Nitrous Oxide Emission Reduction in an Annual Cropping System as a function of Nitrification Inhibitors and Liquid Manure Injection Timings

by

Sisi Lin

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Soil Science

Department of Renewable Resources
University of Alberta

Abstract

Nitrous oxide (N_2O) contributes to global warming and ozone depletion. Two-thirds of the global N_2O emissions are derived from agricultural soils receiving manure or fertilizer applications. The goal of this study was to identify and develop management practices that can decrease N_2O emissions from manured soils. We tested two times of liquid manure injections (early fall versus late spring) and two nitrification inhibitors (NIs; nitrapyrin vs. DMPP). We conducted two field experiments in central Alberta (Lacombe and Edmonton), Canada over a period of 13 months and a 28-day laboratory incubation. Barley (*Hordeum vulgare* L.) for silage was planted, and productivity and N uptake were recorded. Soil ammonium and nitrate concentrations and N_2O fluxes were repeatedly monitored. First-order kinetic models represented well mineral N transformations in both field and incubation experiments, with the exception of fast nitrate depletion rates which were better depicted by second-order models. Compared to the controls, field N_2O emissions were increased by manure application (on average 3.15 vs. 0.48 kg N ha^{-1} yr^{-1} at both sites), but emissions were sharply reduced with NIs. For instance, in our Lacombe site, fall manure treated with DMPP reduced annual N_2O emissions by 81%, and nitrapyrin reduced emissions by 58%. The emission reductions caused by NIs were also evident in the spring manure field treatments, our incubation, and at our Edmonton site, but the reductions were in general smaller possibly due to prevailing drier conditions - in particular during mid spring in Edmonton. Compared to the spring manure timing, fall manure without NIs resulted in an approximate two-fold increase in N_2O emissions, due to major peak fluxes following the early spring snow-melt, which accounted for at least 65% of the annual N_2O emissions. Fall manure timing also reduced plant productivity and N uptake. In summary, spring-applied manure with

NIs can mitigate N_2O emissions in Alberta's agriculture and in regions with comparable agro-ecological conditions.

Table of Contents

List of Tables

List of Figures or Illustrations

1 General Introduction

Nitrous oxide (N_2O) - one of the main biogenic greenhouse gases (GHGs) - plays an important role in climate change with a global warming potential of 298 times higher than carbon dioxide over a 100 year horizon (Myhre et al. 2013). In addition, N_2O is the largest stratospheric ozone destroyer and this detrimental effect is expected to last throughout the 21st century (Ravishankara et al. 2009). Soils amended with manures or chemical nitrogen (N) fertilizers have been reported as an important source for global N_2O budget, accounting for about two-thirds (1851.3 Tg CO_2e) of total N_2O emissions worldwide in 2005 (USEPA 2012). With an increasing use of global N fertilizer (FAO 2015), total N_2O emissions are projected to experience a substantial increase (Kim et al. 2013; Hoben et al. 2011; Velthof and Mosquera 2011), further aggravating global climate change and ozone layer deterioration.

It is commonly accepted that autotrophic nitrification (Bremner and Blackmer 1978; Butterbach-Bahl et al. 2013) and heterotrophic denitrification (Braker and Conrad 2011) are the two major soil microbial transformations responsible for N_2O emissions. NH_4^+ or NH_3 could be oxidized to NH_2OH, NO_2^- and finally to NO_3^- via autotrophic nitrification associated with aerobic microorganisms, and then the NO_3^- could be reduced to NO_2^-, NO, N_2O or N_2 via denitrification mediated by anaerobic microorganisms (Butterbach-Bahl et al. 2013). In the autotrophic nitrification, N_2O can be generated from the oxidation of NH_2OH or NO_2^- (Butterbach-Bahl et al. 2013). Hence, the potential N_2O losses are subject to the factors influencing microbial activities involved in nitrification and denitrification processes, such as soil pH, substrates for nitrification and denitrification (Havlin et al. 2014), soil moisture and temperature (Luo et al. 2013).

The optimum pH range for autotrophic nitrification was reported from 6.5 to 8, and denitrification generates more N_2O as the soil pH decreases (ŠImek and Cooper 2002). Soil moisture plays an important role in controlling N_2O emissions (Zheng et al. 2000), because it is closely related with dissolved oxygen, an essential element for soil microbial community (Schindlbacher et al. 2004; Meixner and Yang 2006). It was reported that 50-70% water-filled pore space (WFPS) caused the largest amount of N_2O fluxes derived by both autotrophic nitrification and denitrification (Davidson et al. 1991; Davidson et al. 2000). Denitrification is favored by an even higher WFPS (Dobbie et al. 1999; Zhu et al. 2013) and completely anaerobic conditions lead to a shift of end products from N_2O to dinitrogen (N_2) (Meixner and Yang 2006; Morley and Baggs 2010). Moreover, soil temperature has a positively quadratic relationship with the quantity of N_2O produced (Smith et al. 2003; Phillips et al. 2015) not only because an increasing temperature can enhance N_2O-related microbial and enzymatic activities but also it accelerates metabolic respiration (Butterbach-Bahl et al. 2013), utilizing the O_2 in the soil and accordingly motivating denitrification and increasing N_2O production.

Nitrification inhibitors (NIs) have been proposed as a means for enhancing N usage efficiency in cropping systems by incorporating them in manure or fertilizer applications (Zerulla et al. 2001). The principle of NIs is to retard the nitrification by inhibiting nitrifying-related microbial activities and accordingly keeping soil mineral N in the form of ammonium instead of nitrate (Subbarao et al. 2006) within a limited duration of this delaying effect (Zerulla et al. 2001). Numerous previous studies have shown that the positive effect of NIs on N usage efficiency in terms of decreased leaching, increased crop yield and reduced N-related emissions (Prasad and Power 1995; Wolt 2004; Hua et al. 2008; Menéndez et al. 2012; Burzaco et al. 2014). Nitrapyrin, dicyandiamide (DCD) and DMPP are three commonly available NIs in agriculture; and due to the high solubility in water of DCD (Subbarao et al. 2006), DMPP and nitrapyrin have been previously mixed with liquid manures applied to the soil (Thompson et al. 1987; Menéndez et al. 2006).

Other possible strategies for mitigating N_2O emissions derived from manures or fertilizers have been previously studied, including application method and timing. No tillage has been shown to not only conserve soil N (Spargo et al. 2008) but also minimize N_2O emissions (Baggs et al. 2003) in particular in relatively dry environments (Rochette et al. 2008). The implementation of shallow manure injection, a minimum soil disturbance practice, is expected to reduce NH_3 volatilization (Laboski et al. 2013), and moreover, it has certain potential to mitigate N_2O emissions (Flessa and Beese 2000), by favoring anaerobic conditions conducive to emitting a greater proportion of denitrified N_2 rather than N_2O (Smith and Mukhtar 2015) especially in wetter soils. The depth of manure injection in the soil profile has been recommended to be at least 5 cm (Nyord et al. 2008); shallower injections can result in increased NH_3 losses (Rodhe and Etana 2005). Besides the conservation of N in soil, manure injection could cause a band effect, and consequently, an unequal spatial distribution of manure nutrients (Chen et al. 2010). Chen et al. (2010) found that the crop rows close to manure bands had greater plant biomass and total N uptake than that farther away from manure bands. This finding could imply the potential uneven N-related emissions between manure band and interband areas.

In temperate regions, spring and fall have been reported as the two most common times of the year for dairy and hog slurry applications (Beaulieu 2004), and this timing effect on N_2O emissions, leaching and ammonia volatilization has been previously investigated by several studies. Some of these studies showed that the fall/winter slurry application caused a higher risk of nitrate leaching (Weslien et al. 1998; van Es et al. 2006; Thorman et al. 2007) and N_2O

emissions (Weslien et al. 1998; Thorman et al. 2007), but lower risk of NH_3 emissions (Weslien et al. 1998). However, their observations were inconsistent with other studies in which higher N_2O emissions were observed in the spring compared to fall manure applications (Rochette et al. 2004; Hernandez-Ramirez et al. 2009a).

Despite the fact that numerous studies have independently addressed the impact of either NIs or manure timing on N-related losses, plant biomass and N uptake, to date, the management effects of manure timing coupled with NIs have not been well documented. In addition, a first-order kinetic model has been proposed to describe ammonium transformations in soils (Irigoyen et al. 2003; Omonode and Vyn 2013); nonetheless, second-order kinetic modeling has never been tested when assessing mineral N transformations in soils. Therefore, the objectives of this thesis were (i) to compare the performance of first- versus second-order kinetic models in representing soil mineral N transformations, (ii) to investigate the key drivers for large N_2O emissions, and (iii) to explore the impact of manure timing (fall versus spring) coupled with NIs (DMPP vs. nitrapyrin) on manure-N usage efficiency in terms of N_2O emissions, barley aboveground biomass and N uptake in an annual cropping system receiving liquid manure inputs in central Alberta, Canada.

2 Times of Manure Injection and Nitrification Inhibitors Impacts on Nitrous Oxide Emissions and Nitrogen Transformations in a Barley Crop

2.1 Abstract

Mitigating greenhouse gas (GHG) emissions that arise during the utilization of livestock manure as a nutrient source in agricultural systems is a crucial environmental challenge. We evaluated the management effects of two times (fall versus spring) of liquid manure injection and the addition of two nitrification inhibitors [NIs; 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) vs. 3, 4-dimethylpyrazole phosphate (DMPP)] on nitrous oxide (N_2O) emissions, soil mineral nitrogen transformations, barley (*Hordeum vulgare* L.) biomass productivity and N uptake, and estimated system N balance. Eight treatments were established in split-plot designs in two field sites located in Lacombe and Edmonton, Alberta, Canada. Repeated measurements included N_2O fluxes by manual static chambers, and soil ammonium and nitrate concentrations. Most of the dynamics of soil mineral N were well described by first-order kinetics although the rapid nitrate depletion in the fall-manured soils was even better depicted by second-order models. Relative to the controls, manured soils without NIs resulted in substantial N_2O emissions, and the use of NIs with fall injections significantly reduced these annual N_2O emissions by 81% with DMPP and 58% with nitrapyrin; spring injections exhibited comparable treatment effects, but with narrower magnitude differences. These beneficial effects were particularly discernable at the Lacombe site which was relatively wetter than Edmonton, indicating the overriding role of varying moisture on N dynamics and fluxes. Following a six-month period of frozen soil over winter, the spring snow-melting caused at least two-thirds of the annual N_2O emissions from the fall-manured soils, and these intense-episodic fluxes also revealed that fall-added NIs still continued active in the early spring. Yet, fall injections without NIs led to about double the N_2O emissions compared to the corresponding spring treatments. Moreover, spring manure increased barley dry matter yield and N uptake. Implementing spring injections with NIs can effectively improve N use by crops and diminish global warming effect. Overall, annual N budgets suggested that up to half of the manure-added N remains in the soil profile in immobilized forms which could be re-mineralized in subsequent years.

Keywords: liquid manure, kinetic models, nitrous oxide, timing, nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), 3, 4-dimethylpyrazole phosphate (DMPP), N balance.

2.2 Introduction

With an increasing global demand of livestock products (Herrero et al. 2009), the appropriate disposal of abundant manure has become a great concern. One feasible solution is to apply the manure to the soil since the manure is conductive to biomass productivity increases as a source of N (Hernandez-Ramirez et al. 2009a). However, this practice can lead to serious environmental issues at the same time (Basso and Ritchie 2005; Webb et al. 2010). It has been reported that manured or synthetic N fertilized soils account for 67% (1851.3 Tg CO_2e) of global total nitrous oxide (N_2O) emissions from agriculture in 2005 (USEPA 2012). N_2O plays a dominant role in global warming with a 100-year global warming potential (GWP) value of 298 (GWP for CO_2 is 1) (Myhre et al. 2013), as well as in stratospheric ozone depletion (Ravishankara et al. 2009).

In soils, autotrophic nitrification (Bremner and Blackmer 1978; Butterbach-Bahl et al. 2013) and denitrification (Braker and Conrad 2011) are two major pathways to generate N_2O. Soil moisture is one of the crucial environmental drivers for N_2O fluxes (Zheng et al. 2000), since it is interrelated with the oxygen consumption by the soil microbial community (Schindlbacher et al. 2004; Meixner and Yang 2006), and oxygen serves as an electron acceptor in nitrification (Velusamy and Krishnani 2013). Autotrophic nitrification tends to be a predominant pathway in producing N_2O under lower water-filled pore space (WFPS) (i.e., 35-60%) (Davidson and Schimel 1995; Bateman and Baggs 2005). It has been found that the heterotrophic denitrification rate increased with a higher soil water content (Luo et al. 2000), and the end product depends on the level of anaerobic conditions. It has been proposed that the maximum amount of dinitrogen (N_2) and greatest decrease of N_2O were observed at an absolutely restricted aeration condition (i.e., ~2% O_2 v/v) (Morley and Baggs 2010). Additionally, soil temperature is another dominant contributor for N_2O fluxes. As the temperature increased by 10 °C, the N_2O production was observed to be two times (Phillips et al. 2015) or even higher times (Dobbie and Smith 2001).

The identification of best management practices for manure applications provides potential opportunities to mitigate N_2O emissions, and address the global warming effect. Liquid manures are best injected into the soil profile instead of surface spreading since this incorporation not only decreases ammonia volatilization (Laboski et al. 2013) but also increases the proportion of N_2 derived from denitrification (Smith and Mukhtar 2015) because of less available O_2 in deeper soil . Moreover, with the purpose of enhancing fertilizer-N usage efficiency, the addition of nitrification inhibitors (NIs) with manure applications has been proposed (Zerulla et al. 2001). Effective NIs widely used in agriculture include nitrapyrin, dicyandiamide (DCD) and DMPP (Subbarao et al. 2006). The function of NIs is to delay the oxidation reaction by depressing the nitrifiers activities (Subbarao et al. 2006); hence the subsequent process, denitrification, would be restricted by a low concentration of nitrate as substrate (Saggar et al. 2013), preventing gaseous N losses. The majority of both dairy and hog manure is typically applied to the soil in the spring and fall seasons in temperate regions (Beaulieu 2004), thus N losses from manure could be hypothetically driven by manure application timing (Chadwick et al. 2011). A study estimated the nitrate losses by leaching and found that a fall/winter slurry application resulted in higher nitrate losses than a spring slurry application (van Es et al. 2006). Another recent study found that the fall/winter cattle slurry application increased direct N_2O emissions from free draining grassland soils in England compared to a spring application (Thorman et al. 2007).

Even though several earlier studies have examined the effect of manure application timing on N_2O emissions (Allen et al. 1996; Weslien et al. 1998; Rochette et al. 2004), it is still unclear if the addition of NIs would amplify, narrow, or even eliminate the difference in N_2O emissions between the fall and spring manure applications. Therefore, the objectives of this study were (i) to investigate the effects of liquid manure application timing (fall versus spring) and NIs (nitrapyrin vs. DMPP) on N_2O emissions, soil mineral N concentrations and transformations, barley biomass productivity and N uptake, and estimated N budgets (ii) to examine the dominant environmental controls on N_2O emissions, and (iii) to evaluate the usefulness of first- vs. second-order kinetic models in depicting and representing the transformations of mineral N in soils receiving high manure inputs and treated with inhibitors.

2.3 Materials and Methods

2.3.1 Site Characteristics and Field Experiment Design

Two field sites were established in Lacombe (52°27’17’’N, 113°44’20’’W) and Edmonton (53°29’30’’N, 113°31’53’’W), Alberta, arranged in a split-plot experimental design. Treatments were replicated four and three in Lacombe and Edmonton, respectively. Soil classifications are Orthic Black Chernozem for Lacombe and Black Chernozem for Edmonton. Prior to treatment establishment, the soil in Lacombe had a sandy clay loam to clay loam texture, a 1.22 g cm^{-1} bulk density and a 7.0 pH, and the soil in Edmonton had a clay to heavy clay texture, a 1.15 g cm^{-1} bulk density and a 6.1 pH. The climate of these two sites is semi-arid continental.

Two types of NIs were admixed and applied with the liquid manure: 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin) and 3, 4-dimethylpyrazole phosphate (DMPP). Six manure treatments and two controls (without any manure added) were established at each experimental site. The eight treatments were: control where the soil disturbed using the manure injector (CT), control without any soil disturbance (CZ), fall-manured soil with no NIs added (FW), fall-manured soil with DMPP (FD), fall-manured soil with nitrapyrin (FN), spring-manured with no NIs added (SW), spring-manured soil with DMPP (SD), and spring-manured soil with nitrapyrin (SN).

A coulter manure injector was used to establish CT and also to apply the liquid manure in the six treatments receiving manure. All injections were conducted at a constant volume rate of 56.17 m^3 manure ha^{-1} and 0.4 kg active compound NIs ha^{-1}. The NIs were evenly added and mechanically agitated with the liquid manure prior to manure injections. The injector created ~2.5 cm width and ~12.7-15.2 cm deep injection bands, as well as ~28 cm spacing between consecutive bands. Manure samples were taken at each time of manure applications for total N, NH_4^+-N and water content analyses. Lacombe received liquid swine manure on 6 Oct 2014, 25 May 2015 and 28 Sep 2015, while Edmonton received liquid dairy manure on 30 Sep 2014, 12 May 2015 and 7 Oct 2015.

Barley for silage was planted at 300 seeds m^{-2} on 24 and 11 May 2015 in Lacombe and Edmonton, respectively. To quantify aboveground biomass and plant N uptake, the crop was harvested on 11 Aug and 28 Jul 2015 in Lacombe and Edmonton using a forage harvester, respectively. Crop phenology was recorded and photos were taken for each treatment on the dates of N_2O flux measurements.

2.3.2 Field Chamber Management

A manual static chamber method (Hernandez-Ramirez et al. 2009a) was used to measure the field N_2O flux at both sites. The chamber bases (15 cm in height, 65.5 cm in length and 17.0 cm in width) were installed in the middle of each plot inserted in the soil with a depth of 5 cm perpendicular to the manure injection rows. Each chamber encompassed two manure injection rows. The flux measurement frequency was two times weekly after manure injections, major precipitations or during early spring-thawing period; otherwise, it was one flux measurement per week. In order to improve the consistency of gaseous flux estimations, gas samples were collected within the period between 11 am and 3 pm. Three gas samples were collected for each chamber at 16, 32 and 48 minutes. To represent the time zero of chamber closure, three ambient gas samples were randomly collected on each date of flux measurements at 10 cm above the ground surface.

The field N_2O flux rate was determined by plotting a linear or a quadratic relationship between measured N_2O concentrations versus time (as mentioned above, concentrations in ambient gas samples were assumed as time 0). Zero N_2O flux rate was assumed if there was a non-significant relationship (this statistical decision followed an alpha critical threshold of 0.20); otherwise the flux rate was calculated by the modified ideal gas law as follows:

$$\boldsymbol{Flux} = \frac{\boldsymbol{S} * \boldsymbol{P} * \boldsymbol{V} * \boldsymbol{A}^{-1}}{\boldsymbol{R} * \boldsymbol{T}} \qquad [2\text{-}1]$$

where Flux is the N_2O flux rate (μmol min^{-1} m^{-2}); S is identified as the slope of the line from a simple linear regression, or the first-order derivative at time zero for a quadratic regression curve (Yates et al. 2006; Pennock et al. 2010) (μL L^{-1} min^{-1}); P is the pressure of the gas (atm); V is the volume of the gas chamber (L); A is the surface area of the gas chamber (m^2); R is the gas constant (atm μL K^{-1} μmol^{-1}) and T is the temperature of the gas (K).

We recorded that 72, 18 and 10 % of the N_2O flux measurements in Lacombe were calculated by linear, quadratic and zero regressions, respectively. Likewise, 79, 13 and 8% of the N_2O flux measurements in Edmonton were calculated by linear, quadratic and zero regressions, respectively. The cumulative emissions between two consecutive sampling dates were assumed to equal the product of the average N_2O flux rate and the time interval between the two dates. For the estimation of annual cumulative emissions (Fig. 2-1), flux quantities were assumed to be negligible during the winter months (e.g., November to March) due to freezing ambient temperatures leading to minimal soil biological activity and gaseous transport processes. The annual cumulative emissions for the spring treatments (Fig. 2-1) were estimated by using the measured fluxes in spring and fall 2015 plus the flux data from the control without disturbance (CZ) in fall 2014 and early spring 2015.

The efficiency of NIs in reducing cumulative N_2O emissions was calculated as a reduction coefficient as follows:

$$\boldsymbol{RC = \frac{E_{control} - E_{inhibitor}}{E_{control}} * 100\%} \qquad [2\text{-}2]$$

where RC is the reduction coefficient (%); $E_{control}$ is the N_2O emissions from the manured soil without inhibitors and $E_{inhibitor}$ is the N_2O emissions from the manured soil with inhibitors.

2.3.3 Soil Sample Collection

We conducted repeated soil samplings at the Edmonton site to determine any differences in the temporal changes of ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentrations as a function of experimental treatments and environmental conditions. Composite (≥ 3) 0-15 cm soil samples were collected from each plot during three periods (i.e., fall 2014, spring 2015 and fall 2015) using a push probe (i.d. 2.5 cm). As the coulter manure injector creates injection bands, soil samples were collected from both the injection row and non-injection areas. Soil samplings were conducted prior to and about one month after the manure injection in fall 2014 and fall 2015. Also, seven soil samplings took place during the spring and early summer over a period of 83 days, including one sampling before and six samplings (about every two weeks) following the spring manure injection on 12 May 2015. The collected soil samples were stored at 5°C. Prior to the laboratorial analyses, soil samples were air-dried and grinded through a 2 mm sieve.

As mentioned above, the distance between manure injection bands was about 28 cm spacing, and the width of the manure band was about 2.5 cm. Due to these injection rows created by the manure injector, the percentage (or area weights) of the manure injection and non-injection rows were estimated and used to as necessary calculate spatially-aggregated soil mineral N concentrations (i.e., 8.2 and 91.8% for injection and non-injection area, respectively).

First- and second-order kinetic models were fitted to our time series of nutrient concentrations with the purpose of describing and interpreting the soil mineral N transformations. Model evaluation was conducted using coefficient of determination (R^2) and p-values as criteria. Because the relationship between the changes in soil ammonium and time is typically a curve, the first-order kinetic model (Irigoyen et al. 2003; Omonode and Vyn 2013) has been proposed to depict the ammonium depletion patterns and rates. Moreover, second-order kinetic modelling has been also conducted to capture and represent both soil ammonium and nitrate transformations with time in an incubation study (See chapter 3). The first-order kinetic model assumes that there is a significant linear relationship between the natural logarithm of mineral N (i.e., NH_4^+-N or NO_3^--N) concentration versus time (Eq. [2-3]), whereas the second kinetic model assumes a significant linear relationship between the reciprocal of mineral N versus time (Eq. [2-4]).

$$\boldsymbol{k_1 = \frac{lnC_o - lnC_t}{t}} \qquad [2\text{-}3]$$

$$\boldsymbol{k_2 = \frac{1}{C_t * t} - \frac{1}{C_o * t}} \qquad [2\text{-}4]$$

where k_1 is the first-order constant rate or kinetic (day^{-1}); k_2 is the second-order constant rate or kinetic (mg^{-1} N kg day^{-1}); C_o is the initial concentration of NH_4^+-N or NO_3^--N (mg kg^{-1}) and C_t is the concentration of NH_4^+-N or NO_3^--N at time t (day).

2.3.4 Soil Sensor Installation and Measurements

We deployed 5TM sensors and Em50 data loggers to measure the average half-hour soil temperature and moisture content data for each field treatment in the Edmonton site. The installation of the sensors and data loggers was accomplished within 24 hours following manure injections. Two 5TM sensors were installed in each plot horizontally at 10 cm and 20 cm below ground surface. The middle prong of the sensor was established at these target soil depths. The ECH2O utility software was used to collect the soil temperature and moisture content data.

2.3.5 Laboratory Analyses

The N_2O concentration in gas samples was measured by an electron capture detector in a Laurier Varian 3800 gas chromatograph. The minimum analytical detectable N_2O flux was 2.84 g N ha^{-1} day^{-1}. A 2M KCl solution was used to extract the NH_4^+ and NO_3^- from the soil samples; subsequently, the filtrate was colorimetrically evaluated using a SmartChem discrete wet chemistry analyzer. Aboveground barley dry matter biomass was determined using oven dry weight, and barley N concentration was quantified using near infrared spectroscopy (Hernandez-Ramirez et al. 2011).

2.3.6 Statistical Analyses

The Shapiro-Wilk test was conducted to examine the data normal distribution. The Bartlett or Levene tests were conducted to examine homogeneous variances. The Box-Cox Power transformation was used if the data did not fulfill these assumptions. One-way analysis of variance (ANOVA) and a Fisher's least significant difference (LSD) test were conducted to assess differences in (1) total N, ammonium and water content of the applied liquid manure among the applications; (2) dry matter yield and plant N uptake among CZ, CT, FW and SW treatments; (3) dry matter yield and plant N uptake among fall-manured soils or spring-manured soils; (4) annual cumulative N_2O emissions among CZ, CT, FW and SW treatments and (5) annual cumulative N_2O emissions among fall-manured soils or spring-manured soils at both sites. The differences in total N, ammonium and water content in the applied liquid manure between two sites were determined by a two sample t test. The linear relationship between the logarithm or reciprocal of soil NH_4^+-N or NO_3^--N concentrations versus time were tested by a simple linear regression model. All statistical tests for treatment effects were performed at a 90 or 95% confidence interval. All analyses were analyzed using the version 3.1.3 R software. A split-plot linear model was applied to test the effect of manure timing, nitrification additives and their interactions on annual cumulative N_2O, plant dry matter yield and plant N uptake.

2.4 Results

2.4.1 Nitrous Oxide Emissions

One-year cumulative N_2O emissions were measured in both Lacombe and Edmonton sites to investigate the effects of manure injection timing and nitrification inhibitors (NIs) (Fig. 2-1). Compared to the control treatments (average 0.3±0.1 and 0.6±0.2 kg N ha^{-1} yr^{-1} in Lacombe and Edmonton, respectively), adding the fall liquid manure without NIs (FW) resulted in a significant increase in annual N_2O emissions (Fig. 2-1). This significant difference, nevertheless, was not found in the spring manure without NIs (SW) at both sites (Fig. 2-1). Even though there was no statistically significant effect of manure injection timing on the annual N_2O emissions (Table 2-1 and Fig. 2-1), the amount of annual N_2O emissions derived from fall manure without NIs was more than double than that from spring manure without NIs at both sites (i.e., 6.2±3.7 vs. 3.1±1.0 in Lacombe and 2.3±0.7 vs. 1.0±0.2 kg N ha^{-1} yr^{-1} in Edmonton, respectively). Even with consideration of the manure N inputs, the larger fraction of annual N_2O emissions to N inputs was still observed in the fall manure without NIs at both sites (Table 2-2).

