Journal*, **84**, 115-130.

Thomas, B.W., Hao, X., Larney, F.J., Goyer, C., Catigny, M.H., and Charles, A (2017). Non-legume cover crops can increase nongrowing season nitrous oxide emissions. *Soil Science Society of America Journal*, **81**, 189-199.

Topp, E. and Pattey, E. (1997). Soils as sources and sinks for atmospheric methane. *Canadian Journal of Soil Science,* **77**, 167-178.

USDA National Agricultural Statistics Service, 2022 Census of Agriculture. Complete data available at https://www.nass.usda.gov/Publications/AgCensus/2022/index.php.

Ussiri, D.A.N., Lal, R., and Jarecki, M. (2009). Nitrous oxide and methane emissions from long- term tillage under a continuous corn cropping system in Ohio. *Soil and Tillage Research,* **104**, 247-255.

van den Pol-van Dasselaar, A., van Beusichem, M.L. and Oenema, O. (1998). Effects of soil moisture content and temperature on methane uptake by grasslands on sandy soils. *Plant and Soil* **204**, 213–222. https://doi.org/10.1023/A:1004371309361

Venterea, R. T., and 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

Venterea, R. T., Coulter, J. A., and Dolan, M. S. (2016). Evaluation of Intensive "4R" Strategies for Decreasing Nitrous Oxide Emissions and Nitrogen Surplus in Rainfed Corn. *Journal of Environmental Quality*, ***45***, 1186-1195. https://doi.org/10.2134/jeq2016.01.0024

Veum, K.S., Lorenz, T., and 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

Wang, T, Xu, Z., Kolady, D., Ulrich-Schad, J.D., and Clay, D.E. (2020). Cover crop usage in South Dakota: Farmer perceived profits and future adoption decisions. *Journal of Soil Water Conservation*. In press.

Wegner, B.R, Chalise, K.S, Lai, S.S, Abagandura, G.O., Kumar, S. Osborne, S.L., Lehman, R.M., and Jagadamma, S. (2018). Response of soil surface greenhouse gas fluxes to crop residue removal and cover crops under corn-soybean rotation. *Journal of Environmental Quality,* **47**, 1146-1154.

Wegner-Riddle, C., Congreves, K.A., Abalos, D, Berg A.A., Brown, S.E., Ambadan, J.T., Gao, X., and Tenuta, M. (2017). Globally important nitrous oxide emissions form croplands induced by freeze-thaw cycles. *Nature Geoscience,* **10**, 279-283,

Weier, K.L., Doran, J.W., Powers, J.F., and Walters, D.T. (1993). Denitrification and the dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. *Soil Science Society Journal*, **57**, 66-72.

Weiler, D.A., Tornquist, C.G., Zschornack, T., Ogle M., Carlos, F.S., and Bayer, C. (2018). Daycent simulation of methane emissions, grain yields, and soil organic carbon in subtropical paddy rice system. Revista Brasileira de Ciência do Solo, DOI: 10.1590/18069657rbcs20170251

Wilson, M.L., Baker, J.M., and Allan, D.L. (2013). Factors affecting successful establishment of aerially seeded winter rye. *Agronomy Journal,* **105**, 1868-1877. doi:10.2134/agronj2013.0133

Xiangygin, N., and Groffman, P.M. (2018). Declines in methane uptake in forest soils *PNAS*, **115**, 8587-8590.