Based on ANOVA results, there was a significant effect of NIs on the annual cumulative N_2O emissions in our Lacombe site ($P<0.05$), whereas there was no such significant effect in Edmonton (Table 2-1 and Fig. 2-1). At the Lacombe site, the magnitude of our calculated reduction coefficients (Eq. [2-2]) for the fall manure treatments with DMPP (FD) and nitrapyrin (FN) were 81.0 and 57.8%, respectively (Fig. 2-1). Furthermore, the reduction coefficients were less pronounced for the spring manure treatments (i.e., 64.3% for DMPP, and 32.7% for nitrapyrin; Fig. 2-1). Overall, it should be highlighted that all soils receiving liquid manure with NIs resulted in annual mean N_2O emissions consistently similar to or lower than 2.6 kg N ha^{-1} yr^{-}

[1](Fig. 2-1). Moreover, when contrasting our field sites, the annual N_2O emissions derived from both the fall and spring manure without NIs (FW and SW treatments) for Lacombe were about three times larger than for Edmonton (Fig. 2-1). Additionally, N_2O emissions for all fall manure treatments (FW, FN and FD) from 27 Mar to 10 Apr 2015 corresponded to 78% of the annual emissions in the Lacombe site (Fig. 2-2b), and emissions from 27 Mar to 14 Apr 2015 represented 65% of the annual emission in the Edmonton site (Fig. 2-3b). Substantial N_2O emissions from all spring manure treatments were also found at both sites within May 2015 following the spring manure injections (Fig. 2-2b and Fig. 2-3b) when the average monthly temperature was around 11°C and the cumulative precipitation was about 21 mm (Fig. 2-4).

2.4.2 Soil Mineral Nitrogen and Kinetics

In order to investigate the amount and temporal changes of NH_4^+ and NO_3^- concentrations in the surface soil (i.e., 0-15 cm depth increment), repeated topsoil samples were collected in the Edmonton site in the spring-summer 2015. From 13 May to 20 Jul 2015, we found a general trend for gradual decline in NH_4^+ concentrations in particular for the treatments exhibiting a high initial concentration, such as the fall manure with DMPP and certain spring manure treatments (Fig. 2-5b and e). Similarly, relatively quicker depletion patterns were observed for NO_3^- concentrations for all fall and spring manure treatments (Fig. 2-5h and k). However, it should be emphasized that these nitrate depletion progressions were delayed by approximately 15 days compared to the ammonium depletion patterns (Fig. 2-5h and k). Unlike most of the treatments, NH_4^+ concentration in the spring manure with DMPP moderately increased during 42 days following the spring manure injection, and subsequently, decreased sharply on 7 Jul 2015 (Fig. 2-5e). In particular, it took longer for the spring manure treatments than for the fall manure treatments to reach asymptotic depletion plateaus for both NH_4^+ and NO_3^- concentrations within the spring 2015 (i.e., about 42 vs. 31 days; Fig. 2-5). During the interval from 13 to 27 May 2015, the amount of NO_3^- present in the soil treated with fall manure injection with DMPP additive was in general three times larger than in the soil receiving spring manure with DMPP (FD ≈ 3 SD; Fig. 2-5h and k).

Our measured, temporal patterns of soil mineral N transformations – ammonium and nitrate depletion progressions – were fitted and modelled by both first- and second-order kinetic models. When focusing on the experimental treatments that had p-values lower than 0.1, the coefficients of determination (R^2) for nitrate depletions using second-order kinetic were relatively higher than when employing first-order kinetic, with the only exception of SW treatment (Table 2-3). Conversely, regarding ammonium depletion progressions, there was no clear differentiation in the performance of first- vs. second-order kinetic when using R^2 and p-values as model evaluation criteria (Table 2-3).

Compared to the two control treatments receiving neither manure nor additive, nearly all soils receiving manure injections either in the fall or spring and with or without NIs required in general longer periods to reach and settle into depletion plateaus for both NH_4^+ and NO_3^- concentrations, with only few noticeable exceptions (FN and SW) (Table 2-3, Fig. 2-5). The control zero (CZ) had comparatively faster soil mineral N depletion rates than the control treated with manure injector disturbance (CT) (Table 2-3). Overall, the fall manure DMPP treatment had the fastest rates of mineral N depletion among the fall manure treatments based on both first- and second-order kinetic models, while the spring manure without NIs exhibited the fastest rates of mineral N depletion among the three spring manure treatments (Table 2-3).

2.4.3 Plant Yield and Nitrogen Uptake

There was a significant effect of the manure injection timing on both aboveground barley dry matter yield and plant N uptake in the Lacombe site (*Ps*<0.05), but there were no significant effects of nitrification inhibitor additions at both sites (Table 2-1). In Lacombe, the soils receiving spring manure amendment resulted in higher aboveground plant dry matter yield and plant N uptake than the soils treated with fall manure (Table 2-4). In further details, when comparing the fall manure treatments, the amount of N uptake under fall manure with DMPP was significantly lower than for fall manure without NIs (FD was 82% of FW; Table 2-4).

2.4.4 Weather and Soil Characteristics

Annual total precipitation in 2015 was 380 mm in Lacombe and 294 mm in Edmonton (Fig. 2-4c and d). The monthly average temperature from Oct 2014 to Nov 2015 was higher than the corresponding normal values in both Edmonton and Lacombe with the exceptions of Nov 2014, as well as Feb and Sep 2015 (Fig. 2-4a and b). The monthly precipitation was generally lower than the normal values during the growing season (Apr – Aug 2015) and higher over the cold months with the exceptions of Oct and Dec 2014, and Oct and Nov 2015 at both sites (Fig. 2-4c and d). A predominantly drier condition over the experimental period was even more obvious in the Edmonton site during the spring 2015, when each monthly precipitation over the growing season (i.e., Apr – Aug) was only about half of the corresponding normal average (Fig. 2-4d). The majority of highest soil temperature occurred through Jun – Sep (Fig. 2-6a), while the highest soil average volumetric water content occurred following the snow melt and soil thawing in April with a range of 0.24-0.28 m^3 m^{-3} (Fig. 2-6b).

2.4.5 Manure Characteristics

Reflecting the variability in manure properties, there was a significant difference in both total N and ammonium application rates across the three different times of manure injection in the Lacombe site ($P<0.05$) in general (Table 2-5). More specifically, the highest total N and ammonium loads were observed in the spring 2015, while the lowest manure-N rates were quantified in the fall 2014 (Table 2-5).

2.5 Discussion

2.5.1 Effectiveness of Manure Timing and Nitrification Inhibitor Choices

Our results showed that the fall manure injection led to a lower N input usage efficiency as indicated by a higher annual cumulative N_2O losses from fall-manured soils compared to the spring manure injection at both sites (Fig. 2-1), lower plant dry matter yield and also lower plant N uptake and utilization particularly in the Lacombe site (

Table 2-4) even though the fall manure total N and ammonium was lower (

Table 2-5Table 2-5). Our finding is consistent with the reports by Weslien et al. (1998), Thorman et al. (2007), but it is opposite to the studies by Rochette et al. (2004) and Hernandez-Ramirez et al. (2009a). The two latter studies considered that the lower N_2O emission derived from the fall manure application was likely attributed to the limited net nitrification under a wet and cool soil condition. Rochette et al. (2004) measured N_2O emissions following fall and spring pig slurry injections in a Le Bras loam soil near Québec City, Canada. As WFPS in their study ranged from 60-80% during the fall N_2O measurements, it was likely that most N was lost via N_2 rather than N_2O (Morley and Baggs 2010). Likewise, the largest contribution to the total annual N_2O emissions in our three fall manure treatments was the N_2O emissions that occurred during the early spring snow-melting and soil thawing (March-April 2015; Fig. 2-2 and Fig. 2-3) in concurrence with an increasing temperature and an abundant soil moisture content (Fig. 2-6). This was also found by Nyborg et al. (1997) when assessing the addition of synthetic N fertilizers. They reported an increased N_2O flux during the spring thaw in a Black Chernozemic soil with high availability of NO_3^--N and moisture.

Adding the liquid manure caused extreme increases in annual N_2O emissions at both field sites, and both inhibitors were effective in reducing N_2O emissions particularly in the Lacombe site (Fig. 2-1). The proportion of N_2O losses to the manure applied total N for the fall and spring manure with no inhibitors added at both sites was 0.26 – 2.54% (Table 2-2). This result is within the range of <0.1 – 3% in an earlier report, which summarized the cumulative N_2O emissions from cattle and pig slurry applications based on a compilation of eight studies (Chadwick et al. 2011). The wide range in this study could be due to the differences in soil texture, season of application, crop types, application method and slurry characteristics.

Contrasting the two NI additives, DMPP was more effective in reducing cumulative N_2O emission than nitrapyrin; this became evident in our wetter Lacombe site (Fig. 2-1). This is because DMPP has a similar mobility as ammonium (Pasda et al. 2001), whereas nitrapyrin has an even lower mobility (Subbarao et al. 2006). Hence, DMPP could more tightly and longer remain in the soil solution in close contact with the soil ammonium ions than nitrapyrin, and hence, decrease the likelihood for ammonium nitrification, and subsequently nitrate denitrification. Our reduction coefficients for the spring-manured soil with NIs in Lacombe (i.e., DMPP: 57.8%; nitrapyrin: 32.7%; Fig. 2-1) are in line with an existing meta-analysis study, in which the reduction coefficients for DMPP were about 55% [95% confidence interval (CI): ~21 to ~60%] and for and nitrapyrin 30% (95% CI: ~17 to ~40%) (Akiyama et al. 2010). However, the reduction coefficients calculated for our fall-manured soil with NIs in Lacombe (i.e., DMPP: 81.0%; nitrapyrin: 64.3%; Fig. 2-1) were much higher than the range compiled by Akiyama et al. (2010). This might be due to the variations and unique combinations of factors such as soil texture, manure composition and rate, crop type and climate across different studies. All of these factors have the scope to interact and influence the effectiveness of NIs.

It is noteworthy that both DMPP and nitrapyrin admixed and injected with liquid manure in fall 2014 were still active in the following early spring (Fig. 2-2b) after the soil had undergone a six-month freezing period (Fig. 2-4a). In contrast, in a related incubation study using the same soils collected from our experimental fields shortly after the spring manure injections (See chapter 3), it was observed that DMPP activity decayed rather quickly within one week after the incubation had begun at a temperature about 3°C higher than that under field conditions (i.e., 20.4 vs. 17.8 °C). This can be explained because the decay in activity of inhibitors is highly dependent on the temperature, and inhibitors could persist over even longer periods under colder temperatures

(Guiraud and Marol 1992; Zerulla et al. 2001). Due to the extended effectiveness of inhibitors under field conditions, our fall-manured soils with DMPP (or nitrapyrin) did not show any obvious distinction in annual N_2O emissions compared to the spring-manured soils with DMPP (or nitrapyrin) (Fig. 2-1a). However, most notably, the fall-manured soil with NIs resulted in lower aboveground plant dry matter yield and plant N uptake than the spring-manured soil with NIs particularly in the Lacombe site (

Table 2-4). This may be explained because a large amount of N_2 losses could have occurred during the early spring snow melting and soil thawing via complete denitrification as soils underwent predominant anaerobic conditions (Meixner and Yang 2006); likewise, leaching and surface runoff could also contribute to this effect. Overall, our data implies that under certain circumstances shifts in plant productivity and variations in N_2O emissions did not necessarily trade off with each other, and hence, this can suggest that best management practices need to be thoroughly identified to jointly address and simultaneously attain both an optimal plant performance and effective mitigation of detrimental environmental effects.

2.5.2 Estimated N Budget

Our holistic examination of system N balances pointed out key N sources and sinks as a function of varying N management options as well as it revealed remaining knowledge gaps and uncertainties for further investigation. At least 96% of N input was from the liquid manure and about ~49 to 66% of total N in the manure was plant available ammonium (Table 2-2) which can also be readily available for N_2O production by soil microbes. The rest of the manure N was in organic form (~34-51%), which can be later mineralized to ammonium under favorable conditions, and subsequently, this additional available N could hypothetically contribute to a delayed N_2O emission. It is likely that due to this delayed mineralization of manure-applied organic N, the large N_2O fluxes in our study (i.e., flux larger than our detection limit of 2.84g N ha^{-1} day^{-1}) consistently lasted for about thirty days following the spring injections at both Lacombe and Edmonton sites (Fig. 2-2b and Fig. 2-3b). Delayed N_2O emissions have been also observed in previous studies, in which N_2O emissions from soils treated with aeration and injection tillage lasted 18 days following a swine effluent application (Sistani et al. 2010). The shorter duration of reported N_2O emissions in their study might be attributed to their lower rates of manure-applied N, more rapid soil drying and plant N uptake, and favorable environmental conditions for soil microbes, such as higher temperature and soil water content.

Crop N uptake was the largest contributor for the total N output among treatments, followed by the denitrified-N losses (i.e., the sum of N_2O and N_2), nitrate leaching and NH_3 volatilization (Table 2-2). Similar N balance results were previously found under a continuous barley cropping system where the dominant N input was also synthetic fertilizers and the main gaseous N output was associated with denitrification (Ross et al. 2008). Based on these N balance results, we recommend developing direct measurements of dinitrogen losses as well as N_2O to N_2 ratios from manured soils to improve overall agroecosystem accounting of N fluxes and pools (Stevens and Laughlin 1998).

To the best of our knowledge, we estimated the N balance for all our experimental settings; however, some uncertainties can be recognized. The positive closures of our mass balances in manured soils (Table 2-2) can be attributed to intense microbial immobilization of manure-derived mineral N (i.e., sourced from either directly-added manure-ammonium or from mineralization of manure-organic N) which could have resulted in increases and accumulations of organic N content in the soil profiles receiving manure injections. This emerging hypothesis can be tested by sampling full soil profiles (~one-meter deep) after multiple years of repeated manure additions and analyzing these soils for changes in total N mass density. Additionally, it is also plausible that a certain amount of manure-applied organic N did not mineralize during our experimental period. On the other hand, we might have overestimated the N losses by leaching, because NIs have been found to reduce nitrate leaching efficiently (Di and Cameron 2007; Hua et al. 2008). The estimations for both dinitrogen from soil and NH_3 volatilization did not reflect the effect of manure timing, which is poorly documented and hence further studies are suggested to address this specific knowledge area.

2.5.3 Soil NH_4^+-N and NO_3^--N Kinetics

The choice of which model to use (i.e., first- vs. second-order kinetic models) to represent and predict the soil mineral transformation rates was discussed in a related incubation study (See chapter 3) using the same treated soils from our field experiment. They found that nitrate accumulation with time was better described by first-order kinetic models, while the time series of ammonium depletion was better depicted by second-order kinetic modelling. However, in our field study, there was no any clear improvement of using second-order over first-order modelling for depicting soil ammonium depletion (

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe†								Edmonton†						
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN
	kg N ha^{-1} yr^{-1}														
position from atmosphere‡	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
symbiotic N fixation§	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
re(Total N) ¶	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1
	0.0	(34.9)¶¶	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)
Manure(Ammonium)¶	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6
	0.0	(0.1)¶¶	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)
N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1
s:															
ported in crop harvest¶	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9
	(1.5)¶¶	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)
ous N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1
Nitrous oxide from soil¶	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4
	(0.3)¶¶	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)
Dinitrogen from soil\\	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5
H_3 volatilization††	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2
te Leaching§§	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9
ce N run-off losses§	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5
N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4
N balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7
	(1.7)\\\	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).

†† NH_3 volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).

§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).

¶¶ Values in parenthesis correspond to one standard error.

\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3). Conversely, in the case of nitrate depletion patterns in our field study, second-order modelling seemed to better describe as substantiated by lower p-values and higher R^2, in particular for the three fall manure treatments (

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe[†]								Edmonton[†]						
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN
	kg N ha^{-1} yr^{-1}														
osition from atmosphere[‡]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
symbiotic N fixation[§]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
re(Total N) [¶]	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1
	0.0	(34.9)[¶¶]	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)
anure(Ammonium)[¶]	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6
	0.0	(0.1)[¶¶]	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)
N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1
s:															
orted in crop harvest[¶]	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9
	(1.5)[¶¶]	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)
us N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1
itrous oxide from soil[¶]	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4
	(0.3)[¶¶]	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)
initrogen from soil[\\]	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5
H$_3$ volatilization[††]	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2
e Leaching[§§]	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9
ce N run-off losses[§]	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5
N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4
balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7
	(1.7)[\\\]	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).

†† NH$_3$ volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).

§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).

¶¶ Values in parenthesis correspond to one standard error.

\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3). Collectively, this might imply that second-order kinetic modelling could be useful for representing and predicting the rather faster and dynamic rates of mineral N patterns in soils receiving high N input; this promising notion can be subject of further study.

As expected, our field results indicated a gradual pattern towards depletion of soil nitrate concentrations following spring manure injection, whereas data shown in chapter 3 from a microcosm incubation using the same soils showed nitrate accumulations. This apparent divergence can be mainly explained as in the fields the barley crop assimilated and made use of the available soil N for biomass growth leading to a net decrease in soil nitrate. Moreover, first- and second-order ammonium depletion rates estimated in this field study (

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe[†]								Edmonton[†]						
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN
	kg N ha^{-1} yr^{-1}														
osition from atmosphere[‡]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
symbiotic N fixation[§]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
re(Total N) [¶]	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1
	0.0	(34.9)[¶¶]	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)
Ianure(Ammonium)[¶]	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6
	0.0	(0.1)[¶¶]	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)
N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1
s:															
orted in crop harvest[¶]	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9
	(1.5)[¶¶]	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)
us N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1
itrous oxide from soil[¶]	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4
	(0.3)[¶¶]	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)
initrogen from soil[\\]	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5
H_3 volatilization[††]	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2
e Leaching[§§]	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9
ce N run-off losses[§]	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5
N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4
I balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7
	(1.7)[\\\]	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).

†† NH_3 volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).

§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).

¶¶ Values in parenthesis correspond to one standard error.

\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3) were lower than those found in the soil microcosms (See chapter 3). We infer this numerical difference is attributable to substantially lower temperature and soil moisture content in the field (during the period from 13 May to 20 Jul 2015) than in the incubation (18.2 vs. 20.4 °C, and 0.11 vs. 0.35-0.41 m^3 m^{-3}). As shown in previous reports, lower temperature limits the soil microbial activities and lower moisture is beneficial for ammonium nitrification (Davidson and Schimel 1995; Bateman and Baggs 2005), thereby leading to reduced ammonium depletion rates in our field study.

The addition of nitrification inhibitors (NIs) clearly impacted the temporal dynamics of soil mineral N. The spring-manured soils treated with NIs exhibited much slower first-order depletion rates of ammonium concentration than the spring manure soils receiving no NIs (

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe†								Edmonton†						
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN
	kg N ha^{-1} yr^{-1}														
osition from atmosphere‡	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
symbiotic N fixation§	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
re(Total N) ¶	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1
	0.0	(34.9)¶¶	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)
anure(Ammonium)¶	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6
	0.0	(0.1)¶¶	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)
N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1
s:															
orted in crop harvest¶	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9
	(1.5)¶¶	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)
ous N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1
itrous oxide from soil¶	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4
	(0.3)¶¶	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)
initrogen from soil\\	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5
H_3 volatilization††	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2
e Leaching§§	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9
ce N run-off losses§	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5
N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4
balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7
	(1.7)\\\	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).

†† NH_3 volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).

§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).

¶¶ Values in parenthesis correspond to one standard error.

\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3). This result is consistent with an earlier report (Omonode and Vyn 2013). Additionally, most of the manure treatments receiving NIs took longer (about 13 extra days) to reach depletion plateaus following their initial peaks in nutrient concentration, with the only exception of fall manure with nitrapyrin and spring manure without NIs (FN and SW) (

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe[†]								Edmonton[†]						
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN
	kg N ha^{-1} yr^{-1}														
position from atmosphere[‡]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
symbiotic N fixation[§]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
re(Total N) [¶]	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1
	0.0	(34.9)[¶¶]	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)
Manure(Ammonium)[¶]	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6
	0.0	(0.1)[¶¶]	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)
N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1
s:															
ported in crop harvest[¶]	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9
	(1.5)[¶¶]	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)
ous N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1
Nitrous oxide from soil[¶]	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4
	(0.3)[¶¶]	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)
Dinitrogen from soil[\\]	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5
NH_3 volatilization[††]	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2
te Leaching[§§]	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9
ce N run-off losses[§]	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5
N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4
N balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7
	(1.7)[\\\]	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).

†† NH_3 volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).

§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).

¶¶ Values in parenthesis correspond to one standard error.

\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3). In general, this observation suggests that the assessed NIs remained still active even under the dry conditions prevailing in the spring 2015 in the Edmonton site. However, these evident effects on nutrient concentration patterns did not translate into strong reduction of N_2O emissions (Fig. 2-1b, Fig. 2-3). The effect of moisture on NIs activity is still not well documented, thus it is suggested to further address this unknown using a wide range of soils under varying inhibitor rate, N addition rate, moisture and temperature.

2.5.4 Drivers for High N_2O Emissions

Butterbach-Bahl et al. (2013) pointed that the soil moisture content was the dominant factor driving N_2O emission as it reflects the soil oxygen availability. This could be also supported by our results. The annual cumulative N_2O emissions for the Lacombe site were almost three times as much as that in Edmonton (Fig. 2-1). This result could be explained by the higher annual precipitation (Oct 2014 – Sep 2015) in Lacombe than Edmonton (i.e., 420 vs. 320 mm; Fig. 2-4) rather than by the differences in annual average temperature (i.e., 3.9 vs. 5.3 °C, respectively; Fig. 2-4). More specifically, the soils did not immediately respond to the manure injections by exhibiting any extreme N_2O emissions, but our observed major N_2O effluxes took place only following several consecutive large rainfalls that occurred in the Lacombe site (Fig. 2-7).

Another key control on our N_2O emissions was the availability of substrate for denitrification (Havlin et al. 2014). For instance, both the fall-manured soils with and without DMPP (FD and FW) exhibited much higher N_2O fluxes than the fall-manured soil treated with nitrapyrin (FN) during the week immediately after the manure injection conducted on 7 Oct 2015 (Fig. 2-3c). This result can be attributed to the fact that both the fall-manured soils with and without DMPP still kept comparatively higher concentration of residual nitrate in the 0-15 cm soil layer than the fall-manured soil with nitrapyrin as quantified on 25 Sep 2015 after the barley growth cycle and harvest had been completed (Fig. 2-5i).

2.6 Conclusion

Compared to the fall application of liquid manure, the spring timing enhanced the overall manure-N use efficiency and utilization by obtaining higher plant N uptake, higher plant dry matter yield, and lower risk of large annual N_2O emissions. Therefore, it is suggested that land injection of liquid manure should be conducted during mid-to-late spring in regions with comparable edaphic and climatic conditions as Alberta. Our study also indicates that the use of nitrification inhibitors (NIs) leads to more consistent N_2O emissions which otherwise are typically very variable, temporally erratic and unpredictable. Along these lines, the evaluated NIs were effective in numerically reducing N_2O emissions, and moreover, this effectiveness of the NIs was still functional in soils following a six-month freezing winter. Additionally, DMPP was even more effective in reducing N_2O emissions than nitrapyrin; however, this apparent advantage in retaining manure-applied N in the soils did not translate into differences in plant N uptake between these two NIs. Soil moisture content and nitrate concentration clearly arose as two key drivers of N_2O emissions. Most of the temporal patterns of N transformations were well described by first-order kinetic modelling, with the exception of nitrate dynamics which was better depicted by second-order models in particular in N-enriched fall-manured soils where very fast transformation rates can commonly occur.

2.7 Acknowledgement

The authors thank Alberta Livestock and Meat Agency Ltd. (ALMA), Climate Change and Emissions Management Corporation (CCEMC), Dow AgroSciences and Eurochem Agro for their funding supports, the Alberta Agriculture and Forestry for its technical support and Lewis Fausak, Craig Sprout and Catherine Pocklington for their assistance during this study.

2.8 References

Akiyama, H., Xiaoyuan, Y., and Yagi, K. (2010). "Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis." *Global Change Biol.,* 16(6), 1837.

Alberta Agriculture and Forestry. (2016). "Current and Historical Alberta Weather Station Data Viewer." http://agriculture.alberta.ca/acis/alberta-weather-data-viewer.jsp (Feb 10, 2016).

Allen, A., Jarvis, S., and Headon, D. (1996). "Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the UK." *Soil Biol.Biochem.,* 28(4), 597-607.

Baggs, E. M., Stevenson, M., Pihlatie, M., Regar, A., Cook, H., and Cadisch, G. (2003). "Nitrous oxide emissions following application of residues and fertiliser under zero and conventional tillage." *Plant Soil,* (2), 361.

Basso, B., and Ritchie, J. T. (2005). "Impact of compost, manure and inorganic fertilizer on nitrate leaching and yield for a 6-year maize–alfalfa rotation in Michigan." *Agric., Ecosyst.Environ.,* 108(4), 329-341.

Bateman, E. J., and Baggs, E. M. (2005). "Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space." *Biol.Fertility Soils,* 41(6), 379-388.

Beaulieu, M. S. (2004). "Manure management in Canada." *Farm Environmental Management in Canada,* 1(2), 1-52.

Braker, G., and Conrad, R. (2011). "Chapter 2 - Diversity, Structure, and Size of N_2O-Producing Microbial Communities in Soils—What Matters for Their Functioning?" *Adv.Appl.Microbiol.,* 75 33-70.

Bremner, J. M., and Blackmer, A. M. (1978). "Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen." *Science,* 199(4326), 295-296.

Burzaco, J. P., Ciampitti, I. A., and Vyn, T. J. (2014). "Nitrapyrin impacts on maize yield and nitrogen use efficiency with spring-applied nitrogen: Field studies vs. meta-analysis comparison." *Agron.J.,* 106(2), 753-760.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S. (2013). "Nitrous oxide emissions from soils: how well do we understand the processes and their controls?" *Philos.Trans.R.Soc.Lond.B.Biol.Sci.,* 368(1621), 20130122.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., and Misselbrook, T. (2011). "Manure management: implications for greenhouse gas emissions." *Anim.Feed Sci.Technol.,* 166 514-531.

Chen, Y., Assefa, B., and Arkinremi, W. (2010). "Soil Nutrient Levels and Crop Performance at Various Lateral Positions Following Liquid Manure Injection." *Agric Eng Int: CIGR Journal,* 12(1), 1-11.

Court, M. N., Stephen, R. C., and Waid, J. S. (1964). "Toxicity as a cause of the inefficiency of urea as a fertilizer." *J.Soil Sci.,* 15(1), 49-65.

Davidson, E. A., Rogers, J. E., and Whitman, W. B. (1991). "Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems." *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes.,* 219-235.

Davidson, E. A., and Schimel, J. P. (1995). "Microbial processes of production and consumption of nitric oxide, nitrous oxide and methane." *Biogenic Trace Gases: Measuring Emissions from Soil and Water,* 327-357.

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., and Veldkamp, E. (2000). "Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides Using two functions based on soil nitrogen availability and soil water content, the hole-in-the-pipe model characterizes a large fraction of the observed variation of nitric oxide and nitrous oxide emissions from soils." *Bioscience,* 50(8), 667-680.

Di, H. J., and Cameron, K. C. (2007). "Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor—a lysimeter study." *Nutr.Cycling Agroecosyst.,* 79(3), 281-290.

Dittert, K., Bol, R., King, R., Chadwick, D., and Hatch, D. (2001). "Use of a novel nitrification inhibitor to reduce nitrous oxide emission from ^{15}N-labelled dairy slurry injected into soil." *Rapid Communications in Mass Spectrometry: RCM,* 15(15), 1291.

Dobbie, K. E., McTaggart, I. P., and Smith, K. A. (1999). "Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors." *Journal of Geophysical Research: Atmospheres (1984–2012),* 104(D21), 26891-26899.

Dobbie, K., and Smith, K. (2001). "The effects of temperature, water- filled pore space and land use on N_2O emissions from an imperfectly drained gleysol." *Eur.J.Soil Sci.,* 52(4), 667-673.

FAO. (2015). "World Fertilizer Trends and Outlook to 2018." Food and Agriculture Organization of the United Nations, Rome.

Flessa, H., and Beese, F. (2000). "Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry." *J.Environ.Qual.,* 29(1), 262-268.

Freney, J. R., Simpson, J. R., and Denmead, O. T. (1983). "Volatilization of ammonia." *Gaseous Loss of Nitrogen from Plant-Soil Systems,* J. R. S. J.R.Freney, ed., Springer, Netherlands, 1-32.

Government of Canada. (2016). "Climate." http://climate.weather.gc.ca/ (Feb 10, 2016).

Guiraud, G., and Marol, C. (1992). "Influence of temperature on mineralization kinetics with a nitrification inhibitor (mixture of dicyandiamide and ammonium thiosulphate)." *Biol.Fertility Soils,* 13(1), 1-5.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D., and Condron, L. M. (2013). "Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions." *Soil Biol.Biochem.,* 57 43-55.

Havlin, J., Beaton, J. D., Tisdale, S. L., and Nelson, W. L. (2014). *Soil fertility and fertilizers: An introduction to nutrient management.* Pearson Prentice Hall Upper Saddle River, New Jersey, USA, .

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., and Van Scoyoc, G. E. (2011). "Nitrogen partitioning and utilization in corn cropping systems: Rotation, N source, and N timing." *Eur.J.Agron.,* 34(3), 190-195.

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., and Van Scoyoc, G. E. (2009a). "Greenhouse gas fluxes in an eastern corn belt soil: Weather, nitrogen source, and rotation." *J.Environ.Qual.,* 38(3), 841-854.

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., Van Scoyoc, G. E., and Michalski, G. (2009b). "Nitrous oxide production in an eastern corn belt soil: sources and redox range." *Soil Sci.Soc.Am.J.,* 73(4), 1182-1191.

Herrero, M., Thornton, P. K., Gerber, P., and Reid, R. S. (2009). "Livestock, livelihoods and the environment: understanding the trade-offs." *Current Opinion in Environmental Sustainability,* 1(2), 111-120.

Hoben, J. P., Gehl, R. J., Millar, N., Grace, P. R., and Robertson, G. P. (2011). "Nonlinear nitrous oxide (N_2O) response to nitrogen fertilizer in on- farm corn crops of the US Midwest." *Global Change Biol.,* 17(2), 1140-1152.

Hua, L., LIANG, X., Yingxu, C., Yanfeng, L., Guangming, T., and Wuzhong, N. (2008). "Effect of nitrification inhibitor DMPP on nitrogen leaching, nitrifying organisms, and enzyme activities in a rice-oilseed rape cropping system." *Journal of Environmental Sciences,* 20(2), 149-155.

Irigoyen, I., Muro, J., Azpilikueta, M., Aparicio-Tejo., P., and Lamsfus, C. (2003). "Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures." *Aust.J.Soil Res.,* (6), 1177.

Janzen, H., Beauchemin, K., Bruinsma, Y., Campbell, C., Desjardins, R., Ellert, B., and Smith, E. (2003). "The fate of nitrogen in agroecosystems: An illustration using Canadian estimates." *Nutr.Cycling Agroecosyst.,* 67(1), 85-102.

Kim, D., Hernandez-Ramirez, G., and Giltrap, D. (2013). "Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis." *Agric., Ecosyst.Environ.,* 168 53-65.

Laboski, C., Jokela, W., and Andraski, T. (2013). "Dairy manure application methods: N credits, gaseous N losses, and corn yield." *Proceedings of the Wisconsin Crop Management Conference,* Citeseer, 20-31.

Luo, G. J., Kiese, R., Wolf, B., and Butterbach-Bahl, K. (2013). "Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types." *Biogeosciences,* 10(5), 3205-3219.

Luo, J., Tillman, R. W., and Ball, P. R. (2000). "Nitrogen loss through denitrification in a soil under pasture in New Zealand." *Soil Biol.Biochem.,* 32 497-509.

Maguire, R. O., Kleinman, P. J. A., Dell, C. J., Beegle, D. B., Brandt, R. C., McGrath, J. M., and Ketterings, Q. M. (2011). "Manure Application Technology in Reduced Tillage and Forage Systems: A Review." *J.Environ.Qual.,* 40(2), 292-301.

Meixner, F. X., and Yang, W. X. (2006). "Biogenic emissions of nitric oxide and nitrous oxide from arid and semi-arid land." *Dryland ecohydrology,* Springer, 233-255.

Menéndez, S., Merino, P., Pinto, M., González-Murua, C., and Estavillo, J. (2006). "3, 4-Dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands." *J.Environ.Qual.,* 35(4), 973-981.

Menéndez, S., Barrena, I., Setien, I., González-Murua, C., and Estavillo, J. M. (2012). "Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions." *Soil Biol.Biochem.,* 53 82-89.

Minet, E. P., Jahangir, M. M. R., Krol, D. J., Rochford, N., Fenton, O., Rooney, D., Lanigan, G., Forrestal, P. J., Breslin, C., and Richards, K. G. (2016). "Amendment of cattle slurry with the nitrification inhibitor dicyandiamide during storage: A new effective and practical N_2O mitigation measure for landspreading." *Agric., Ecosyst.Environ.,* 215 68-75.

Misselbrook, T., Smith, K., Johnson, R., and Pain, B. (2002). "SE—Structures and environment: Slurry application techniques to reduce ammonia emissions: Results of some UK field-scale experiments." *Biosystems Engineering,* 81(3), 313-321.

Morley, N., and Baggs, E. (2010). "Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction." *Soil Biol.Biochem.,* 42(10), 1864-1871.

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J. F., Lee, D., and Mendoza, B. (2013). "Anthropogenic and natural radiative forcing." *Climate Change,* 423.

Nyborg, M., Laidlaw, J. W., Solberg, E. D., and Malhi, S. S. (1997). "Denitrification and nitrous oxide emissions from a Black Chernozemic soil during spring thaw in Alberta." *Can.J.Soil Sci.,* 77(2), 153-160.

Nyord, T., Søgaard, H. T., Hansen, M. N., and Jensen, L. S. (2008). "Injection methods to reduce ammonia emission from volatile liquid fertilisers applied to growing crops." *Biosystems Engineering,* 100(2), 235-244.

Omonode, R. A., and Vyn, T. J. (2013). "Nitrification Kinetics and Nitrous Oxide Emissions when Nitrapyrin is Coapplied with Urea--Ammonium Nitrate." *Agron.J.,* 105(6), 1475-1486.

Pasda, G., Hahndel, R., and Zerulla, W. (2001). "Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops." *Biol.Fertility Soils,* 34 85-97.

Pennock, D., Yates, T., Bedard-Haughn, A., Phipps, K., Farrell, R., and McDougal, R. (2010). "Landscape controls on N_2O and CH_4 emissions from freshwater mineral soil wetlands of the Canadian Prairie Pothole region." *Geoderma,* 155(3), 308-319.

Phillips, R. L., McMillan, A. M. S., Palmada, T., Dando, J., and Giltrap, D. (2015). "Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil." *N.Z.J.Agric.Res.,* 58(1), 89-95.

Prasad, R., and Power, J. (1995). "Nitrification inhibitors for agriculture, health, and the environment." *Advances in Agronomy (USA),* .

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. (2009). "Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century." *Science,* 326(5949), 123-125.

Rochette, P., Angers, D. A., Chantigny, M. H., Bertrand, N., and Côté, D. (2004). "Carbon dioxide and nitrous oxide emissions following fall and spring applications of pig slurry to an agricultural soil." *Soil Sci.Soc.Am.J.,* 68(4), 1410-1420.

Rochette, P., Worth, D. E., Lemke, R. L., McConkey, B. G., Pennock, D. J., Wagner-Riddle, C., and Desjardins, R. (2008). "Estimation of N_2O emissions from agricultural soils in Canada. I. Development of a country-specific methodology." *Can.J.Soil Sci.,* 88(5), 641-654.

Rodhe, L., and Etana, A. (2005). "Performance of slurry injectors compared with band spreading on three Swedish soils with ley." *Biosystems Engineering,* (1),.

Ross, S. M., Izaurralde, R. C., Janzen, H., Robertson, J., and McGill, W. B. (2008). "The nitrogen balance of three long-term agroecosystems on a boreal soil in western Canada." *Agric., Ecosyst.Environ.,* 127(3), 241-250.

Saarnio, S., Heimonen, K., and Kettunen, R. (2013). "Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake." *Soil Biol.Biochem.,* 58 99-106.

Saggar, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., Kim, D., Zaman, M., and Tillman, R. (2013). "Denitrification and N_2O: N_2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts." *Sci.Total Environ.,* 465 173-195.

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., and Zechmeister-Boltenstern, S. (2010). "Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature." *Eur.J.Soil Sci.,* 61(5), 683-696.

Schindlbacher, A., Zechmeister- Boltenstern, S., and Butterbach- Bahl, K. (2004). "Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils." *Journal of Geophysical Research: Atmospheres (1984–2012),* 109(D17),.

ŠImek, M., and Cooper, J. (2002). "The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years." *Eur.J.Soil Sci.,* 53(3), 345-354.

Sistani, K., Warren, J., Lovanh, N., Higgins, S., and Shearer, S. (2010). "Greenhouse gas emissions from swine effluent applied to soil by different methods." *Soil Sci.Soc.Am.J.,* 74(2), 429-435.

Smith, D. W., and Mukhtar, S. (2015). "Estimation and attribution of nitrous oxide emissions following subsurface application of animal manure: a review." *Transactions of the ASABE,* 58(2), 429.

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., and Rey, A. (2003). "Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes." *Eur.J.Soil Sci.,* 54(4), 779.

Spargo, J. T., Alley, M. M., Follett, R. F., and Wallace, J. V. (2008). "Soil nitrogen conservation with continuous no-till management." *Nutr.Cycling Agroecosyst.,* 82(3), 283-297.

Stevens, R., and Laughlin, R. (1998). "Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils." *Nutr.Cycling Agroecosyst.,* 52(2-3), 131-139.

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., and Rao, I. (2006). "Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities." *Crit.Rev.Plant Sci.,* 25(4), 303.

Team, R. C. (2015). "R: A Language and Environment for Statistical Computing." *R Foundation for Statistical Computing,Vienna, Austria,* URL http://www.R-project.org/.

Thompson, R. B., Ryden, J. C., and Lockyer, D. R. (1987). "Fate of nitrogen in cattle slurry following surface application or injection to grassland." *J.Soil Sci.,* 38(4), 689-700.

Thorman, R., Sagoo, E., Williams, J., Chambers, B., Chadwick, D., Laws, J., Yamulki, S., Bosch, A., Teira, M., and Villar, J. E. M. (2007). "The effect of slurry application timings on direct and indirect N_2O emissions from free draining grassland." *Proceedings of the 15th Nitrogen Workshop: Towards a Better Efficiency in N Use. Editorial Milenio, Lleida (Spain),* 297-299.

U.S. Environmental Protection Agency. (2012). "Summary Report: Global Anthropogenic Non-CO_2 Greenhouse Gas Emissions: 1990 - 2030." Office of Atmospheric Programs, Climate Change Division, U.S. Environmental Protection Agency, Washington DC.

Uchida, Y., Clough, T. J., Kelliher, F. M., and Sherlock, R. R. (2008). "Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil." *Soil Biol.Biochem.,* 40(4), 924-931.

van Es, H. M., Sogbedji, J. M., and Schindelbeck, R. R. (2006). "Effect of manure application timing, crop, and soil type on nitrate leaching." *J.Environ.Qual.,* 35(2), 670-679.

Velthof, G. L., and Mosquera, J. (2011). "The impact of slurry application technique on nitrous oxide emission from agricultural soils." *Agric., Ecosyst.Environ.,* 140(1), 298-308.

Velusamy, K., and Krishnani, K. K. (2013). "Heterotrophic nitrifying and oxygen tolerant denitrifying bacteria from greenwater system of coastal aquaculture." *Appl.Biochem.Biotechnol.,* 169(6), 1978-1992.

Webb, J., Pain, B., Bittman, S., and Morgan, J. (2010). "The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—A review." *Agric., Ecosyst.Environ.,* 137(1), 39-46.

Weslien, P., Klemedtsson, L., Svensson, L., Galle, B., Kasimir- Klemedtsson, Å, and Gustafsson, A. (1998). "Nitrogen losses following application of pig slurry to arable land." *Soil use Manage.,* 14(4), 200-208.

Wolt, J. D. (2004). "A meta-evaluation of nitrapyrin agronomic and environmental effectiveness with emphasis on corn production in the Midwestern USA." *Nutr.Cycling Agroecosyst.,* 69(1), 23-41.

Yates, T. T., Si, B. C., Farrell, R. E., and Pennock, D. J. (2006). "Wavelet Spectra of Nitrous Oxide Emission from Hummocky Terrain during Spring Snowmelt." *Soil Sci.Soc.Am.J.,* 70(4), 1110-1120.

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., von Locquenghien, K. H., Pasda, G., Rädle, M., and Wissemeier, A. (2001). "3, 4-Dimethylpyrazole phosphate (DMPP)–a new nitrification inhibitor for agriculture and horticulture." *Biol.Fertility Soils,* 34(2), 79-84.

Zheng, X., Wang, M., Wang, Y., Shen, R., Gou, J., Li, J., Jin, J., and Li, L. (2000). "Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agro-ecosystem in Southeast China." *Chemosphere-Global Change Science,* 2(2), 207-224.

Zhu, X., Burger, M., Doane, T. A., and Horwath, W. R. (2013). "Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability." *Proc.Natl.Acad.Sci.U.S.A.,* 110(16), 6328-6333.

2.9 Tables

Table 2-1. ANOVA results for annual cumulative N_2O emissions, barley dry matter yield and nitrogen uptake in the Lacombe and Edmonton sites.

	Lacombe		Edmonton	
	F-value	p-value	F-value	p-value
	Annual N_2O Emission			
Timing†	1.083	0.375	1.775	0.314
Additive‡	4.212	0.041	1.546	0.271
Timing:Additive	0.902	0.432	0.553	0.596
	Plant Dry Matter Yield			
Timing	22.710	0.018	7.408	0.113
Additive	1.008	0.394	0.592	0.576
Timing:Additive	2.766	0.103	0.552	0.596
	Plant Nitrogen Uptake			
Timing	87.300	0.003	5.770	0.138
Additive	0.838	0.456	0.823	0.473
Timing:Additive	2.605	0.115	1.348	0.313

† Timing factor includes fall 2014 and spring 2015 manure injection treatments.
‡ Additive factor includes manure without nitrification inhibitors, manure with DMPP, and manure with nitrapyrin treatments.

Table 2-2. Estimated annual N budget for Lacombe and Edmonton sites.

	Lacombe[†]								Edmonton[†]							
	CZ	FD	FN	FW	CT	SD	SN	SW	CZ	FD	FN	FW	CT	SD	SN	SW
	kg N ha^{-1} yr^{-1}															
N inputs:																
N deposition from atmosphere[‡]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Non-symbiotic N fixation[§]	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Manure(Total N)[¶]	0.0	245.2	245.2	245.2	0.0	405.8	405.8	405.8	0.0	380.7	380.7	380.7	0.0	399.1	399.1	399.1
	0.0	(34.9)[¶¶]	(34.9)	(34.9)	0.0	(5.4)	(5.4)	(5.4)	0.0	(6.8)	(6.8)	(6.8)	0.0	(11.5)	(11.5)	(11.5)
Manure(Ammonium)[¶]	0.0	213.8	213.8	213.8	0.0	304.4	304.4	304.4	0.0	286.1	286.1	286.1	0.0	255.6	255.6	255.6
	0.0	(0.1)[¶¶]	(0.1)	(0.1)	0.0	(0.1)	(0.1)	(0.1)	0.0	(0.8)	(0.8)	(0.8)	0.0	(1.6)	(1.6)	(1.6)
Total N inputs	10.0	255.2	255.2	255.2	10.0	415.8	415.8	415.8	10.0	390.7	390.7	390.7	10.0	409.1	409.1	409.1
N outputs:																
N exported in crop harvest[¶]	35.4	78.7	88.9	95.9	33.1	138.6	126.7	133.2	63.8	143.3	134.1	143.8	56.3	132.8	126.9	110.5
	(1.5)[¶¶]	(3.3)	(6.2)	(2.9)	(3.6)	(5.8)	(8.4)	(8.3)	(8.8)	(8.8)	(13.5)	(2.9)	(1.4)	(4.8)	(17.6)	(6.9)
Gaseous N losses	1.0	46.6	46.6	46.6	1.0	71.6	71.6	71.6	1.0	67.2	67.2	67.2	1.0	66.1	66.1	66.1
Nitrous oxide from soil[¶]	0.3	1.2	2.6	6.2	0.3	1.1	2.1	3.1	0.6	1.8	2.2	2.3	0.6	1.0	1.4	1.0
	(0.3)[¶¶]	(0.3)	(1.4)	(6.4)	(0.0)	(0.4)	(0.8)	(1.5)	(0.8)	(1.0)	(1.8)	(1.3)	(0.0)	(0.1)	(0.3)	(0.4)
Dinitrogen from soil[\\]	0.7	24.3	22.9	19.3	0.7	40.5	39.5	38.5	0.4	37.3	36.8	36.8	0.4	39.9	39.5	39.9
NH_3 volatilization[††]	ngb	21.1	21.1	21.1	ngb	30.0	30.0	30.0	ngb	28.2	28.2	28.2	ngb	25.2	25.2	25.2
Nitrate Leaching[§§]	ngb	24.5	24.5	24.5	ngb	28.4	28.4	28.4	ngb	38.1	38.1	38.1	ngb	27.9	27.9	27.9
Surface N run-off losses[§]	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5	ngb	1.5	1.5	1.5
Total N outputs	36.4	151.3	161.5	168.5	34.1	240.1	228.2	234.6	64.8	250.1	240.9	250.6	57.3	228.3	222.4	206.0
System N balance	-26.4	103.9	93.7	86.7	-24.1	175.7	187.6	181.2	-54.8	140.6	149.8	140.1	-47.3	180.8	186.7	203.1
	(1.7)[\\\]	(38.6)	(42.5)	(44.3)	(3.7)	(11.7)	(14.7)	(15.2)	(9.6)	(17.3)	(22.8)	(11.7)	(1.4)	(18.0)	(31.1)	(20.3)

ngb = negligible.

† The time period for the annual N budget in Lacombe corresponds to 7 Oct 2014 – 6 Oct 2015; the time period in Edmonton corresponds to 1 Oct 2014 – 30 Sep 2015.

‡ Deposition from atmosphere was assumed to be 5.0 kg N ha^{-1} yr^{-1} (Janzen et al. 2003).

§ Non-symbiotic N fixation and surface run-off losses were assumed to be 5.0 and 1.5 kg N ha^{-1} yr^{-1}, respectively (Ross et al. 2008).

¶ The values were directly measured in this study.

\\Dinitrogen from soil assume: 10% gaseous N losses from total N in manure (i.e., dinitrogen from soil = 10%*Total N in manure – nitrous oxide from soil) (Janzen et al. 2003).
†† NH_3 volatilization assume: 13% of soluble ammonium in manure (Misselbrook et al. 2002).
§§ Nitrate leaching assume: 10 and 7% of total N in manure for the fall and spring treatments, respectively (Janzen et al. 2003; van Es et al. 2006).
¶¶ Values in parenthesis correspond to one standard error.
\\\These propagated errors for the system N balance closure were estimated by simple addition of the standard errors derived from the direct measurements in this study: manure (total N), manure (ammonium), N exported in crop harvest, and nitrous oxide from soil.

Table 2-3. Rates (k) of soil mineral nitrogen concentration changes with time in the Edmonton site based on first- and second-order kinetic models. P-values (*P*) and coefficient of determination (R^2) for each model and data subset are shown as criteria for model evaluation.

Treatment	Time interval†	First-Order Kinetics‡						Second-Order Kinetics§					
		Ammonium Depletion			Nitrate Depletion			Ammonium Depletion			Nitrate Depletion		
		P	R^2	k	P	R^2	k	P	R^2	k	P	R^2	k
				day^{-1}×1000			day^{-1}×1000			μg^{-1} N kg day^{-1}			μg^{-1} N kg day^{-1}
CZ	1	0.001	0.980	-8.821**	0.011	0.978	16.68**	0.001	0.978	-2.161**	0.005	0.990	1.299**
FD	2	0.010	0.838	27.83**	0.008	0.930	42.06**	0.010	0.838	3.658**	0.006	0.940	1.064**
FN	1	0.284	0.361	-3.637	0.082	0.843	26.53*	0.242	0.413	-0.769	0.043	0.917	0.761**
FW	2	0.174	0.405	5.718	0.074	0.707	24.41*	0.174	0.405	1.307	0.044	0.790	0.743**
CT	1	0.647	0.079	0.001	0.038	0.925	13.32**	0.618	0.093	0.187	0.024	0.953	0.972**
SD	2	0.275	0.286	4.700	0.219	0.445	5.569	0.275	0.286	0.397	0.241	0.414	0.244
SN	2	0.022	0.767	13.93**	0.288	0.356	7.590	0.022	0.767	1.865**	0.401	0.241	0.264
SW	1	0.015	0.896	21.38**	0.055	0.893	32.19*	0.040	0.800	3.252**	0.095	0.819	1.109*
Mean		-	-	7.637	-	-	21.044	-	-	0.967	-	-	0.807
S.E.		-	-	4.600	-	-	4.793	-	-	0.710	-	-	0.150

S.E. = one standard error; * = significantly different at *P*<0.1; ** = significantly different at *P*<0.05.

† The time interval 1 corresponds to the period from 13 May throughout 4 Jul 2015 (time series of 5 sampling dates and data points) for ammonium, and 24 May throughout 4 Jul 2015 (4 data points) for nitrate. The time interval 2 corresponds to the period from 13 May throughout 20 Jul 2015 (6 data points) for ammonium, and throughout 20 Jul 2015 (5 data points) for nitrate. The lapse between the selected intervals for ammonium and nitrate can indicate the time necessary for an N transformation via nitrification in these soils. Temporal patterns of ammonium and nitrate concentrations are shown in Fig. 2-4.

‡ The first-order kinetic was calculated based on Eq. [2-3].

§ The second-order kinetic was calculated based on Eq. [2-4].

Table 2-4. Aboveground barley dry matter yield and nitrogen uptake with one standard error in the Lacombe and Edmonton sites.

Treatment	Lacombe		Edmonton	
	kg ha^{-1}			
	Dry Matter Yield			
CZ	2946.4 ± 260.8	Bb†	4410.6 ± 440.4	Cb
FD	5865.5 ± 200.4	a	7996.4 ± 559.5	a
FN	6042.4 ± 393.2	a	7494.4 ± 891.2	a
FW	6691.2 ± 384.5	Aa	7896.4 ± 99.5	Aa
CT	2416.5 ± 365.4	Bb	3688.9 ± 189.5	Cb
SD	7414.4 ± 295.5	a	7042.5 ± 348.9	a
SN	7018.4 ± 401.1	a	6908.5 ± 893.0	a
SW	7048.9 ± 452.9	Aa	5942.5 ± 256.5	Ba
	Aboveground Nitrogen Uptake			
CZ	35.3 ± 1.4	Cc	63.8 ± 8.8	Cb
FD	78.7 ± 3.2	b	143.2 ± 8.4	a
FN	88.8 ± 6.1	ab	134.0 ± 13.4	a
FW	95.8 ± 2.8	Ba	143.8 ± 2.8	Aa
CT	33.1 ± 3.6	Cb	56.3 ± 1.3	Cb
SD	138.6 ± 5.7	a	132.4 ± 4.4	a
SN	126.6 ± 8.3	a	126.8 ± 14.6	a
SW	133.1 ± 8.2	Aa	110.4 ± 6.8	Ba

† Values followed by different capital letters indicate significant differences among control zero (CZ), control disturbance (CT), fall manure without nitrification inhibitor (FW) and spring manure without nitrification inhibitor (SW) treatments based on LSD test (P <0.05); values followed by different lowercase letters indicate significant differences among three fall manure treatments (FD,FN and FW) and control zero (CZ) or three spring manure treatments (SD,SN and SW) and control disturbance (CT) based on LSD test (P <0.05).

Table 2-5. Average total N, ammonium and water contents with one standard error in the manure applied in fall 2014, spring 2015 and fall 2015. Different capital letters indicate significant differences among the manure applications based on LSD test (*P*<0.05), and different lowercase letters indicate significant differences between field locations (Lacombe vs. Edmonton) based on the two sample t test (*P*<0.05).

	Lacombe		Edmonton	
	Total N (kg ha^{-1})			
Fall 2014	245.2 ± 34.8	Ba	380.7 ± 6.7	Ab
Spring 2015	405.8 ± 5.4	Aa	399.1 ± 11.4	Aa
Fall 2015	315.6 ± 54.2	ABa	402.7 ± 21.4	Aa
	NH_4^+-N (kg ha^{-1})			
Fall 2014	161.9 ± 0.1	Ca	216.7 ± 0.7	Ab
Spring 2015	230.6 ± 0.1	Aa	193.6 ± 1.5	Ab
Fall 2015	199.8 ± 2.8	Ba	212.9 ± 0.8	Bb
	H_2O m/m (%)			
Fall 2014	98.7 ± 0.1	Aa	91.8 ± 0.8	Bb
Spring 2015	97.7 ± 0.3	Aa	92.9 ± 0.1	Ab
Fall 2015	97.9 ± 0.7	Aa	93.6 ± 0.1	Ab

2.10 Figures

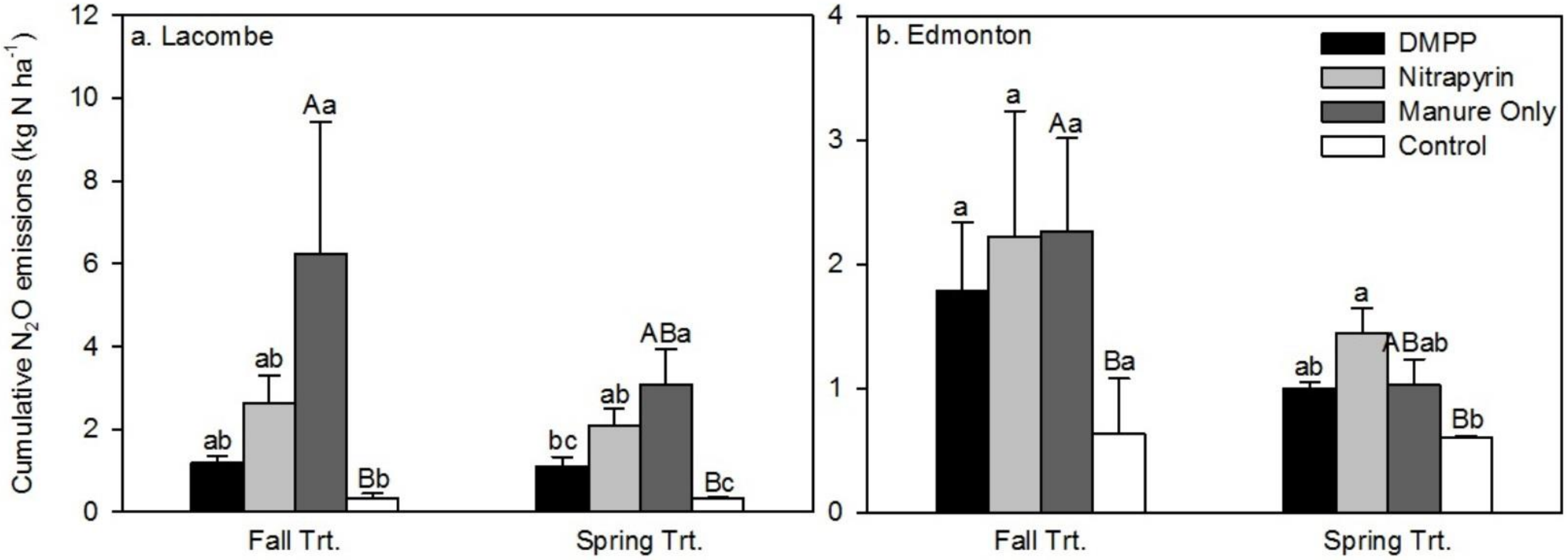


Fig. 2-1. Annual cumulative N_2O emissions in the (a) Lacombe and (b) Edmonton sites. We assumed negligible N_2O emission from 1 Nov 2014 to 26 Mar 2015 for both sites. Different capital letters indicate significant differences among control zero (CZ), control disturbance (CT), fall manure without inhibitors (FW) and spring manure without inhibitors (SW) treatments based on LSD test ($P<0.05$); different lowercase letters indicate significant differences among three fall manure treatments (FD, FN and FW) and the control zero (CZ), or three spring manure treatments (SD, SN and SW) and the control disturbance (CT) based on LSD test ($P<0.05$). Note the different y-axis scales across panels.

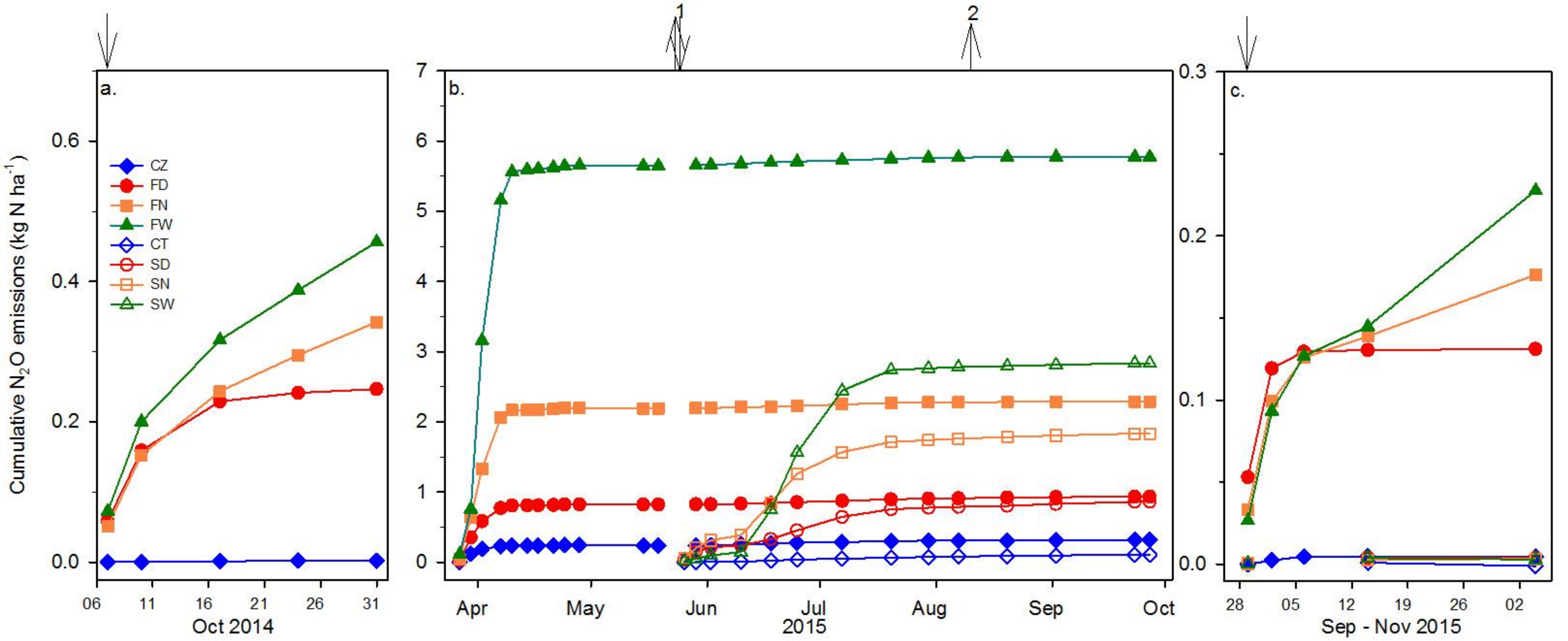


Fig. 2-2. Cumulative N_2O emission patterns in the Lacombe site during the periods from (a) 4 Oct to 31 Oct 2014, (b) 27 Mar to 28 Set 2015 and (c) 29 Sep to 4 Nov 2015. The upward arrows indicate the dates of seeding (↑^{1}) and harvest (↑^{2}), and the downward (↓) arrows indicate the dates of manure injections. Standard errors were not included for clarity. Note the different y-axis scales across panels.

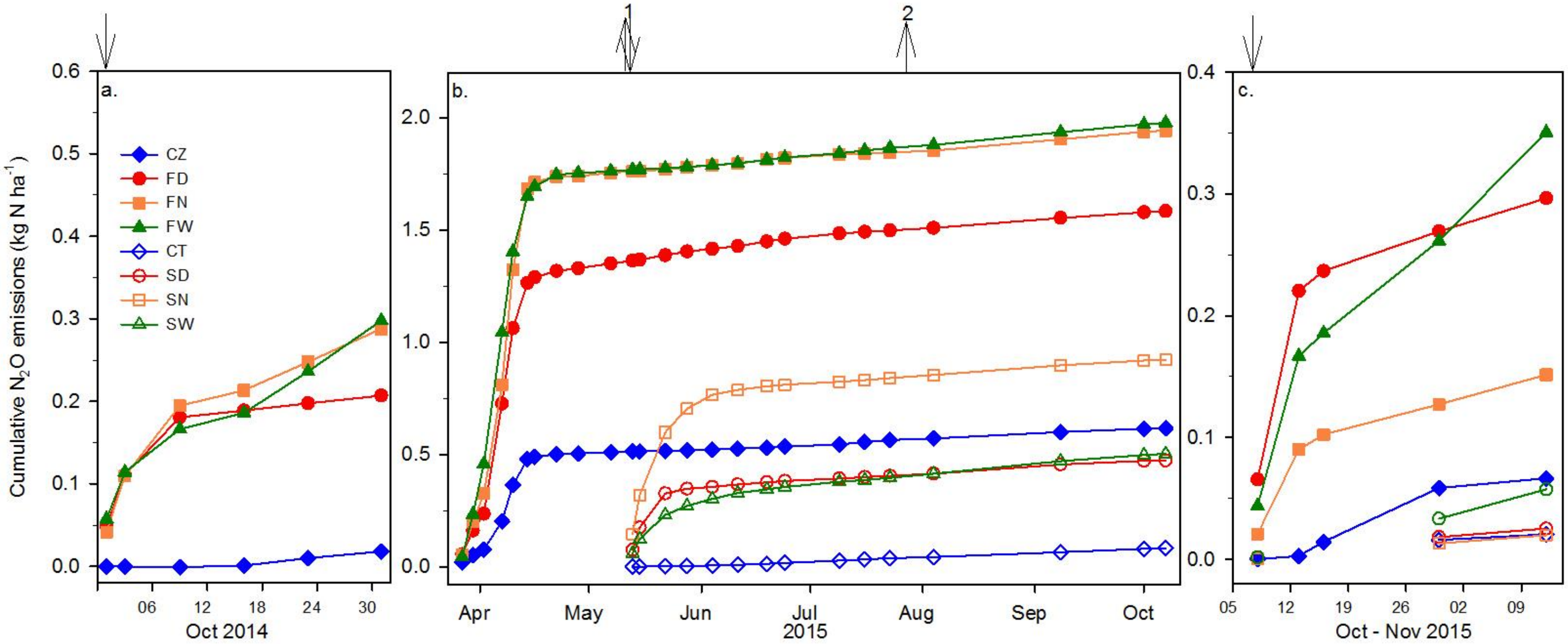


Fig. 2-3. Cumulative N$_2$O emission patterns in the Edmonton site during the periods from (a) 1 Oct to 31 Oct 2014, (b) 27 Mar to 7 Oct 2015 and (c) 8 Oct to 11 Dec 2015. The upward arrows indicate the dates of seeding (↑^{1}) and harvest (↑^{2}), and the downward (↓) arrows indicate the dates of manure injections. Standard errors were not included for clarity. Note the different y-axis scales across panels.

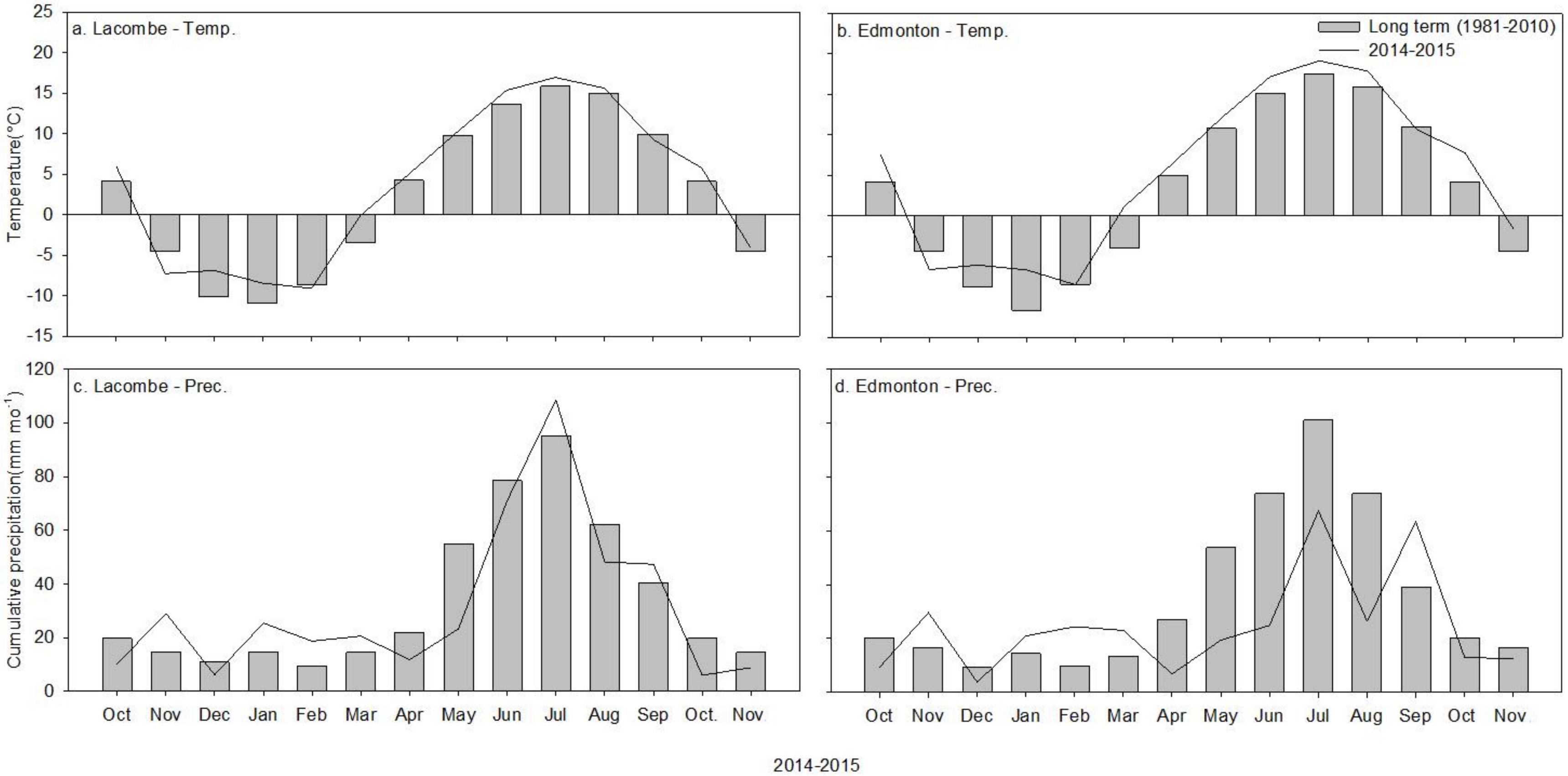


Fig. 2-4. Monthly average air temperature for (a) Lacombe and (b) Edmonton sites, and cumulative precipitation for (c) Lacombe and (d) Edmonton sites during the experimental period. The 30-year normal monthly averages are also shown. Monthly average temperature and cumulative precipitation data is derived from Alberta Agriculture and Forestry (2016). The 30-year normal monthly temperature and cumulative precipitation data is derived from Government of Canada (2016).

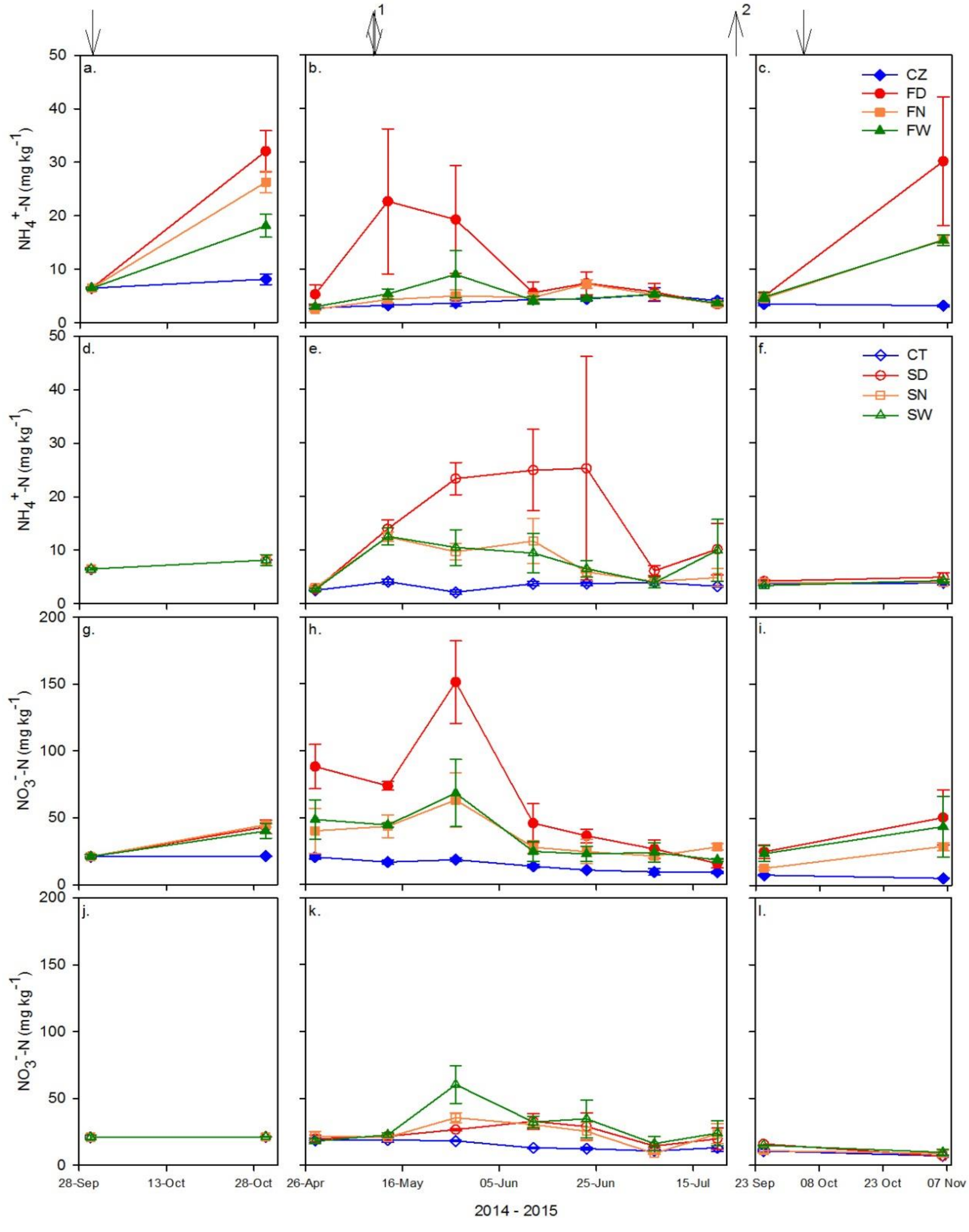


Fig. 2-5. Soil (a, b, c, d, e, and f) ammonium and (g, h, i, j, k and l) nitrate concentrations at the 0-15 cm depth increment during the experimental period in the Edmonton site. Error bars correspond to one standard error. The ammonium and nitrate values for all spring manure and control treatments (SD, SN, SW and CT) from 13 May to 20 Jul 2015 were derived by using weighted averages of the measurements taken from the band and interband zones in the field plots. The same spatial zone sampling and weighted calculation were applied to derived the ammonium and nitrate values for all fall manure treatments (FD, FN and FW) on 30 Oct 2014 and 6 Nov 2015. The ammonium and nitrate values for all fall manure treatments (FD, FN and FW) on 30 Oct 2014 were the average for the 0-20 cm soil layer. The upward arrows indicate the dates of seeding (↑^{1}) and harvest (↑^{2}), and the downward (↓) arrows indicate the dates of manure injections.

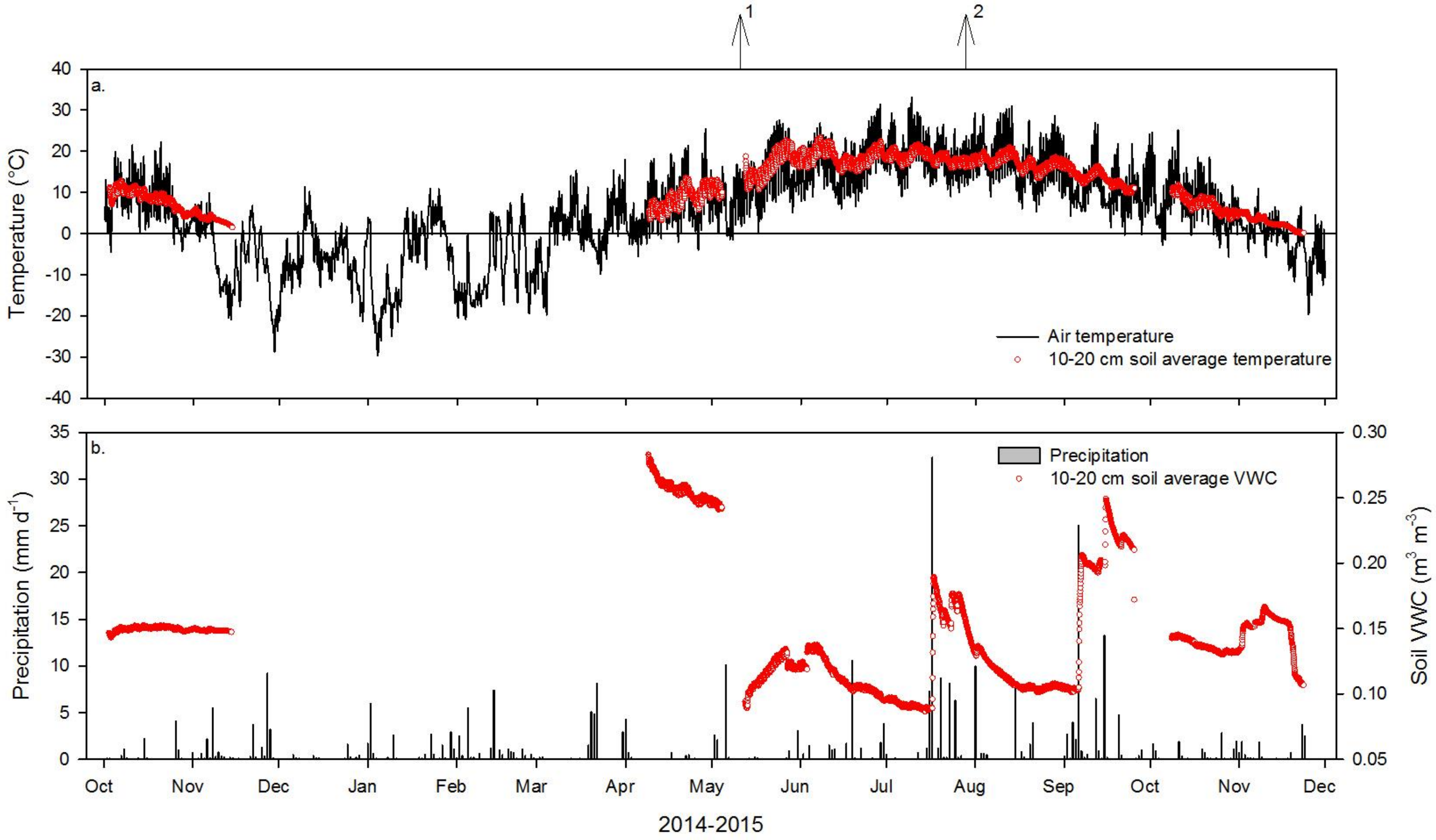


Fig. 2-6. (a) Hourly average air temperature and hourly average 10-20 cm soil temperature and (b) daily cumulative precipitation and hourly average 0-20 cm soil volumetric water content in the Edmonton site during the experimental period. The upward arrows ($\uparrow^1$ or $\uparrow^2$) indicate the dates of seeding ($\uparrow^1$) and harvest ($\uparrow^2$), respectively. VWC = volumetric water content. Average hourly air temperature and cumulative daily precipitation data is derived from Alberta Agriculture and Forestry (2016).

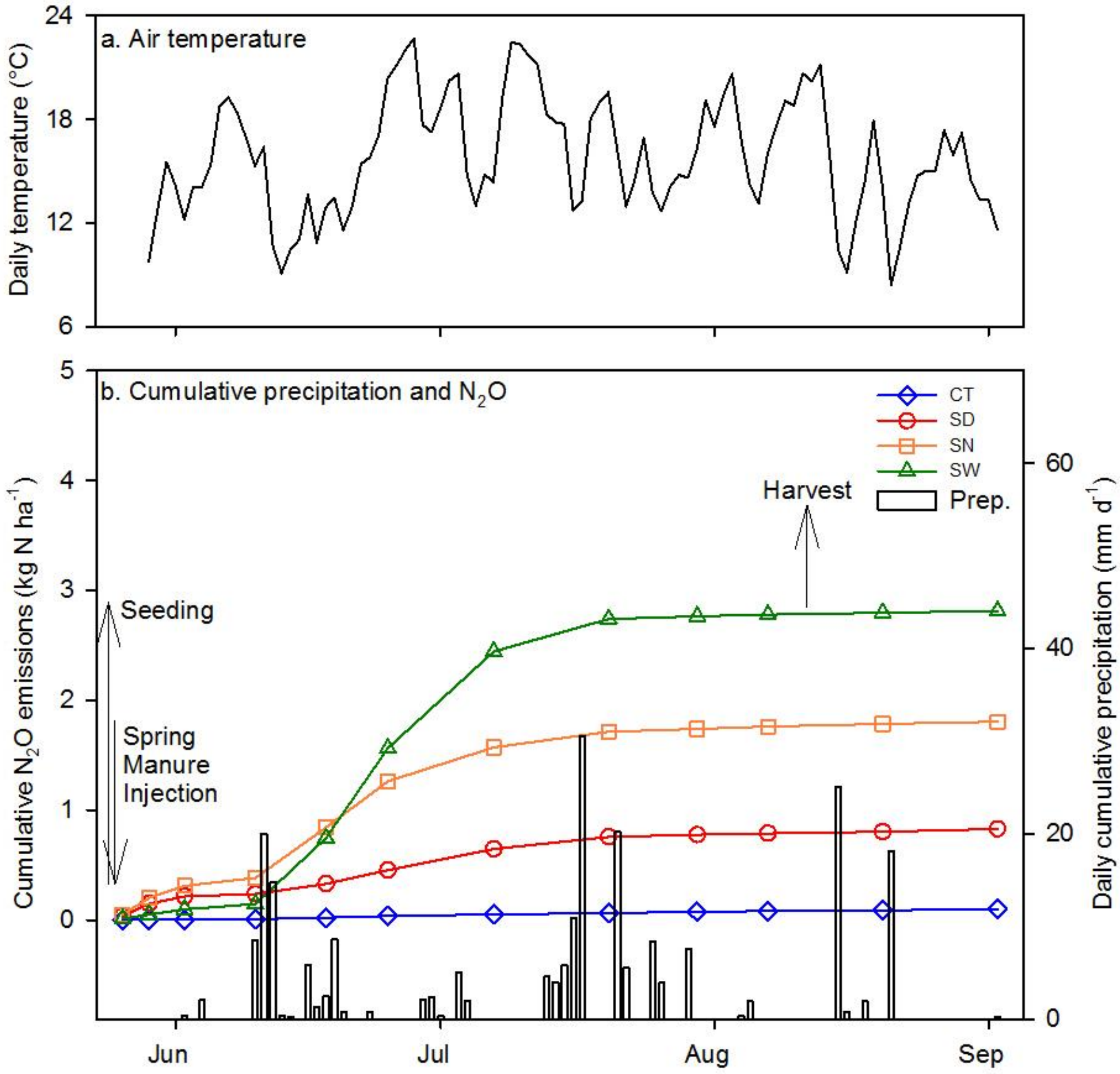


Fig. 2-7. (a) Average daily air temperature and (b) cumulative daily precipitation and N_2O emissions in the Lacombe site during the period from 26 May to 2 Sep 2015 after the spring manure injection. Error bars were not included for clarity. Average daily air temperature data is derived from Alberta Agriculture and Forestry (2016).

3 Nitrogen Kinetics and Nitrous Oxide Emissions from Manured Soils: Comparisons of Field versus Incubation Experiments and Nitrification Inhibitor Types

3.1 Abstract

Agricultural soils recurrently receiving manure or synthetic nitrogen fertilizer inputs constitute major sources of nitrous oxide (N_2O) – a potent greenhouse gas (GHG) of critical environmental concern. We examined N_2O fluxes and mineral N transformations in soils receiving dairy liquid manure injections and the addition of nitrification inhibitors (NIs; nitrapyrin and DMPP) in both a field experiment and a laboratory incubation. Seven field treatments including fall and spring manure injections were established in a split-plot design, and N_2O fluxes were measured using manual static chambers at least weekly. For the incubation experiment, we used soils from the same field treatments collected shortly after the spring manure injection. Soil ammonium (NH_4^+), nitrate (NO_3^-) and N_2O fluxes were measured at least weekly during this 28-day incubation. Relative to the control with no manure, field N_2O fluxes were largely increased by the manure injections without NIs, while the addition of NIs to the liquid manure showed some modest N_2O flux reductions during cold periods. For instance, DMPP additive tended to decrease the N_2O emissions by 31% during the month following the fall manure injection. When comparing our field versus incubation experiments, incubated soils typically showed higher N_2O production (after accounting for differences in thermal times). This apparent divergence can be partially attributed to higher soil water content in the incubation. Moreover, N input and substrate availability also showed to drive N_2O production and the rates of N transformations such as ammonification and nitrification. This became self-evident from our spatial contrast of manure injection band vs. interband zones; the band soils exhibited one-order of magnitude larger production of N_2O in clear linkage with variations in NH_4^+ and NO_3^- concentrations. Additionally, model fitting of these nutrient changes with time revealed that the kinetics of ammonium depletion can be better depicted by second order models, while nitrate accumulation was well described by first order on the basis on statistical criteria (frequency of *Ps*<0.05 and R^2s>0.85). An improved knowledge of these biophysical controls and processes can support quantitative modeling that accurately represents N dynamics and N_2O fluxes in manured soils with and without NIs additions.

Keywords: liquid manure, kinetic model, denitrification, ammonification, nitrification, mineralization, nitrous oxide, nitrification inhibitor, 2-chloro-6-(trichloromethyl) pyridine (nitrapyrin), 3, 4-dimethylpyrazole phosphate (DMPP).

3.2 Introduction

Nitrous oxide is a crucial contributor to global warming with a 100-year time horizon global warming potential 298 times greater than carbon dioxide on a mass basis (Myhre et al. 2013), and also contributes to the ozone depletion (Ravishankara et al. 2009). On a global scale, the agriculture sector is responsible for approximately 82% (2763.1 Tg CO_2e) of total N_2O emissions in 2005 and about 67% of these emissions originate from agriculture soils receiving repeat applications of manures or synthetic N fertilizers (USEPA 2012). The global nitrogen fertilizer demand is expected to increase by 1.4% in the period of 2014-2018 (FAO 2015), and consequently, N_2O emissions to the atmosphere are predicted to continue increasing (Kim et al. 2013; Hoben et al. 2011; Velthof and Mosquera 2011). Thus, it is necessary to enhance N input management and investigate GHG mitigation strategies.

Autotrophic nitrification and heterotrophic denitrification are the two major sources of N_2O production in the soil (Braker and Conrad 2011). Autotrophic nitrification is an oxidation process converting NH_4^+ to NH_2OH, NO_2^- and eventually to NO_3^- by aerobic microorganisms, whereas the denitrification is a reduction reaction converting NO_3^- to NO_2^-, NO, N_2O or N_2 by anaerobic microorganisms (Butterbach-Bahl et al. 2013). The intermediate products in the autotrophic nitrification, NH_2OH or NO_2^-, could be further oxidized to N_2O (Butterbach-Bahl et al. 2013). Any factors impacting autotrophic nitrification and denitrification can affect the magnitude of N_2O emissions, including soil texture (Harrison-Kirk et al. 2013), manure application methods (Maguire et al. 2011), soil moisture and temperature (Luo et al. 2013), soil respiration (Butterbach-Bahl et al. 2013) and plant N uptake (Saarnio et al. 2013).

Soil moisture has major effects on N_2O emissions as it is not only a substrate for soil nitrifying microbes (Schaufler et al. 2010), but also controls the microbe-available oxygen diffusion (Schindlbacher et al. 2004; Meixner and Yang 2006). It was proposed that the maximum N_2O emissions happened at around 50-70% water-filled pore space (WFPS) where both nitrifier and denitrifier activities occur (Davidson et al. 1991; Davidson et al. 2000). Below 35-60% WFPS, N_2O production is mainly derived from autotrophic nitrification (Davidson and Schimel 1995; Bateman and Baggs 2005), whereas denitrification dominates at higher WFPS (Dobbie et al. 1999; Zhu et al. 2013). However, under completely anaerobic conditions, the main end product of denitrification is dinitrogen (N_2) rather than N_2O (Meixner and Yang 2006).

Along with soil water content, soil temperature is another dominant environmental factor for N_2O emissions. N_2O production is positively associated with soil temperature (Smith et al. 2003) and the amount of N_2O production has been found to double with a rise of 10 °C in temperature (Phillips et al. 2015) following a curvilinear Q_{10} relationship (Hernandez-Ramirez et al. 2009a). This is due to acceleration of N_2O-related enzymatic processes, and also to a reduction in oxygen concentrations because of enhanced respiration (Butterbach-Bahl et al. 2013).

It has been suggested that injecting slurry manure into soil can shift denitrification towards production of dinitrogen instead of N_2O (Smith and Mukhtar 2015), and this practice can also prevent ammonia N losses that happened following surface broadcast application (Laboski et al. 2013). An effective injection depth is required to achieve this benefits (i.e., deeper than 5 cm) (Nyord et al. 2008). In practice, nevertheless, manure injections could induce a banding effect, which refers to the uneven distribution of manure in the fields within and between injection rows owing to large machine spacing and high manure volume to band spacing ratio (Chen et al. 2010).

With the purpose of reducing anthropogenic N_2O emissions, NIs can be incorporated with fertilizer or manure applications. NIs are able to slow down the nitrification enzymatic activities, delaying nitrification oxidation and reducing the potential for high N_2O emissions and other N losses (Subbarao et al. 2006). Nitrapyrin, dicyandiamide (DCD) and DMPP are three commonly used NIs in agriculture, but DCD is more suitable to be used as a coating on solid fertilizers due to its high solubility in water and low volatility (Subbarao et al. 2006).Therefore, DMPP and nitrapyrin may be effective NIs with liquid manure applications (Thompson et al. 1987; Menéndez et al. 2006).

Very few studies have addressed comparisons of N_2O production in incubation versus field experiments using the same soils and treatments (Minet et al. 2016). Moreover, to date, there have been no studies quantifying and describing nitrification kinetics by testing second order model. The objectives of this project were to: 1) evaluate the performance of NIs and the key biophysical controls on N_2O emissions in soils receiving liquid dairy manure, 2) compare soil N_2O production from laboratory incubation versus field measurements, and 3) assess various mathematical models to describe the soil mineral N transformation rates.

3.3 Materials and Methods

3.3.1 Site Characteristics and Field Experiment Design

The study site was located in Edmonton, Alberta (53°29’30’’N, 113°31’53’’W). The soil is classified as Black Chernozem, with a clay to heavy clay texture, a bulk density of 1.15 g cm^{-1}, a 66.7 g organic carbon kg^{-1} soil, an electrical conductivity of 0.6 dS m^{-1} and a pH of 6.1. The climate of this area is semi-arid continental.

The field experimental design for this site was a split-plot with three replicates. There were seven treatments in total, including control (no manure added) with a pass of the manure injector to simulate disturbance (CT), fall manure without any inhibitors (FW), fall manure with DMPP (FD), fall manure with nitrapyrin (FN), spring manure without any inhibitors (SW), spring manure with DMPP (SD) and spring manure with nitrapyrin (SN). Barley (*Hordeum vulgare* L.) for silage was planted on 11 May 2015. The fall and spring liquid dairy manure was applied on 30 Sep 2014 and 12 May 2015 respectively with a uniform volume rate of 56.17 m^3 ha^{-1} using a coulter manure injector. To avoid disrupting the seed beds, the direction of manure application was perpendicular to the direction of seed beds. The NI (i.e., nitrapyrin or DMPP) was mechanically mixed with the liquid manure at a rate of 0.4 kg active ingredient ha^{-1} prior to field injection. The characteristics of the liquid manure were measured in every manure batch.

3.3.2 Laboratory Incubation Experiment

The 0-15 cm depth of the soil was collected using a push probe on 13 May 2015. Composite samples of 9 or 10 soil cores were collected from each field plot. Due to the injection and non-injection areas created by the coulter manure injector, soil samples were collected from band (B) and interband (I) areas separately only for the control and spring manure treatments. The collected soil samples were stored in a refrigerator at 5°C until the pre-incubation.

Each field plot was replicated six times in the laboratory incubation (i.e., 18 replicates for each treatment in total). 90 g of soil were placed in each incubation container and the average bulk density was 1.01 g cm^{-1}. All the soil samples were incubated under a constant 60% WFPS (Eq. [3-1]) and room temperature throughout both the 2-day pre-incubation and 28-day aerobic incubation. The room temperature was recorded by a temperature data logger (UX100-001, HOBO Onset™). To keep water content constant, microcosms were weighed daily and deionized water was added when required. To reduce water loss and protect from direct solar radiation, a cardboard cover was placed on the top of the microcosms, which allowed for air circulation.

$$WFPS = \frac{GWC * BD}{f} * 100\% \qquad [3\text{-}1]$$

where WFPS is the water-filled pore space (%); GWC is the gravimetric water content (g g^{-1}); BD is the bulk density (g cm^{-3}) and f is the total porosity (cm^3 cm^{-3}).

To measure N_2O and CO_2 production rate, each soil incubation container was placed inside a 500 mL mason jar equipped with a rubber septum on the top to collect gas samples. These gas samplings took place on days 0, 3, 8, 10, 15, 21 and 28 of the incubation. For every gas production measurement, gas samples were taken at 17, 34 and 51 minutes after sealing the mason jars. Six ambient gas samples were also taken on every sampling date. The calculation of gas productions is shown below in the field flux method section (Eq. [3-5]).

We also conducted destructive sampling to measure soil N transformations. Soil samples were randomly selected from each treatment and stored in the refrigerator at 5°C on days 7, 14, 21 and

28 of the incubation for conducting soil NH_4^+-N and NO_3^--N analyses. At the end of the experiment, all the soil samples were air dried and ground to pass through a 2 mm sieve.

In general, soil N transformation rates were interpreted by the linear rate, first order kinetic and second order kinetic models with three scenarios (three, four and five points representing the first three, four and five sampling dates, respectively). The selection of the best fitted model was determined based on statistical criteria [i.e., p-values (*P*) <0.05 and coefficient of determination (R^2) > 0.85) of the linear or nonlinear regressions. The linear rate model assumed that the soil mineral N concentrations followed a simple linear relationship with time. Mineralization, ammonification and nitrification rates were calculated by Eq. [3-2] according to the linear model assumption. However, the soil mineral N changes versus time typically follow curvilinear patterns, thus we also conducted first order and second order kinetic models to better represent these curvatures and estimate both ammonium depletion and nitrate accumulation rates. Others studies have previously conducted the first order kinetic model to determine ammonium depletion (Irigoyen et al. 2003; Omonode and Vyn 2013) and they observed a significant linear relationship between the natural logarithm NH_4^+-N concentration and time. In this study, we extended the application of first order kinetic to also model nitrate accumulation rate (Eq. [3-3]). We conducted the first and second order numerical modelling for both ammonium depletion and nitrate accumulation rates (Eq. [3-4]) as follows:

$$r = \frac{C_t - C_o}{t} \tag{3-2}$$

$$k_1 = \frac{lnC_o - lnC_t}{t} \tag{3-3}$$

$$k_2 = \frac{1}{C_t * t} - \frac{1}{C_o * t} \tag{3-4}$$

where r is the rate of transformations (mg N $kg^{-1}day^{-1}$); k_1 is the first-order constant rate or kinetic (day^{-1}); k_2 is the second-order constant rate or kinetic (mg^{-1} N kg day^{-1}); C_o is the initial concentration of NH_4^+-N or NO_3^--N (mg kg^{-1}) and C_t is the concentration of NH_4^+-N or NO_3^--N at time t (day).

3.3.3 Field Flux Method

The field flux measurements were conducted following a common manual static chamber method (Hernandez-Ramirez et al. 2009a) twice a week after manure injections, major precipitation and during the spring-thawing period; otherwise, flux measurements were done once a week. The static chambers (10 cm height above the ground and inserted 5 cm deep in the soil) were located in the middle of the plot encompassing two manure injection rows. The distance between injections was about 28 cm, and the width of the manure band was about 2.5 cm. Based on these dimensions, the manure injection and non-injection rows were estimated to contribute 7.6% and 92.4% of total area within the chamber, respectively, and these percentages (or area weights) were used to spatially aggregate the band and interband data. The gas sampling time was between 11 am and 3 pm and gas samples were taken at 16, 32 and 48 minutes after the chamber was closed. Three ambient gas samples were randomly taken at the chamber height level.

All N_2O and CO_2 production rates (incubation and field) were determined by plotting gas concentration versus time and assessing a linear (incubation) or a quadratic fitting relationship (incubation and field). The cumulative productions were calculated by multiplying the average gas production rate between two consecutive sampling dates with the time interval. The N_2O and CO_2 production rates have been considered as zero if non-significant relationship (P>0.2) was observed; otherwise the production rate was calculated by Eq. [3-5] which is an extended expression of the Ideal Gas Law. If both linear and quadratic fitting relationships were significant ($P<0.2$), the one with higher R^2 value would be considered as the production rate. 74%, 15% and 11% of the total gas measurements in the field were calculated by linear, quadratic and zero regressions, respectively. A 94% and 6% of the total measurements in the laboratory incubation were calculated by linear and zero regressions, respectively. The slope (S) used in Eq. [3-5] was the regression coefficient of a simple linear regression or the first derivative of the quadratic regression curve at time zero (Yates et al. 2006; Pennock et al. 2010).

$$PR = \frac{S * P * V * A^{-1}}{R * T} \quad [3\text{-}5]$$

where PR is the production rate of the gas (μmol min^{-1} m^{-2}), S is the slope (μL L^{-1} min^{-1}), P is the pressure of the gas (atm), V is the volume of the gas chamber (L), A is the surface area of the gas chamber (m^2), R is the gas constant (atm μL K^{-1} μmol^{-1}) and T is the temperature of the gas (K).

3.3.4 Gas Flux and Soil N Balance Analyses

Both incubation and field gas samples were analyzed by using a Laurier Varian 3800 gas chromatograph with an electron capture detector for N_2O and a thermal conductivity detector for CO_2. The estimated flux detection limit was 2.84 g N ha^{-1} day^{-1}. To quantify the concentrations of soil NH_4^+-N and NO_3^--N, soil samples were extracted with 2M KCl, and subsequently, the filtrate was measured colorimetrically on a SmartChem discrete wet chemistry analyzer.

3.3.5 Statistical Analyses

Data normality was tested by the Shapiro-Wilk test. The homogeneity of variances was tested by the Bartlett or Levene tests. The data were transformed if necessary using the Box-Cox Power transformation. The difference in the total N, NH_4^+-N, water content and pH of the liquid manure between the two times of application was assessed by a two sample t test. The differences in the N_2O, CO_2, soil NH_4^+-N and NO_3^--N among treatments were determined by one-way analysis of variance (ANOVA) followed by a Fisher's least significant difference (LSD) test; these differences were assessed by Kruskal-Wallis or Welch's ANOVA tests if the assumptions were not satisfied after transformations. The relationship between the soil mineral N and time were tested by regression analyses, such as simple linear, multiple linear and nonlinear regressions. All analyses were tested at a p- value of 0.05 with the exception of the GHG regression tests (i.e., 0.20; GHG data is presented in Fig. 3-1, Fig. 3-2 and Fig. 3-3) and were performed using the version 3.1.3 of R software (Team 2015).

3.4 Results

3.4.1 Laboratory Incubation N_2O & CO_2 Production Rates

Soil samples used in the laboratory incubation were collected from our field plots on 13 May 2015, one day after spring manure injections and 225 days after fall manure injections. Injection band and interband areas were sampled separately in the three spring manure treatments and the control plots; while soils from the three fall manure treatments were collected as composited samples from these plots without separating band and interband areas. These samples were then incubated for a period of 28 days under controlled environment conditions (20.41°C mean air temperature, 0.35-0.40 cm^3 cm^{-3} soil volumetric water content).

Cumulative N_2O and CO_2 production rates were sharply impacted by manure band versus interband (Fig. 3-1). Aggregating 4 weeks of incubation, all the band soils for the spring manure treatments showed higher cumulative fluxes for both N_2O (Fig. 3-1a vs. b) and CO_2 (Fig. 3-1c vs. d) compared to their corresponding interband soils. The spring manure only (SW-B) had the highest N_2O flux (4.63 mg N kg^{-1} soil) followed by the spring nitrapyrin (SN-B) (2.66 mg N kg^{-1} soil) and the spring DMPP (SD-B) (2.00 mg N kg^{-1}soil) (Fig. 3-1a). In contrast, there were no significant differences in cumulative CO_2 among the three spring band treatments (Fig. 3-1c). Across the fall treatments, the fall manure with DMPP (FD) had the highest N_2O flux, followed by the fall manure only (FW) and the fall manure with nitrapyrin (FN) (Fig. 3-1a). The general N_2O flux ranking of the treatments in the incubation was: spring band > fall manure > spring interband ≈ control treatments (Fig. 3-1a and b). The spring manure interband treatments did not show any differences in both cumulative N_2O and CO_2 flux from the control treatments (Fig. 3-1).

When band and interband data were aggregated, there were no significant differences in cumulative CO_2 flux in the incubation (Fig. 3-2b). However, there were significant differences in cumulative N_2O flux observed among certain treatments ($P<0.05$ as LSD test) (Fig. 3-2a). Compared with the control (0.18 kg N ha^{-1}), both the fall and the spring manure only treatments induced an extremely high cumulative N_2O flux (0.39 and 0.38 kg N ha^{-1}, respectively) (Fig. 3-2a). For the spring manure treatments, the treatments with NIs showed a reduction in N_2O flux. The spring manure with DMPP and with nitrapyrin trended to reduce cumulative N_2O by 32 and 42%, respectively, compared to the treatment with manure only (Fig. 3-2a). However, NIs did not lead to a significant reduction of N_2O production in the fall manure treatments (Fig. 3-2a). The highest N_2O flux was observed in the fall manure with DMPP treatment (0.58 kg N ha^{-1}), and the cumulative N_2O fluxes of the fall manure without NIs were similar with that of the fall manure with nitrapyrin (Fig. 3-2a).

3.4.2 Field N_2O emissions

Cumulative N_2O emissions in the field were affected by inhibitor type and time of manure addition. When comparing the three periods of field flux measurements, the highest cumulative N_2O production for the fall manure treatments was found in the early spring season prior to the manure injection on 12 May 2015 with an average value of 1.63 kg N ha^{-1} (Fig. 3-3b), which was more than 6 times larger than the average cumulative emissions after the fall manure injection (Fig. 3-3a) and approximately 37 times larger than that after the spring manure injection (Fig. 3-3c). Compared to the fall manure without NIs (FW treatment), the fall manure with DMPP (FD) numerically reduced cumulative N_2O emissions by 31 and 23% during the Oct 2014 (Fig. 3-3a) and 27 Mar to 12 May 2015 periods (Fig. 3-3b), respectively, but did not reduce emissions during

15 May to 11 Jun 2015 period (Fig. 3-3c). Unlike the fall DMPP, the fall manure with nitrapyrin did not reduce cumulative N_2O production during any sampling period (Fig. 3-3).

3.4.3 Selection of Models for Soil Mineral Nitrogen Transformations

Three scenarios of simple linear regression were tested to find the best simple linear model for the changes in mineral N, NH_4^+-N and NO_3^--N versus time. For NO_3^--N, the optimum simple linear relationship was observed in the model with four points (within the first 21 days of incubation) because of the maximum count of fitted models (i.e., $P<0.05$ and $R^2>0.85$) compared to the three-point and five-point models (Table 3-1). Similarly, the best fitted models for NH_4^+-N were the four-point models due to relatively lower p-values and higher R^2 value although there was no statistically significant relationship between NH_4^+-N and time (Table 3-1). Thus, the four-point model was used to calculate the rate of the mineral N, NH_4^+-N and NO_3^--N concentration changes for all soil treatments (i.e., linear rates as Eq. 3-2; Table 3-2).

Additionally, three scenarios (three, four and five points) were studied for the natural logarithm and reciprocal of the soil mineral N [ln (NH_4^+-N) and ln (NO_3^--N) as Eq. 3-3; $1/NH_4^+$-N and $1/NO_3^-$-N as Eq. 3-4]. It seemed that the relationship between the time versus the natural logarithm of NH_4^+-N concentration in the two scenarios with four and five points could be better described by a quadratic curve rather than a straight line or a cubic curve as indicated by a maximum count of fitted models (

Table 3-3). This was also found in the relationship between the time versus the reciprocal of NH_4^+-N concentration in the scenario with five points (Table 3-4). The same condition was found in the relationship between the time versus the natural logarithm or reciprocal of NO_3^--N concentration in the scenario with five points (

Table 3-3 and Table 3-4). However, no studies have previously used the kinetic equation to calculate the soil mineral N transformations for a quadratic relationship between the logarithm or reciprocal of soil mineral N concentrations versus time. This could be further examined in the future studies. Hence, we were only able to calculate the soil N transformation rates based on the linear relationship between the natural logarithm and reciprocal versus time in this paper, which are known as first order and second order models, respectively.

Based on the highest frequency of fitted models fulfilling our model selection criteria ($P< 0.05$ and R^2s > 0.85), the linear models with four points for both ammonium depletion and nitrate accumulation were the best scenarios (

Table 3-3 and Table 3-4), which therefore were used to calculate the first order and second order kinetic N rates (Table 3-2). It should be noted that the p-values for the ammonium depletion in the four-point model were lower than 0.25 for first-order (

Table 3-3) and 0.28 for second-order (Table 3-4), while the p-values for the nitrate accumulation in the four-point model were even lower (< 0.09 and 0.11, respectively) with the exception of the control treatment (

Table 3-3 and Table 3-4).

3.4.4 Soil Mineral Nitrogen and Kinetics

The simple linear N transformation rates were calculated for four separate phases with one duration each phase (

Table 3-5) and for the whole incubation period (28 days) (i.e., linear rates in Table 3-2). There were a NH_4^+-N depletion and a NO_3^--N accumulation during the first 3 weeks of incubation (

Table 3-5). It was observed that the highest average net mineralization and net nitrification rates among all treatments took place during the second phase (8-14 days), while the highest average net ammonification rate took place during the first phase (0-7 days) in the stages of the incubation (

Table 3-5).

According to the linear N transformation rates, apparent mineralization and nitrification processes were evident in the soil during the 4 weeks of incubation (Table 3-2). Both fall and spring manure treatments exhibited more rapid ammonium depletion and nitrate accumulation kinetics than the control with an exception of second order kinetics for nitrate accumulation (Table 3-2). The fall manure with DMPP had the highest ammonium depletion kinetic rate across the fall manure treatments (78.14 day^{-1} × 1000 for first order and 8.75 μg^{-1} N kg day^{-1} for second order). There was no clear difference in the ammonium depletion kinetic among the spring manure treatments for both first order and second order kinetics (Table 3-2). For the first order nitrate accumulation kinetic, a relatively higher kinetic rate was observed in the spring manure without NIs compared to the spring manure with NIs (Table 3-2). A similar outcome of the kinetic rates was found among the fall manure treatments where the soils with NIs exhibited slower N transformations (Table 3-2).

3.4.5 Manure Characteristics

Manure characteristics were variable (Table 3-6). There was no significant difference in total N, NH_4^+-N, water content and pH in the manure application in fall 2014 versus spring 2015.

3.5 Discussion

3.5.1 Nitrification Inhibitor Effects on N_2O Production

Both inhibitors appeared to numerically reduce N_2O production immediately after seasonal injections for both the fall (Fig. 3-3a) and the spring manure treatments (Fig. 3-2a) even though these trends were not statistically significant. However, we observed more pronounced N_2O fluxes from the incubated soil treated with DMPP than with nitrapyrin for the fall manure treatments (Fig. 3-2a). This could be explained by the time of manure injection. All samples for the incubation experiment including the fall treatments were field collected on 13 May 2015, but these fall manure soils had received the manure addition on 1 Oct 2014 (about six months prior to soil sample collection). Our results indicate that the fall DMPP treatment kept much more N in the soil during Oct 2014 than the soils received only manure without any additives (data not shown). Furthermore, from Nov 2014 until the soil samples collected, the N retained by the inhibitor DMPP was still in the soil because the soil was frozen and few microbial activities were assumed to happen in the field during this time. Therefore, once the soil with the fall DMPP additive was incubated under optimal conditions (i.e., temperature of 20.4°C and 60% WFPS), the N retained by the DMPP since the previous fall was rapidly transformed leading to intense N_2O production. This result could also imply the quick decay of the DMPP inhibitor due to sudden exposure to higher temperatures in the incubation relative to the field temperatures (20.41 vs. 17.79°C).

Compared to other inhibitors, the high effectiveness of DMPP has been previously found by other researchers. Dittert et al. (2001) demonstrated the benefits of DMPP in the mitigation of N_2O in the grassland ecosystem. Furthermore, Akiyama et al. (2010) summarized N_2O available data as a function of NIs with the goal of evaluating a range of inhibitors in agricultural systems. They reported that DMPP (1-2 kg ha^{-1}) and nitrapyrin (7.5-12.5 kg ha^{-1}) reduced N_2O production from a grassland system by ~55% (2 observations) and ~30% (7 observations), respectively, compared to conventional fertilizer without any NIs. In our study, the fall DMPP and fall nitrapyrin only numerically reduced N_2O production by ~31% and ~0.03%, respectively (Fig. 3-3a). The lower N_2O reduction coefficients found in our study could be due to the predominance of cold and dry weather during Oct 2014 as these climatic-edaphic conditions may have led to lower nitrification and denitrification activities, possibly resulting in lower N_2O production. DMPP showed capacity to reduce N_2O production, but this response may vary as a function of the specific soil, climate and management conditions. However, it should be noted that, in the spring incubation treatments (Fig. 3-2a), nitrapyrin and DMPP reduced N_2O production by a similar amount relative to manure without NIs. This could be a consequence of the incubation containers which prevented nitrapyrin from moving and dissipating as they effectively kept the active ingredient concentrated in the soil microcosms.

A difference in the effectiveness of DMPP and nitrapyrin could possibly be explained by their different mobility relative to that of NH_4^+-N (Fig. 3-3a). A lower effectiveness of nitrapyrin can be associated with its lower mobility compared to NH_4^+-N (Subbarao et al. 2006), while higher effectiveness of DMPP can be directly linked with its movement speed similar to NH_4^+-N (Pasda et al. 2001). Thus, DMPP could interact with the NH_4^+-N much more tightly than nitrapyrin, becoming more effective and reducing much more potential N_2O production. On the other hand, the fall nitrapyrin treatment did not show any obvious N_2O reduction in the field in Oct 2014 (Fig. 3-3a), and any major N_2O fluxes in the incubation (Fig. 3-2a) as the fall DMPP treatment performed. This might be explained because nitrapyrin could not be highly effective under the relatively low soil water content (Fig. 3-3a) that can typically occur in the falls in Central Alberta (i.e., 0.15±0.01 m^3 m^{-3}). This relationship is not completely clear yet, and further research could focus on assessing potential thresholds of soil water content to enable the effectiveness of nitrification inhibitors (e.g., nitrapyrin).

To a certain extent, our incubation experimental setting represented an environmental change (i.e., from cold-dry conditions in the fields prior to soil sample collection to warm-wet during the incubation) which can imitate a similar magnitude of change as the spring thawing, where the soils commonly experience a sudden increasing soil water content and temperature during the early spring after approximately six months of freezing conditions under the Alberta winter. We could further infer from our incubation data that the fall manure application could have an increased risk for high N_2O emissions in the field following the spring thaw in the subsequent year (See Chapter0). A previous field study recorded high N_2O emissions during early snow melting periods in Central Alberta (Nyborg et al. 1997). Their results showed that during the beginning of the spring thaw, a greater amount of N_2O was emitted from the soils receiving chemical N inputs compared to the control without N addition.

Contrary to our initial hypothesis, NIs in this study only showed modest N_2O emission reduction (Fig. 3-2a and Fig. 3-3a). This nearly null response can be explained by relatively dry field conditions in the study site during the growing season 2015 (data not shown) and intrinsic separation of the band and interband soils in the incubation protocols as discussed below.

3.5.2 Band vs. Interband N Effects on N_2O outputs

Remarkably higher cumulative N_2O fluxes occurred in the spring manure band soils than corresponding interband soils (Fig. 3-1a vs. b). This clear spatial effect of manure banding explained because the band zones had significantly higher initial mineral N concentration from the manure injection (Table 3-7) and also due to the accelerated ammonification and nitrification rates during the incubation (band vs. interband ammonification and are not shown). Under field conditions, concentrated mineral N, including NH_4^+-N and could be lost via nitrate leaching, plant uptake, ammonia volatilization, immobilization denitrification. However, it is noteworthy that the incubation containers prevented these N substrates from diluting or dispersing as it typically occurs in fields where mineral N concentrations in band and interband soil zones intermixed and gradually converged with following N additions. Unlike in the field where barley growth was an important N sink, N by plants did not occur in the incubation. Also, only a small portion of NH_4^+-N can be be transferred to NH_3 in the incubation through volatilization. This is because the pH of used in the incubation was from 6 to 7, and as previous literature showed, the possibility ammonia volatilization above 16°C would be only about 0.1% at pH 6 and 1% at pH 7 in receiving urea fertilizer application (Court et al. 1964; Freney et al. 1983). Likewise, N immobilization rarely happened in the spring manure band samples except during the first of incubation (

Table 3-5). Therefore, nitrification and denitrification appeared to be the two dominant mineral N transformations accountable for the excessive NH_4^+-N and NO_3^--N in the spring manure band samples. The amount of NH_4^+-N and NO_3^--N substrates can influence the nitrification and denitrification, respectively (Havlin et al. 2014), and these two processes contribute to N_2O production (Hernandez-Ramirez et al. 2009b). As a consequence, the concentrated mineral N in the spring manure band soils was most likely nitrified and denitrified leading to high N_2O (Fig. 3-1a) as well as high losses of N_2 gas associated with complete denitrification (Havlin et al. 2014).

3.5.3 Comparing Incubation and Field N_2O Results

Many studies have previously assessed GHG emissions using the laboratory incubation experiments; however, comparisons between incubation versus field settings have been rarely conducted when using the same soils and treatments. In our study, we had the opportunity to conduct such comparison for N_2O production. This comparison was done by normalizing the cumulative fluxes from incubation versus field measurements to account for differences in thermal times (Table 3-8). For the control and fall treatments, the N_2O results in the laboratory incubation had, in general, the same treatment mean ranking as the field measurements, but the N_2O production quantity in the incubation were much higher compared to the field (Fig. 3-2a vs. Fig. 3-3c, respectively). This is can be in part attributed to the fact that the incubation provides optimum biophysical conditions compared with the variable conditions prevalent in the field (e.g., soil moisture content; Fig. 3-2a vs. Fig. 3-3c). Optimal and constant conditions in soil incubations could stimulate the activities of nitrifiers and denitrifiers, revealing the optimum potential for these processes. Moreover, the impact of soil moisture seemed to be more influential than the temperature effects on N_2O production since our estimations had already accounted for thermal time differences between incubation vs. field experiments (Table 3-8). This overriding moisture impact on N_2O was also observed in the field study (See Chapter0).

Contrary to our expectations, the incubation and the field results provided an unanticipated contrast across the spring treatments. Initially, we presumed that lower N_2O production would be found in the field versus incubation for all treatments; however, the reverse outcome was unexpectedly observed for the spring treatments (Table 3-8). Larger or similar thermal-time corrected N_2O production for spring treatments under field conditions relative to laboratory incubation could be attributed to N dispersion with time from the manure injection band into the interband zone in the field plots. As time progressed, the area and the volume of the soil influenced by the liquid manure band in the field should increase, possibly leading to more N_2O emissions in the spring treatments which had recently received manure injection. Conversely, this intermixing effect could not happen in the incubation setting because the band and interband soil samples were kept separately by the incubation containers without any cross-exposure between band and interband microcosms. For this reason, the calculation for the aggregating band and interband N_2O output in the incubation data could underestimate the N_2O production specifically for the spring treatments even after accounting for the thermal time difference. Additionally, there is another reason to explain the noted reverse N_2O output for the spring treatments when comparing the averages of the three spring treatments in the field versus incubation (i.e., field > incubation) even when focusing on the flux results prior to any thermal time correction – field fluxes were about two times higher than incubation fluxes (Fig. 3-3c vs. Fig. 3-2a, respectively). In further detail, it should be noted that the field N_2O flux even one day after manure injection was still much higher than that in the incubation (data not shown). This unanticipated result can be explained because the denitrification rates within small soil aggregates are typically much higher than in the interaggregate soil volume due to the uneven gas diffusion inside small aggregates, and this restricted diffusion rates can lead to a loss of soil NO_3^--N and N_2O production (Uchida et al. 2008). When our soil was removed from the field to the laboratory, the small aggregates in our soils could have been broken, resulting in lower N_2O production rate in the incubation than in the field conditions where the soils remained undisturbed and hence N_2O production could continue unaffected.

3.5.4 Transformation from NH_4^+-N to NO_3^--N and its Kinetics

The NH_4^+-N depletion observed during the first 3 weeks of our incubation (

Table 3-5) can be directly attributed to faster nitrification than ammonification rates; likewise NH_4^+ immobilization and ammonia volatilization could also add to this net effect. During the same period of time, the pronounced NO_3^--N accumulation can be explained by a vigorous nitrification compared to both denitrification and any NO_3^- immobilization. As evidenced by the much higher magnitude of NO_3^--N accumulation than NH_4^+-N depletion within the first 3 weeks of incubation, net mineralization dominated in all our soils (

Table 3-5). However, the speed of net mineralization largely varied as a function of time of manure injection and nitrification inhibitor type (Table 3-2). High mineralization rates were found for both fall and spring manure treatments (Table 3-2), and this could be because of a high N input of the dairy manure decreasing the soil C:N ratio. Havlin et al. (2014) indicated that if the soil C:N ratio is narrower than 20:1, there would be a net mineralization during the decomposition process.

Our observation of similar first-order ammonium depletion kinetics among the spring manure only and the spring manure with nitrification inhibitor treatments (i.e., SW, SD and SN; Table 3-2) contrasts with results in an earlier report by Omonode and Vyn (2013). They found that there was an effect of nitrification inhibitors on the patterns of ammonium concentration with time (this response is referred in our study as ammonium depletion kinetic) as evidenced by a lower nitrification rate in the soil with nitrification inhibitors compared to the soil with N input only, and they postulated this effect should be due to a decrease in the soil nitrifier population or a slow reactivation of nitrifiers. Omonode and Vyn (2013) further indicate that nitrification inhibitors would not have an impact on nitrification rate if the N application rate was beyond 100 to 160 kg N ha^{-1}. In our experiment, the N manure input (i.e., > 502.5 kg N ha^{-1}, Table 3-6) was much higher than their N fertilizer ranges. Thus, we inferred that the first-order ammonium depletion kinetic for our spring manure soils was only affected by the high N input rather than NIs. Similarly, the first-order nitrate accumulation kinetic for the fall manure treatments mainly depended on the concentration of NH_4^+-N instead of an effect of NIs. This is because the fall inhibitor treatments (FD and FN) seemed to be completely inactive or degraded in the spring-taken soil samples as demonstrated by the lack of reduction in the N_2O production in these fall inhibitor treatments during the incubation experiment (Fig. 3-2a).

The use of a second order kinetic model to describe and represent soil N transformations has not been previously explored. In our study, we compared the first order kinetic models with the second order kinetic models to examine their capacity and performance in describing soil N transformation rates. It is noteworthy that the second order models seemed to improve the description of the ammonium depletion with time as demonstrated by lower p-values and higher R^2 values compared to the first order model. However, this improved performance by using second order models did not occur in the case of the nitrate accumulation (

Table 3-3, Table 3-4 and Fig. 3-4).

3.5.5 Soil Mineral Nitrogen Changes leading to Nitrous Oxide Production

Changes in cumulative N_2O production as a function of the changes in concentrations of NH_4^+-N and NO_3^--N during every 7-day periods in the incubation (i.e., 0-7d, 8-14d, 15-21d, 22-28d) were estimated to test how soil mineral N affected the N_2O production (Table 3-7, Fig. 3-1a and b). This result showed that there was a significant multiple linear relationship between the ΔN_2O^a cumulative production versus the $\Delta NH_4^+\text{-}N^b$ and $\Delta NO_3^-\text{-}N^c$ {i.e., a, b and c are the lambda values from Box Cox transformations and they are 0.110, -0.004 and 0.102, respectively. The back transformed equation is

$$\Delta N_2O(mg\ N\ kg^{-1}) =$$

$$[4.979 - 4.495 * \Delta NH_4^+ - N^{-0.004}(mg\ N\ kg^{-1}) + 0.251 * \Delta NO_3^- – N^{0.102}(mg\ N\ kg^{-1})]^{1/0.110},$$

Ps<0.01, adjusted R^2= 21%}. Both ΔNH_4^+-N and ΔNO_3^--N act as substrates and contribute to N_2O production through nitrification and denitrification. Hence, according to our derived linear equation with negative regression coefficient and exponent for ΔNH_4^+-N as well as positive regression coefficient and exponent for ΔNO_3^--N, the depletion of NH_4^+-N and the accumulation of NO_3^--N had both a positive feedback on N_2O production. In further details, one unit change in nitrate accumulation appears to have a relatively stronger impact on increasing N_2O production than one unit change in ammonium depletion. This is shown by the exponent for ΔNO_3^--N which absolute magnitude is larger of the exponent for the ΔNH_4^+-N (i.e., 0.102 > -0.004). In other words, this linear analysis can suggest that denitrification of nitrate substrate might be the major contributor to the N_2O production in our laboratory incubation. The remaining 79% of the variability in the ΔN_2O response of this relationship could be in part explained by the different management (i.e., time of manure addition and inhibitor type treatment) and the spatial variation of several soil properties (texture, organic carbon, water content) which likely existed across our field plots.

3.6 Conclusion

Nitrification inhibitors may play a positive role in reducing N_2O production. More specifically, DMPP seemed to perform better than nitrapyrin during the periods immediately following fall injections and during the early spring. In spite of this, the fall manure injection with DMPP appeared to keep substantial amounts of fall manure-N in the soils which could create a significant risk for large soil N_2O emissions during the following early spring when sufficient water sources flow into the soil from either snow-melting or rainfall. To mitigate the probability for this high N_2O emission and use the plant-available N in the soil, perennial and winter crops could be grown to produce biomass instead of annual barley. Such winter cereals (e.g., winter wheat) with active growth from the early spring to the late fall could generate plant N uptake as an important sink for available N in fields.

Based on our comparison of N_2O production between the incubation versus the field measurements, soil water content was ascertained as a key biophysical control of the N_2O production. Hence, the management of irrigation or drainage as well as the water uptake by plants in fertilized agricultural systems should receive consideration as GHG reduction strategies. Furthermore, our comparison reveals that the applicability and interpretation of laboratory incubations could be limited as they can lead to underestimations of N_2O production potential in certain specific cases. Our study showed that soil N dynamics and transformations can be captured and quantified using incubation protocols of three weeks duration. In our comparison of N transformation rates derived from three numerical fitting approaches (simple linear, first order and second order models), the first- and second-order kinetics resulted promising to better depict nitrate accumulation and ammonium depletion processes in N-amended soils, respectively.

3.7 Acknowledgement

The authors thank Alberta Livestock and Meat Agency Ltd. (ALMA), Climate Change and Emissions Management Corporation (CCEMC), Dow AgroSciences and Eurochem Agro for their funding supports, the Alberta Agriculture and Forestry for its technical support and Lewis Fausak, Craig Sprout and Catherine Pocklington for their assistance during this study.

3.8 References

Akiyama, H., Xiaoyuan, Y., and Yagi, K. (2010). "Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis." *Global Change Biol.,* 16(6), 1837.

Bateman, E. J., and Baggs, E. M. (2005). "Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space." *Biol.Fertility Soils,* 41(6), 379-388.

Braker, G., and Conrad, R. (2011). "Chapter 2 - Diversity, Structure, and Size of N_2O-Producing Microbial Communities in Soils—What Matters for Their Functioning?" *Adv.Appl.Microbiol.,* 75 33-70.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S. (2013). "Nitrous oxide emissions from soils: how well do we understand the processes and their controls?" *Philos.Trans.R.Soc.Lond.B.Biol.Sci.,* 368(1621), 20130122.

Chen, Y., Assefa, B., and Arkinremi, W. (2010). "Soil Nutrient Levels and Crop Performance at Various Lateral Positions Following Liquid Manure Injection." *Agric Eng Int: CIGR Journal,* 12(1), 1-11.

Court, M. N., Stephen, R. C., and Waid, J. S. (1964). "Toxicity as a cause of the inefficiency of urea as a fertilizer." *J.Soil Sci.,* 15(1), 49-65.

Davidson, E. A., Rogers, J. E., and Whitman, W. B. (1991). "Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems." *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes.,* 219-235.

Davidson, E. A., and Schimel, J. P. (1995). "Microbial processes of production and consumption of nitric oxide, nitrous oxide and methane." *Biogenic Trace Gases: Measuring Emissions from Soil and Water,* 327-357.

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., and Veldkamp, E. (2000). "Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides Using two functions based on soil nitrogen availability and soil water content, the hole-in-the-pipe model characterizes a large fraction of the observed variation of nitric oxide and nitrous oxide emissions from soils." *Bioscience,* 50(8), 667-680.

Dittert, K., Bol, R., King, R., Chadwick, D., and Hatch, D. (2001). "Use of a novel nitrification inhibitor to reduce nitrous oxide emission from ^{15}N-labelled dairy slurry injected into soil." *Rapid Communications in Mass Spectrometry: RCM,* 15(15), 1291.

Dobbie, K. E., McTaggart, I. P., and Smith, K. A. (1999). "Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors." *Journal of Geophysical Research: Atmospheres (1984–2012),* 104(D21), 26891-26899.

FAO. (2015). "World Fertilizer Trends and Outlook to 2018." Food and Agriculture Organization of the United Nations, Rome.

Freney, J. R., Simpson, J. R., and Denmead, O. T. (1983). "Volatilization of ammonia." *Gaseous Loss of Nitrogen from Plant-Soil Systems,* J. R. S. J.R.Freney, ed., Springer, Netherlands, 1-32.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D., and Condron, L. M. (2013). "Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions." *Soil Biol.Biochem.,* 57 43-55.

Havlin, J., Beaton, J. D., Tisdale, S. L., and Nelson, W. L. (2014). *Soil fertility and fertilizers: An introduction to nutrient management.* Pearson Prentice Hall Upper Saddle River, New Jersey, USA, .

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., and Van Scoyoc, G. E. (2009a). "Greenhouse gas fluxes in an eastern corn belt soil: Weather, nitrogen source, and rotation." *J.Environ.Qual.,* 38(3), 841-854.

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., Van Scoyoc, G. E., and Michalski, G. (2009b). "Nitrous oxide production in an eastern corn belt soil: sources and redox range." *Soil Sci.Soc.Am.J.,* 73(4), 1182-1191.

Hoben, J. P., Gehl, R. J., Millar, N., Grace, P. R., and Robertson, G. P. (2011). "Nonlinear nitrous oxide (N_2O) response to nitrogen fertilizer in on- farm corn crops of the US Midwest." *Global Change Biol.,* 17(2), 1140-1152.

Irigoyen, I., Muro, J., Azpilikueta, M., Aparicio-Tejo., P., and Lamsfus, C. (2003). "Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures." *Aust.J.Soil Res.,* (6), 1177.

Kim, D., Hernandez-Ramirez, G., and Giltrap, D. (2013). "Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis." *Agric., Ecosyst.Environ.,* 168 53-65.

Laboski, C., Jokela, W., and Andraski, T. (2013). "Dairy manure application methods: N credits, gaseous N losses, and corn yield." *Proceedings of the Wisconsin Crop Management Conference,* Citeseer, 20-31.

Luo, G. J., Kiese, R., Wolf, B., and Butterbach-Bahl, K. (2013). "Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types." *Biogeosciences,* 10(5), 3205-3219.

Maguire, R. O., Kleinman, P. J. A., Dell, C. J., Beegle, D. B., Brandt, R. C., McGrath, J. M., and Ketterings, Q. M. (2011). "Manure Application Technology in Reduced Tillage and Forage Systems: A Review." *J.Environ.Qual.,* 40(2), 292-301.

Meixner, F. X., and Yang, W. X. (2006). "Biogenic emissions of nitric oxide and nitrous oxide from arid and semi-arid land." *Dryland ecohydrology,* Springer, 233-255.

Menéndez, S., Merino, P., Pinto, M., González-Murua, C., and Estavillo, J. (2006). "3, 4-Dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands." *J.Environ.Qual.,* 35(4), 973-981.

Minet, E. P., Jahangir, M. M. R., Krol, D. J., Rochford, N., Fenton, O., Rooney, D., Lanigan, G., Forrestal, P. J., Breslin, C., and Richards, K. G. (2016). "Amendment of cattle slurry with the nitrification inhibitor dicyandiamide during storage: A new effective and practical N_2O mitigation measure for landspreading." *Agric., Ecosyst.Environ.,* 215 68-75.

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J. F., Lee, D., and Mendoza, B. (2013). "Anthropogenic and natural radiative forcing." *Climate Change,* 423.

Nyborg, M., Laidlaw, J. W., Solberg, E. D., and Malhi, S. S. (1997). "Denitrification and nitrous oxide emissions from a Black Chernozemic soil during spring thaw in Alberta." *Can.J.Soil Sci.,* 77(2), 153-160.

Nyord, T., Søgaard, H. T., Hansen, M. N., and Jensen, L. S. (2008). "Injection methods to reduce ammonia emission from volatile liquid fertilisers applied to growing crops." *Biosystems Engineering,* 100(2), 235-244.

Omonode, R. A., and Vyn, T. J. (2013). "Nitrification Kinetics and Nitrous Oxide Emissions when Nitrapyrin is Coapplied with Urea--Ammonium Nitrate." *Agron.J.,* 105(6), 1475-1486.

Pasda, G., Hahndel, R., and Zerulla, W. (2001). "Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops." *Biol.Fertility Soils,* 34 85-97.

Pennock, D., Yates, T., Bedard-Haughn, A., Phipps, K., Farrell, R., and McDougal, R. (2010). "Landscape controls on N_2O and CH_4 emissions from freshwater mineral soil wetlands of the Canadian Prairie Pothole region." *Geoderma,* 155(3), 308-319.

Phillips, R. L., McMillan, A. M. S., Palmada, T., Dando, J., and Giltrap, D. (2015). "Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil." *N.Z.J.Agric.Res.,* 58(1), 89-95.

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. (2009). "Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century." *Science,* 326(5949), 123-125.

Saarnio, S., Heimonen, K., and Kettunen, R. (2013). "Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake." *Soil Biol.Biochem.,* 58 99-106.

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., and Zechmeister-Boltenstern, S. (2010). "Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature." *Eur.J.Soil Sci.,* 61(5), 683-696.

Schindlbacher, A., Zechmeister-Boltenstern, S., and Butterbach-Bahl, K. (2004). "Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils." *Journal of Geophysical Research: Atmospheres (1984–2012),* 109(D17),.

Smith, D. W., and Mukhtar, S. (2015). "Estimation and attribution of nitrous oxide emissions following subsurface application of animal manure: a review." *Transactions of the ASABE,* 58(2), 429.

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., and Rey, A. (2003). "Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes." *Eur.J.Soil Sci.,* 54(4), 779.

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., and Rao, I. (2006). "Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities." *Crit.Rev.Plant Sci.,* 25(4), 303.

Team, R. C. (2015). "R: A Language and Environment for Statistical Computing." *R Foundation for Statistical Computing,Vienna, Austria,* URL http://www.R-project.org/.

Thompson, R. B., Ryden, J. C., and Lockyer, D. R. (1987). "Fate of nitrogen in cattle slurry following surface application or injection to grassland." *J.Soil Sci.,* 38(4), 689-700.

U.S. Environmental Protection Agency. (2012). "Summary Report: Global Anthropogenic Non-CO_2 Greenhouse Gas Emissions: 1990 - 2030." Office of Atmospheric Programs, Climate Change Division, U.S. Environmental Protection Agency, Washington DC.

Uchida, Y., Clough, T. J., Kelliher, F. M., and Sherlock, R. R. (2008). "Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil." *Soil Biol.Biochem.,* 40(4), 924-931.

Velthof, G. L., and Mosquera, J. (2011). "The impact of slurry application technique on nitrous oxide emission from agricultural soils." *Agric., Ecosyst.Environ.,* 140(1), 298-308.

Yates, T. T., Si, B. C., Farrell, R. E., and Pennock, D. J. (2006). "Wavelet Spectra of Nitrous Oxide Emission from Hummocky Terrain during Spring Snowmelt." *Soil Sci.Soc.Am.J.,* 70(4), 1110-1120.

Zhu, X., Burger, M., Doane, T. A., and Horwath, W. R. (2013). "Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability." *Proc.Natl.Acad.Sci.U.S.A.,* 110(16), 6328-6333.

3.9 Tables

Table 3-1. Linear regression p-values and coefficients of determination (R^2) for the net ammonium (NH_4^+-N) depletion and nitrate (NO_3^--N) accumulation with three, four and five points.

Treatment	Three points[†]		Four points[‡]		Five points[§]	
	p-value	R^2	p-value	R^2	p-value	R^2
			NH_4^+-N			
CT[‖]	0.90	0.02	0.49	0.26	0.51	0.16
FD	0.34	0.75	0.24	0.59	0.22	0.44
FN	0.32	0.77	0.23	0.59	0.70	0.06
FW	0.54	0.44	0.20	0.64	0.46	0.19
SD[‖]	0.17	0.93	0.10	0.81	0.16	0.54
SN[‖]	0.30	0.79	0.14	0.73	0.23	0.42
SW[‖]	0.24	0.86	0.18	0.68	0.26	0.39
Number of fitted models[¶]	0		0		0	
			NO_3^--N			
CT[‖]	0.83	0.07	0.58	0.18	0.51	0.16
FD	0.20	0.90	0.07	0.86	0.04	0.81
FN	0.05	0.99	0.03	0.95	0.03	0.83
FW	0.20	0.91	0.02	0.96	0.02	0.87
SD[‖]	0.25	0.86	0.06	0.89	0.04	0.80
SN[‖]	0.01	1.00	0.00	1.00	0.04	0.80
SW[‖]	0.31	0.78	0.07	0.87	0.01	0.93
Number of fitted models[¶]	1		3		2	

[†] This calculation used data from the first three sampling dates in the 28-day incubation (0, 7 and 14 days).

[‡] This calculation used data from the first four sampling dates in the 28-day incubation (0, 7, 14 and 21 days).

[§] This calculation used data all sampling dates in the 28-day incubation (0, 7, 14, 21 and 28 days).

[‖] Aggregated values were derived by combining the data from manure band and interband zones in Table 3-7 based on area weights.

[¶] Count of fitted models fulfilling our model selection criteria of $P < 0.05$ and $R^2 > 0.85$.

Table 3-2. Nitrogen transformation rates based on simple linear, first order and second order models.

Treatment	Linear Rates[†]			First Order Kinetics[‡]		Second Order Kinetics[§]	
	Net Mineralization [Δ(NH_4^+-N+ NO_3^--N)]	Net Ammonification [Δ(NH_4^+-N)]	Net Nitrification [Δ(NO_3^--N)]	Ammonium Depletion	Nitrate Accumulation	Ammonium Depletion	Nitrate Accumulation
	μg N kg^{-1} day^{-1}			day^{-1}×1000		μg^{-1} N kg day^{-1}	
CT[‖]	128	-24	151	6.19	-7.42	1.62	-0.365
FD	1229	-870	2098*	78.14	-22.33*	8.75	-0.242
FN	921	-139	1060**	27.83	-20.68**	5.73	-0.410**
FW	1488	-67	1555**	14.62	-26.25**	3.20	-0.454**
SD[‖]	205	-464	669*	62.92	-23.88*	9.84**	-0.870*
SN[‖]	263	-402	664**	58.79	-24.48**	9.75*	-0.922**
SW[‖]	938	-403	1341*	58.51	-38.58*	9.62	-1.171*
Mean	739	-338	1077	43.86	-23.37	7.81	-0.634
S.E.	205	111	246	10.36	3.46	1.12	0.132

S.E. = one standard error; * = significantly different at $P<0.1$; ** = significantly different at $P<0.05$.

[†] Linear rates were calculated based on Eq. [3-2] using four points (the first four sampling dates in the 28-day incubation; 0, 7, 14 and 21 days).

[‡] The first order kinetics were calculated based on Eq. [3-3] with four points using fitting with linear regression (the first four sampling dates in the 28-day incubation; 0, 7, 14 and 21 days).

[§] The second order kinetics were calculated based on Eq. [3-4] with four points using fitting with linear regression (the first four sampling dates in the 28-day incubation; 0, 7, 14 and 21 days).

[‖] Aggregated values were derived by combining the data from manure band and interband zones in Table 3-7 based on area weights.

Table 3-3. The linear, quadratic and cubic regression results [p-value and coefficient of determination (R^2)] for the natural logarithms of ammonium [ln(NH_4^+-N)] and nitrate [ln(NO_3^--N)] versus time with three scenarios (three, four and five points).

Treatment	Three points[†]		Four points[‡]				Five points[§]					
	Linear		Linear[Δ]		Quadratic		Linear[Δ]		Quadratic		Cubic	
	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2
	NH_4^+-N											
CT[ǁ]	0.89	0.03	0.45	0.30	0.62	0.62	0.58	0.11	0.64	0.36	0.12	0.99
FD	0.34	0.74	0.25	0.56	0.26	0.93	0.29	0.36	0.14	0.86	0.19	0.98
FN	0.31	0.78	0.23	0.59	0.22	0.95	0.76	0.04	0.04	0.96	0.22	0.97
FW	0.51	0.49	0.18	0.68	0.53	0.71	0.47	0.18	0.57	0.43	0.37	0.91
SD[ǁ]	0.07	0.99	0.06	0.89	0.04	1.00	0.18	0.50	0.00	1.00	0.03	1.00
SN[ǁ]	0.28	0.82	0.09	0.82	0.22	0.95	0.27	0.38	0.05	0.95	0.29	0.95
SW[ǁ]	0.17	0.93	0.15	0.72	0.03	1.00	0.33	0.31	0.01	0.99	0.02	1.00
Count of fitted models[¶]	0		0		2		0		3		2	
	NO_3^--N											
CT[ǁ]	0.86	0.05	0.62	0.15	0.48	0.77	0.51	0.15	0.76	0.24	0.23	0.97
FD	0.22	0.88	0.09	0.82	0.15	0.98	0.05	0.77	0.04	0.96	0.09	0.99
FN	0.02	1.00	0.03	0.94	0.12	0.99	0.04	0.82	0.01	0.99	0.12	0.99
FW	0.17	0.93	0.02	0.96	0.21	0.96	0.02	0.87	0.06	0.94	0.15	0.99
SD[ǁ]	0.23	0.88	0.05	0.90	0.32	0.90	0.04	0.80	0.11	0.89	0.28	0.95
SN[ǁ]	0.04	1.00	0.00	0.99	0.01	1.00	0.04	0.80	0.02	0.98	0.08	1.00
SW[ǁ]	0.30	0.79	0.07	0.86	0.37	0.86	0.01	0.91	0.88	0.91	0.32	0.94
Count of fitted models[¶]	2		3		1		2		3		0	

† This calculation used data from the first three sampling dates in the 28-day incubation (0, 7 and 14 days).

‡ This calculation used data from the first four sampling dates in the 28-day incubation (0, 7, 14 and 21 days).

§ This calculation used data all sampling dates in the 28-day incubation (0, 7, 14, 21 and 28 days).

ǁ Aggregated values were derived by combining the data from manure band and interband zones in Table 3-7 based on area weights.

¶ Count of fitted models fulfilling our model selection criteria of $P < 0.05$ and $R^2 > 0.85$.

Δ The linear models with four points correspond to the first order kinetic model.

Table 3-4. The linear, quadratic and cubic regression results [p-value and coefficient (R^2) values] for the reciprocal of ammonium [$(NH_4^+\text{-N})^{-1}$] and nitrate [$(NO_3^-\text{-N})^{-1}$] versus time with three scenarios (three, four and five points).

Treatment	Three points[†]		Four points[‡]				Five points[§]					
	Linear		Linear[Δ]		Quadratic		Linear[Δ]		Quadratic		Cubic	
	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value	R^2
	NH_4^+-N											
CT[‖]	0.87	0.04	0.41	0.34	0.58	0.66	0.65	0.08	0.69	0.31	0.11	0.99
FD	0.35	0.73	0.28	0.52	0.26	0.93	0.40	0.24	0.13	0.87	0.19	0.98
FN	0.30	0.79	0.23	0.59	0.20	0.96	0.82	0.02	0.03	0.97	0.20	0.97
FW	0.48	0.53	0.16	0.71	0.51	0.74	0.49	0.17	0.54	0.46	0.34	0.93
SD[‖]	0.04	1.00	0.03	0.93	0.15	0.98	0.24	0.42	0.06	0.94	0.09	1.00
SN[‖]	0.25	0.86	0.05	0.91	0.21	0.96	0.32	0.32	0.10	0.90	0.35	0.92
SW[‖]	0.07	0.99	0.14	0.74	0.11	0.99	0.43	0.22	0.01	0.99	0.14	0.99
Count of fitted models[¶]	1		2		0		0		2		0	
	NO_3^--N											
CT[‖]	0.89	0.03	0.65	0.12	0.55	0.69	0.52	0.15	0.75	0.25	0.34	0.93
FD	0.24	0.86	0.11	0.79	0.16	0.97	0.07	0.72	0.04	0.96	0.09	0.99
FN	0.02	1.00	0.04	0.92	0.09	0.99	0.04	0.80	0.01	0.99	0.12	0.99
FW	0.14	0.95	0.02	0.96	0.20	0.96	0.02	0.86	0.06	0.95	0.16	0.98
SD[‖]	0.20	0.90	0.05	0.90	0.31	0.91	0.04	0.80	0.11	0.89	0.28	0.95
SN[‖]	0.08	0.99	0.01	0.97	0.02	1.00	0.04	0.79	0.01	0.99	0.06	1.00
SW[‖]	0.29	0.81	0.07	0.86	0.38	0.86	0.02	0.87	0.11	0.89	0.33	0.93
Count of fitted models[¶]	1		3		1		2		3		0	

† This calculation used data from the first three sampling dates in the 28-day incubation (0, 7 and 14 days).

‡ This calculation used data from the first four sampling dates in the 28-day incubation (0, 7, 14 and 21 days).

§ This calculation used data all sampling dates in the 28-day incubation (0, 7, 14, 21 and 28 days).

‖ Aggregated values were derived by combining the data from manure band and interband zones in Table 3-7 based on area weights.

¶ Count of fitted models fulfilling our model selection criteria of $P < 0.05$ and $R^2 > 0.85$.

Δ The linear models with four points correspond to the second order kinetic model.

Table 3-5. The linear rates of mineralization, ammonification and nitrification for the four separate periods (weeks) during the 28-day incubation.

Treatment	Linear Rates†											
	Net Mineralization $[\Delta(NH_4^+\text{-}N+NO_3^-\text{-}N)]$				Net Ammonification $[\Delta(NH_4^+\text{-}N)]$				Net Nitrification $[\Delta(NO_3^-\text{-}N)]$			
	0-7d	8-14d	15-21d	22-28d	0-7d	8-14d	15-21d	22-28d	0-7d	8-14d	15-21d	22-28d
	$mg \cdot kg^{-1} \cdot day^{-1}$											
CT‡	-0.968	0.623	0.728	-0.120	0.162	-0.194	-0.039	0.391	-1.130	0.817	0.767	-0.511
FD	1.603	1.189	0.893	0.537	-2.658	0.011	0.038	0.145	4.261	1.178	0.855	0.392
FN	0.790	1.618	0.353	0.099	-0.421	-0.014	0.017	0.267	1.211	1.632	0.336	-0.168
FW	0.855	2.448	1.160	-0.168	0.087	-0.272	-0.017	0.173	0.768	2.720	1.177	-0.341
SD‡	-0.745	1.191	0.168	0.141	-1.011	-0.354	-0.028	0.258	0.266	1.545	0.196	-0.116
SN‡	-0.314	0.601	0.500	-0.123	-1.011	-0.064	-0.130	0.312	0.697	0.666	0.630	-0.434
SW‡	-0.953	3.107	0.661	2.327	-1.080	-0.190	0.062	0.247	0.128	3.297	0.599	2.080
Mean	0.039	1.540	0.638	0.385	-0.847	-0.154	-0.014	0.256	0.886	1.694	0.652	0.129
S.E.	0.391	0.354	0.126	0.336	0.361	0.052	0.024	0.031	0.628	0.370	0.123	0.344

† Linear rates were calculated based on Eq. [3-2] to reveal the changes in concentration every 7 days.
‡ Aggregated values were derived by combining the data from manure band and interband zones in Table 3-7 based on area weights.

Table 3-6. The average total N, ammonium, water content and pH with one standard error in the manure applied in fall 2014 and spring 2015. Different letters indicate significant differences between the manure applications based on two sample t test (*P*<0.05).

Season	Total N	NH_4^+-N	H_2O	pH
	kg ha^{-1}	kg ha^{-1}	%(m/m)	
Fall 2014	380.7±6.7 a	216.7±0.7 a	91.8±0.8 a	7.1±0.0 a
Spring 2015	399.1±11.4 a	193.6±1.5 a	92.9±0.1 a	7.0±0.0 a

Table 3-7. Mineral nitrogen, ammonium and nitrate concentrations in the soil during the incubation experiment.

Treatment[†]	Days of Incubation				
	0	7	14	21	28
			mg N kg^{-1}		
			Mineral N		
CT[‡]	22.93±1.01 cd[§]	16.16±1.56 b	20.52±2.35 f	25.61±6.46 f	24.77±3.18 ef
FD	96.30±1.90 b	107.5±20.6 a	115.8±16.3 b	122.1±25.2 bc	125.8±21.1 bc
FN	47.51±4.92 cd	53.04±16.3 b	64.37±18.4 cd	66.84±21.4 de	67.53±23.4 de
FW	49.75±0.80 c	55.73±5.15 b	72.87±2.75 c	80.99±3.59 cd	79.82±2.00 cd
SD[‡]	34.88±0.69 cd	29.66±6.95 b	38.00±10.2 ef	39.18±11.3 def	40.17±13.9 def
SN[‡]	32.66±0.76 cd	30.46±9.24 b	34.67±6.99 ef	38.17±9.55 def	37.31±11.8 def
SW[‡]	34.53±0.91 cd	27.86±2.76 b	49.61±3.18 cde	54.24±3.19 def	70.52±12.1 def
SD-B	151.34±19.88 a	139.01±26.24 a	150.02±16.49 a	167.61±27.08 a	178.47±36.68 a
SN-B	145.99±11.26 a	117.15±2.33 a	145.78±4.53 a	142.53±11.50 ab	141.99±14.41 ab
SW-B	135.18±20.75 a	115.26±35.52 a	157.16±18.90 a	168.70±34.46 a	155.09±28.48 ab
SD-I	25.30±0.83 cd	20.67±5.47 b	28.79±9.97 ef	28.62±10.1 ef	28.80±12.1 ef
SN-I	23.34±3.04 cd	23.33±9.94 b	25.53±7.32 ef	29.59±9.81 ef	28.70±12.1 ef
SW-I	26.25±1.24 cd	20.67±1.43 b	40.77±2.12 def	44.82±0.77 def	63.57±14.1 def
			NH_4^+-N		
CT[‡]	4.09±0.46 b	5.22±1.02 c	3.86±0.20 bcd	3.59±0.07 c	6.32±1.08 a
FD	22.65±13.5 b	4.04±0.23 c	4.12±0.15 cd	4.39±0.38 c	5.40±0.17 a
FN	6.60±1.82 b	3.65±0.08 c	3.56±0.11 cd	3.67±0.16 c	5.55±0.24 a
FW	5.34±0.94 b	5.95±1.30 c	4.05±0.28 bcd	3.93±0.45 abc	5.14±0.33 a
SD[‡]	13.29±1.54 b	6.21±1.34 c	3.74±0.25 cd	3.54±0.14 abc	5.35±0.07 a
SN[‡]	11.90±0.91 b	4.82±1.10 c	4.37±0.54 bcd	3.46±0.06 bc	5.64±0.52 a
SW[‡]	11.96±1.48 b	4.40±1.40 c	3.07±0.91 cd	3.50±0.32 c	5.23±0.16 a
SD-B	115.32±19.61 b	30.37±13.6 c	9.66±2.10 bcd	5.07±0.91 c	6.10±0.42 a
SN-B	95.50±8.88 b	15.34±7.39 c	6.28±1.30 cd	4.90±0.32 c	5.72±0.19 a
SW-B	98.33±19.16 a	22.57±9.16 a	5.24±0.33 a	4.95±0.23 a	6.23±0.12 a
SD-I	4.90±0.63 a	4.23±0.44 bc	3.25±0.43 b	3.42±0.16 ab	5.29±0.11 a
SN-I	5.02±1.43 a	3.95±0.58 ab	4.21±0.50 bc	3.34±0.08 ab	5.63±0.57 a
SW-I	4.86±0.12 b	2.90±0.89 c	2.89±0.96 cd	3.38±0.33 c	5.14±0.16 a
			NO_3^--N		
CT[‡]	18.84±1.71 b	10.93±0.58 c	16.65±2.19 bcd	22.02±6.53 c	18.44±3.79 a
FD	73.64±3.29 b	103.47±20.67 c	111.7±16.39 d	117.7±24.90 c	120.4±21.16 a
FN	40.91±11.00 bc	49.38±16.24 b	60.81±18.31 cd	63.16±21.33 de	61.98±23.65 de
FW	44.40±1.38 bc	49.78±6.06 b	68.82±2.97 c	77.06±3.98 cd	74.67±2.26 cd
SD[‡]	21.58±1.19 d	23.45±5.70 b	34.26±10.42 ef	35.64±11.34 def	34.82±14.05 def
SN[‡]	20.76±1.17 d	25.64±9.70 b	30.30±6.89 ef	34.71±9.55 def	31.67±12.38 def
SW[‡]	22.57±1.69 d	23.46±4.15 b	46.54±3.86 cde	50.74±3.38 def	65.29±11.9 def
SD-B	36.01±0.82 c	108.64±14.38 a	140.36±14.41 a	162.53±26.25 a	172.36±36.31 a
SN-B	50.48±4.53 b	101.81±9.72 a	139.50±4.14 ab	137.63±11.73 ab	136.27±14.23 ab

SW-B	36.84±6.77 c	92.69±40.44 a	151.92±19.21 a	163.74±34.58 a	148.86±28.47 ab
SD-I	20.39±1.23 d	16.44±5.19 b	25.53±10.23 ef	25.20±10.21 ef	23.51±12.25 ef
SN-I	18.31±1.60 d	19.37±10.15 b	21.32±7.19 ef	26.24±9.79 ef	23.06±12.73 ef
SW-I	21.39±1.35 d	17.77±1.78 b	37.87±2.68 def	41.44±0.84 def	58.42±14.0 def

† CT=control with no manure added; FW=fall manure without any inhibitors; FD= fall manure with DMPP; FN=fall manure with nitrapyrin; SW=spring manure without any inhibitors; SD=spring manure with DMPP; SN=spring manure with nitrapyrin; B= manure band area; I=interband area.

‡ Aggregated values were derived by combining the data from manure band and interband zones based on area weights.

§ Values followed by different letters are significantly different based on LSD test (*P*<0.05).

Table 3-8. The value of cumulative N_2O fluxes divided by thermal times for the soil incubation and field measurements.

Treatment	Incubation[†]	Field[‡]
	——mg N_2O-N ha^{-1} $°C^{-1}$ hr^{-1}——	
CT	19.05[§]	0.90
FD	63.34	7.26
FN	38.12	3.69
FW	41.98	3.25
SD	27.81[§]	39.71
SN	23.78[§]	85.48
SW	40.75[§]	35.63

† The thermal time (10725.7 °C hr) for incubation was calculated as the sum of the time multiplied by the average air temperature for 28 days (25 May - 22 Jun 2015). Temperature baseline is 5°C as considered biological zero.
‡ The thermal time (9207.7 °C hr) for field was calculated as the sum of the time multiplied by the average soil temperature below 10cm and 20 cm for 29 days (13 May – 11 Jun 2015). Temperature baseline is 5°C.
§ Cumulative N_2O production used to calculate the incubation values were derived from data combining manure band and interband areas.

3.10 Figures

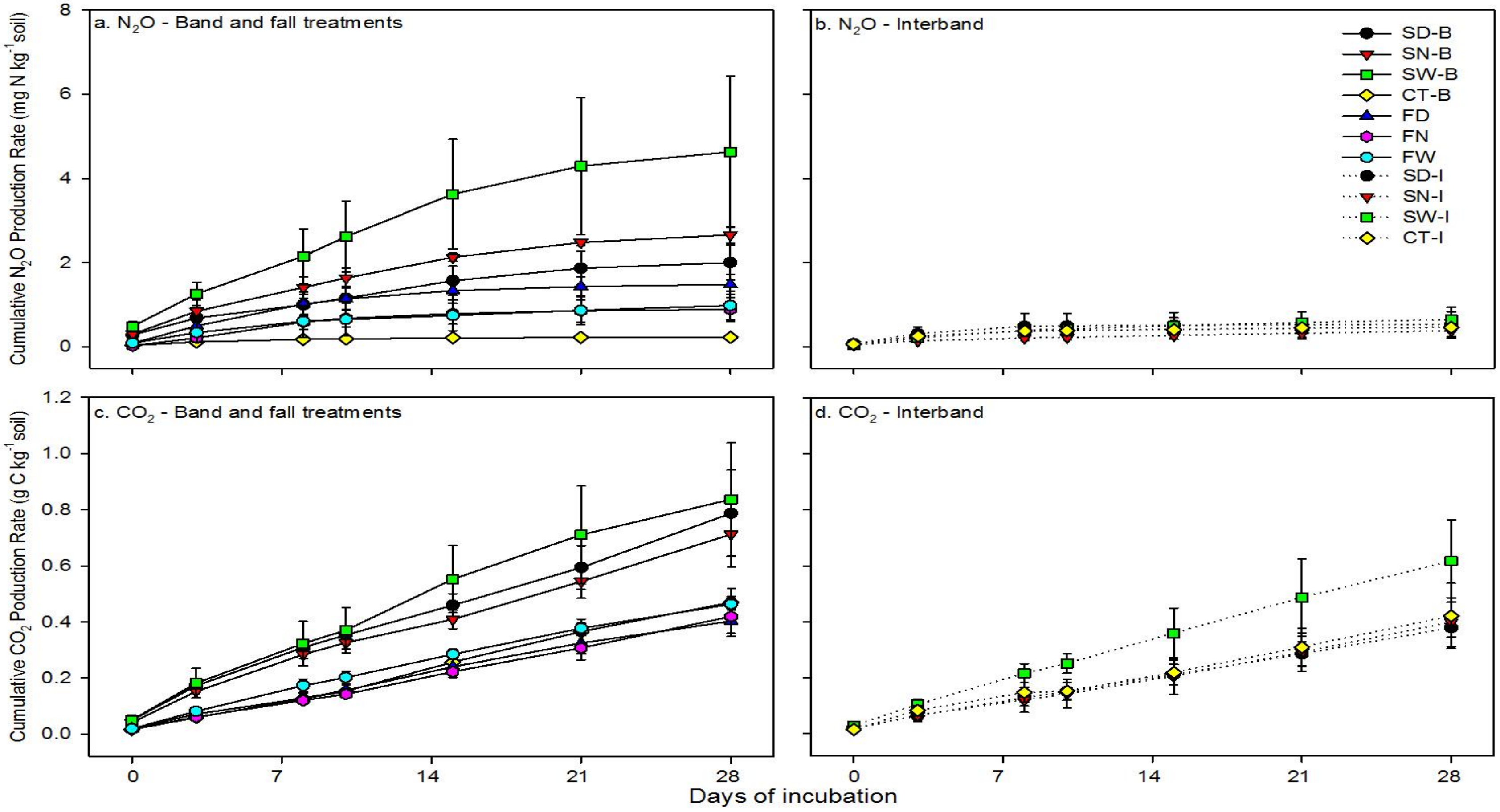


Fig. 3-1. Cumulative N_2O production (a) for the bands (B) of spring manure and control treatments as well as the fall manure treatments, and (b) for the interbands (I) of spring manure and control treatments; cumulative CO_2 production (c) for the bands (B) of spring manure and control treatments as well as the fall manure treatments, and (d) for the interbands (I) of spring manure and control treatments. Error bars correspond to one standard error. Soil samples used in this laboratory incubation were collected from our field plots on 13 May 2015, one day after the spring manure injection and 140 days after the fall manure injection. Injection band and interband areas were sampled separately in the three spring manure treatments and control plots; while soils from the three fall manure treatments were collected as composited samples from the plots without separating band and interband areas.

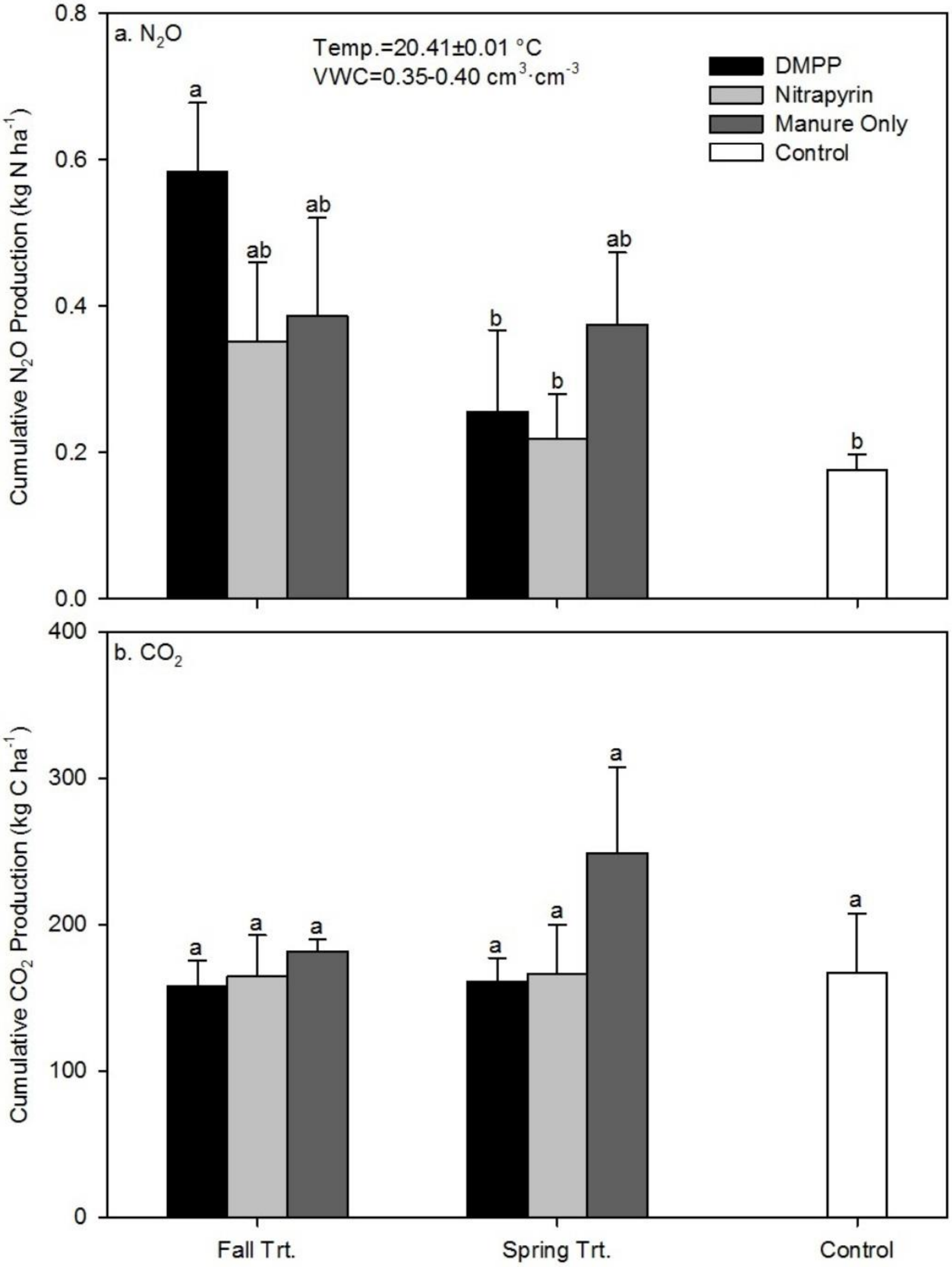


Fig. 3-2. Cumulative (a) N_2O and (b) CO_2 production during the soil incubation showing aggregated data after combining both band and interband areas. Error bars correspond to one standard error. Different letters indicate significant differences among treatments based on LSD test ($P<0.05$). VWC= volumetric water content.

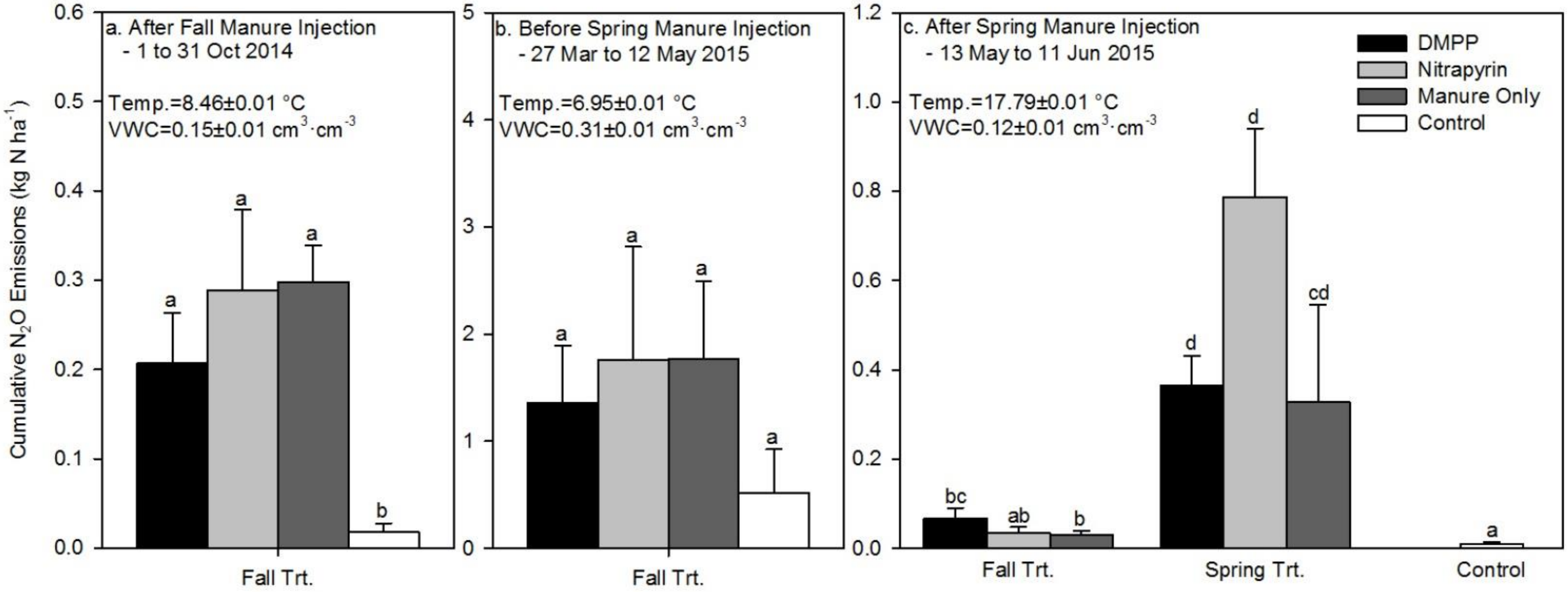


Fig. 3-3. Field cumulative N_2O emissions (a) after fall manure injection (1-31 Oct 2014), (b) before spring manure injection (27 Mar to 12 May 2015) and (c) after spring manure injection (13 May to 11 Jun 2015). Error bars correspond to one standard error. Different letters indicate significant differences among treatments based on LSD test (*P*<0.05). VWC= volumetric water content.

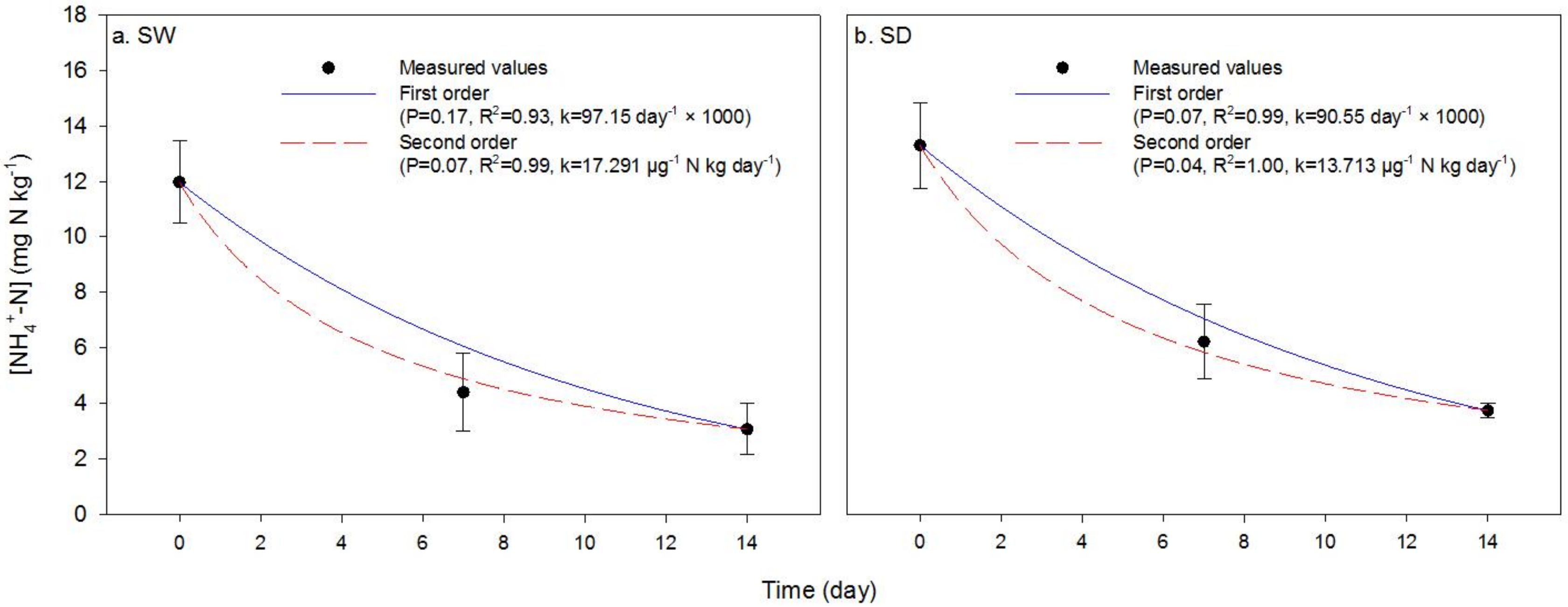


Fig. 3-4. First order and second order fitted models of the net ammonium (NH_4^+-N) concentration depletion with time during the first two weeks of the laboratory incubation for (a) spring manure only without inhibitor (SW) and (b) spring manure DMPP (SD) treatments. Error bars correspond to one standard error. The first order and second order kinetics (k) were derived using the Eqs. [3-3] and [3-4], respectively. VWC= volumetric water content.

4 General Conclusions and Recommendations

Both incubation and field results indicated that soil mineral N transformation rates can be well described by either the first- or second-order kinetic model, but rapid nitrate transformation rates in manured soils were better represented by the second-order model.

Findings and interpretations derived from laboratory incubation for quantification of soil N_2O emission and prediction of management effects could be inherently limited, because an incubation experiment can undervalue N_2O fluxes under certain specific situations, as shown by our comparison of incubation versus field fluxes. In addition, our result showed that key soil mineral N alterations can be well documented by a laboratory incubation lasting a three week period.

Our study shows that when the amount of nitrate available as a substrate was substantial, the increasing soil moisture acted as a key driver for peak N_2O flux. This synergistic interaction suggests that irrigation and drainage managements should be given high priority while designing GHG mitigation strategies.

Estimated annual N balance showed that the plant N uptake was the largest manure-N sink in our annual crop agricultural system, followed by denitrified N_2O and N_2 losses, NO_3^--N leaching and NH_3 volatilization. Compared to the control soils, the manure-amended soils seemed to gain total N mass as an effect of manure applications. This increased manure-induced N could have contributed to raise the soil organic N levels via an accelerated immobilization.

From an environmental perspective, spring manure injection enhanced the manure-N use efficiency relative to the fall timing as indicated by lower annual N_2O emissions, higher barley biomass and N uptake in the spring-manured fields. However, fall manure timing is commonly considered to be more economically efficient due to the greater availability of time, labor and equipment in the fall seasons after intensive agricultural activities and harvest in the summer. To mitigate N_2O emissions, increase farming flexibility, and reduce capital cost, perennial and winter crops could hypothetically be beneficial alternatives to annual crops (barley) in particular if fall manure applications are to be undertaken; nevertheless, the implementation of manure injections in perennial crop fields could interact detrimentally with plant root systems which would perhaps affect agronomic and economic performances. These hypotheses and practical effects deserve experimental examination.

Both nitrification inhibitors (NIs; DMPP and nitrapyrin) successfully decreased annual N_2O emissions at Lacombe, and they effectively maintained their inhibiting activity on emissions after a six-months freezing winter. DMPP was relatively more effective in reducing N_2O emissions than nitrapyrin at the same active compound rate, but this environmental advantage did not translate into differences in barley biomass productivity and N uptake between these two inhibitors.

Overall, the incorporation of NIs with manure applications in the spring season can be considered as a powerful GHG mitigation strategy in Alberta and other geographic areas with comparable edaphic and climatic conditions. A specific example could illustrate this point – a farmer decides to switch his/her liquid manure management for a 100-hectare barley from fall manure injection without NIs to spring manure injection with DMPP. Based on our measured data at Lacombe in 2014-2015, this farmer would reduce N_2O emissions by a net 5.1 kg N_2O-N ha^{-1} yr^{-1}. Assuming a conversion of global warming potentials as one kg N_2O equal to 298 kg CO_2 in a 100-year projection, this amount of net N_2O emission reduction contributes to a decrease of 2388 kg ha^{-1} global warming potential in CO_2 equivalents. Based on the cap price of

\$30 per Mg (ton) of CO_2 emissions in the Alberta Carbon Offset Market in 2017, the reduced N_2O emissions derived from this improved management in 100 Ha are equivalent to \$7164 in tradable carbon credits per year. Moreover, this farmer will also see the benefit of increasing barley biomass productivity by a net 723.2 kg ha^{-1} yr^{-1} and increasing plant N uptake by 42.7 kg N ha^{-1} yr^{-1}. It is noteworthy that our field measurements represent drought conditions. If a manure management change (conversion to spring timing with NIs) is implemented on a normal rainfall year or on a wet year with relatively higher precipitations, this farmer could gain even more environmental and economic benefits from these enhanced management practices.

Based on the data and experiences from this experimental research, future investigations can focus on (i) examining the effect of manure timing coupled with NIs on denitrified N_2, nitrate leaching and NH_3 volatilization, (ii) investigating the optimal effective rate of various NIs for reducing N_2O emissions, nitrate leaching and NH_3 volatilization, (iii) evaluating the effects of environmental factors and their thresholds on NIs, such as temperature, moisture and their combined action, (iv) assessing the changes of total N derived from successive liquid manure applications by collecting one-meter soil profile samples, and (v) exploring the impacts of N input rate on the fitting performance of first- versus second-order kinetic modeling.

5 Bibliography

Akiyama, H., Xiaoyuan, Y., and Yagi, K. (2010). "Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N_2O and NO emissions from agricultural soils: meta-analysis." *Global Change Biol.,* 16(6), 1837.

Alberta Agriculture and Forestry. (2016). "Current and Historical Alberta Weather Station Data Viewer." http://agriculture.alberta.ca/acis/alberta-weather-data-viewer.jsp (Feb 10, 2016).

Allen, A., Jarvis, S., and Headon, D. (1996). "Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the UK." *Soil Biol.Biochem.,* 28(4), 597-607.

Baggs, E. M., Stevenson, M., Pihlatie, M., Regar, A., Cook, H., and Cadisch, G. (2003). "Nitrous oxide emissions following application of residues and fertiliser under zero and conventional tillage." *Plant Soil,* (2), 361.

Basso, B., and Ritchie, J. T. (2005). "Impact of compost, manure and inorganic fertilizer on nitrate leaching and yield for a 6-year maize–alfalfa rotation in Michigan." *Agric., Ecosyst.Environ.,* 108(4), 329-341.

Bateman, E. J., and Baggs, E. M. (2005). "Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space." *Biol.Fertility Soils,* 41(6), 379-388.

Beaulieu, M. S. (2004). "Manure management in Canada." *Farm Environmental Management in Canada,* 1(2), 1-52.

Braker, G., and Conrad, R. (2011). "Chapter 2 - Diversity, Structure, and Size of N_2O-Producing Microbial Communities in Soils—What Matters for Their Functioning?" *Adv.Appl.Microbiol.,* 75 33-70.

Bremner, J. M., and Blackmer, A. M. (1978). "Nitrous oxide: emission from soils during nitrification of fertilizer nitrogen." *Science,* 199(4326), 295-296.

Burzaco, J. P., Ciampitti, I. A., and Vyn, T. J. (2014). "Nitrapyrin impacts on maize yield and nitrogen use efficiency with spring-applied nitrogen: Field studies vs. meta-analysis comparison." *Agron.J.,* 106(2), 753-760.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., and Zechmeister-Boltenstern, S. (2013). "Nitrous oxide emissions from soils: how well do we understand the processes and their controls?" *Philos.Trans.R.Soc.Lond.B.Biol.Sci.,* 368(1621), 20130122.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., and Misselbrook, T. (2011). "Manure management: implications for greenhouse gas emissions." *Anim.Feed Sci.Technol.,* 166 514-531.

Chen, Y., Assefa, B., and Arkinremi, W. (2010). "Soil Nutrient Levels and Crop Performance at Various Lateral Positions Following Liquid Manure Injection." *CIGR Ejournal,* (1),.

Court, M. N., Stephen, R. C., and Waid, J. S. (1964). "Toxicity as a cause of the inefficiency of urea as a fertilizer." *J.Soil Sci.,* 15(1), 49-65.

Davidson, E. A., Rogers, J. E., and Whitman, W. B. (1991). "Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems." *Microbial Production and Consumption of Greenhouse Gases: Methane, Nitrogen Oxides, and Halomethanes.,* 219-235.

Davidson, E. A., and Schimel, J. P. (1995). "Microbial processes of production and consumption of nitric oxide, nitrous oxide and methane." *Biogenic Trace Gases: Measuring Emissions from Soil and Water,* 327-357.

Davidson, E. A., Keller, M., Erickson, H. E., Verchot, L. V., and Veldkamp, E. (2000). "Testing a Conceptual Model of Soil Emissions of Nitrous and Nitric Oxides Using two functions based on soil nitrogen availability and soil water content, the hole-in-the-pipe model characterizes a large fraction of the observed variation of nitric oxide and nitrous oxide emissions from soils." *Bioscience,* 50(8), 667-680.

Di, H. J., and Cameron, K. C. (2007). "Nitrate leaching losses and pasture yields as affected by different rates of animal urine nitrogen returns and application of a nitrification inhibitor—a lysimeter study." *Nutr.Cycling Agroecosyst.,* 79(3), 281-290.

Dittert, K., Bol, R., King, R., Chadwick, D., and Hatch, D. (2001). "Use of a novel nitrification inhibitor to reduce nitrous oxide emission from ^{15}N-labelled dairy slurry injected into soil." *Rapid Communications in Mass Spectrometry: RCM,* 15(15), 1291.

Dobbie, K. E., McTaggart, I. P., and Smith, K. A. (1999). "Nitrous oxide emissions from intensive agricultural systems: variations between crops and seasons, key driving variables, and mean emission factors." *Journal of Geophysical Research: Atmospheres (1984–2012),* 104(D21), 26891-26899.

Dobbie, K., and Smith, K. (2001). "The effects of temperature, water- filled pore space and land use on N_2O emissions from an imperfectly drained gleysol." *Eur.J.Soil Sci.,* 52(4), 667-673.

FAO. (2015). "World Fertilizer Trends and Outlook to 2018." Food and Agriculture Organization of the United Nations, Rome.

Flessa, H., and Beese, F. (2000). "Laboratory estimates of trace gas emissions following surface application and injection of cattle slurry." *J.Environ.Qual.,* 29(1), 262-268.

Freney, J. R., Simpson, J. R., and Denmead, O. T. (1983). "Volatilization of ammonia." *Gaseous Loss of Nitrogen from Plant-Soil Systems,* J. R. S. J.R.Freney, ed., Springer, Netherlands, pp 1-32.

Government of Canada. (2016). "Climate." http://climate.weather.gc.ca/ (Feb 10, 2016).

Guiraud, G., and Marol, C. (1992). "Influence of temperature on mineralization kinetics with a nitrification inhibitor (mixture of dicyandiamide and ammonium thiosulphate)." *Biol.Fertility Soils,* 13(1), 1-5.

Harrison-Kirk, T., Beare, M. H., Meenken, E. D., and Condron, L. M. (2013). "Soil organic matter and texture affect responses to dry/wet cycles: Effects on carbon dioxide and nitrous oxide emissions." *Soil Biol.Biochem.,* 57 43-55.

Havlin, J., Beaton, J. D., Tisdale, S. L., and Nelson, W. L. (2014). *Soil fertility and fertilizers: An introduction to nutrient management.* Pearson Prentice Hall Upper Saddle River, New Jersey, USA, .

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., and Van Scoyoc, G. E. (2011). "Nitrogen partitioning and utilization in corn cropping systems: Rotation, N source, and N timing." *Eur.J.Agron.,* 34(3), 190-195.

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., and Van Scoyoc, G. E. (2009a). "Greenhouse gas fluxes in an eastern corn belt soil: Weather, nitrogen source, and rotation." *J.Environ.Qual.,* 38(3), 841-854.

Hernandez-Ramirez, G., Brouder, S. M., Smith, D. R., Van Scoyoc, G. E., and Michalski, G. (2009b). "Nitrous oxide production in an eastern corn belt soil: sources and redox range." *Soil Sci.Soc.Am.J.,* 73(4), 1182-1191.

Herrero, M., Thornton, P. K., Gerber, P., and Reid, R. S. (2009). "Livestock, livelihoods and the environment: understanding the trade-offs." *Current Opinion in Environmental Sustainability,* 1(2), 111-120.

Hoben, J. P., Gehl, R. J., Millar, N., Grace, P. R., and Robertson, G. P. (2011). "Nonlinear nitrous oxide (N_2O) response to nitrogen fertilizer in on-farm corn crops of the US Midwest." *Global Change Biol.,* 17(2), 1140-1152.

Hua, L., LIANG, X., Yingxu, C., Yanfeng, L., Guangming, T., and Wuzhong, N. (2008). "Effect of nitrification inhibitor DMPP on nitrogen leaching, nitrifying organisms, and enzyme activities in a rice-oilseed rape cropping system." *Journal of Environmental Sciences,* 20(2), 149-155.

Irigoyen, I., Muro, J., Azpilikueta, M., Aparicio-Tejo., P., and Lamsfus, C. (2003). "Ammonium oxidation kinetics in the presence of nitrification inhibitors DCD and DMPP at various temperatures." *Aust.J.Soil Res.,* (6), 1177.

Janzen, H., Beauchemin, K., Bruinsma, Y., Campbell, C., Desjardins, R., Ellert, B., and Smith, E. (2003). "The fate of nitrogen in agroecosystems: An illustration using Canadian estimates." *Nutr.Cycling Agroecosyst.,* 67(1), 85-102.

Kim, D., Hernandez-Ramirez, G., and Giltrap, D. (2013). "Linear and nonlinear dependency of direct nitrous oxide emissions on fertilizer nitrogen input: A meta-analysis." *Agric., Ecosyst.Environ.,* 168 53-65.

Laboski, C., Jokela, W., and Andraski, T. (2013). "Dairy manure application methods: N credits, gaseous N losses, and corn yield." *Proceedings of the Wisconsin Crop Management Conference,* Citeseer, 20-31.

Luo, G. J., Kiese, R., Wolf, B., and Butterbach-Bahl, K. (2013). "Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types." *Biogeosciences,* 10(5), 3205-3219.

Luo, J., Tillman, R. W., and Ball, P. R. (2000). "Nitrogen loss through denitrification in a soil under pasture in New Zealand." *Soil Biol.Biochem.,* 32 497-509.

Maguire, R. O., Kleinman, P. J. A., Dell, C. J., Beegle, D. B., Brandt, R. C., McGrath, J. M., and Ketterings, Q. M. (2011). "Manure Application Technology in Reduced Tillage and Forage Systems: A Review." *J.Environ.Qual.,* (2),.

Meixner, F. X., and Yang, W. X. (2006). "Biogenic emissions of nitric oxide and nitrous oxide from arid and semi-arid land." *Dryland ecohydrology,* Springer, 233-255.

Menéndez, S., Merino, P., Pinto, M., González-Murua, C., and Estavillo, J. (2006). "3, 4-Dimethylpyrazol phosphate effect on nitrous oxide, nitric oxide, ammonia, and carbon dioxide emissions from grasslands." *J.Environ.Qual.,* 35(4), 973-981.

Menéndez, S., Barrena, I., Setien, I., González-Murua, C., and Estavillo, J. M. (2012). "Efficiency of nitrification inhibitor DMPP to reduce nitrous oxide emissions under different temperature and moisture conditions." *Soil Biol.Biochem.,* 53 82-89.

Minet, E. P., Jahangir, M. M. R., Krol, D. J., Rochford, N., Fenton, O., Rooney, D., Lanigan, G., Forrestal, P. J., Breslin, C., and Richards, K. G. (2016). "Amendment of cattle slurry with the nitrification inhibitor dicyandiamide during storage: A new effective and practical N_2O mitigation measure for landspreading." *Agric., Ecosyst.Environ.,* 215 68-75.

Misselbrook, T., Smith, K., Johnson, R., and Pain, B. (2002). "SE—Structures and environment: Slurry application techniques to reduce ammonia emissions: Results of some UK field-scale experiments." *Biosystems Engineering,* 81(3), 313-321.

Morley, N., and Baggs, E. (2010). "Carbon and oxygen controls on N_2O and N_2 production during nitrate reduction." *Soil Biol.Biochem.,* 42(10), 1864-1871.

Myhre, G., Shindell, D., Bréon, F. M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J. F., Lee, D., and Mendoza, B. (2013). "Anthropogenic and natural radiative forcing." *Climate Change,* 423.

Nyborg, M., Laidlaw, J. W., Solberg, E. D., and Malhi, S. S. (1997). "Denitrification and nitrous oxide emissions from a Black Chernozemic soil during spring thaw in Alberta." *Can.J.Soil Sci.,* (2),.

Nyord, T., Søgaard, H. T., Hansen, M. N., and Jensen, L. S. (2008). "Injection methods to reduce ammonia emission from volatile liquid fertilisers applied to growing crops." *Biosystems Engineering,* 100(2), 235-244.

Omonode, R. A., and Vyn, T. J. (2013). "Nitrification Kinetics and Nitrous Oxide Emissions when Nitrapyrin is Coapplied with Urea--Ammonium Nitrate." *Agron.J.,* 105(6), 1475-1486.

Pasda, G., Hahndel, R., and Zerulla, W. (2001). "Effect of fertilizers with the new nitrification inhibitor DMPP (3,4-dimethylpyrazole phosphate) on yield and quality of agricultural and horticultural crops." *Biol.Fertility Soils,* 34 85-97.

Pennock, D., Yates, T., Bedard-Haughn, A., Phipps, K., Farrell, R., and McDougal, R. (2010). "Landscape controls on N_2O and CH_4 emissions from freshwater mineral soil wetlands of the Canadian Prairie Pothole region." *Geoderma,* 155(3), 308-319.

Phillips, R. L., McMillan, A. M. S., Palmada, T., Dando, J., and Giltrap, D. (2015). "Temperature effects on N_2O and N_2 denitrification end-products for a New Zealand pasture soil." *N.Z.J.Agric.Res.,* 58(1), 89-95.

Prasad, R., and Power, J. (1995). "Nitrification inhibitors for agriculture, health, and the environment." *Advances in Agronomy (USA),* .

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. (2009). "Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century." *Science,* 326(5949), 123-125.

Rochette, P., Angers, D. A., Chantigny, M. H., Bertrand, N., and Côté, D. (2004). "Carbon dioxide and nitrous oxide emissions following fall and spring applications of pig slurry to an agricultural soil." *Soil Sci.Soc.Am.J.,* 68(4), 1410-1420.

Rochette, P., Worth, D. E., Lemke, R. L., McConkey, B. G., Pennock, D. J., Wagner-Riddle, C., and Desjardins, R. (2008). "Estimation of N_2O emissions from agricultural soils in Canada. I. Development of a country-specific methodology." *Can.J.Soil Sci.,* 88(5), 641-654.

Rodhe, L., and Etana, A. (2005). "Performance of slurry injectors compared with band spreading on three Swedish soils with ley." *Biosystems Engineering,* (1),.

Ross, S. M., Izaurralde, R. C., Janzen, H., Robertson, J., and McGill, W. B. (2008). "The nitrogen balance of three long-term agroecosystems on a boreal soil in western Canada." *Agric., Ecosyst.Environ.,* 127(3), 241-250.

Saarnio, S., Heimonen, K., and Kettunen, R. (2013). "Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake." *Soil Biol.Biochem.,* 58 99-106.

Saggar, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., Kim, D., Zaman, M., and Tillman, R. (2013). "Denitrification and N_2O: N_2 production in temperate grasslands: processes, measurements, modelling and mitigating negative impacts." *Sci.Total Environ.,* 465 173-195.

Schaufler, G., Kitzler, B., Schindlbacher, A., Skiba, U., Sutton, M. A., and Zechmeister-Boltenstern, S. (2010). "Greenhouse gas emissions from European soils under different land use: effects of soil moisture and temperature." *Eur.J.Soil Sci.,* 61(5), 683-696.

Schindlbacher, A., Zechmeister-Boltenstern, S., and Butterbach-Bahl, K. (2004). "Effects of soil moisture and temperature on NO, NO_2, and N_2O emissions from European forest soils." *Journal of Geophysical Research: Atmospheres (1984–2012),* 109(D17),.

ŠImek, M., and Cooper, J. (2002). "The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years." *Eur.J.Soil Sci.,* 53(3), 345-354.

Sistani, K., Warren, J., Lovanh, N., Higgins, S., and Shearer, S. (2010). "Greenhouse gas emissions from swine effluent applied to soil by different methods." *Soil Sci.Soc.Am.J.,* 74(2), 429-435.

Smith, D. W., and Mukhtar, S. (2015). "Estimation and attribution of nitrous oxide emissions following subsurface application of animal manure: a review." *Transactions of the ASABE,* 58(2), 429.

Smith, K. A., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., and Rey, A. (2003). "Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes." *Eur.J.Soil Sci.,* 54(4), 779.

Spargo, J. T., Alley, M. M., Follett, R. F., and Wallace, J. V. (2008). "Soil nitrogen conservation with continuous no-till management." *Nutr.Cycling Agroecosyst.,* 82(3), 283-297.

Stevens, R., and Laughlin, R. (1998). "Measurement of nitrous oxide and di-nitrogen emissions from agricultural soils." *Nutr.Cycling Agroecosyst.,* 52(2-3), 131-139.

Subbarao, G., Ito, O., Sahrawat, K., Berry, W., Nakahara, K., Ishikawa, T., Watanabe, T., Suenaga, K., Rondon, M., and Rao, I. (2006). "Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities." *Crit.Rev.Plant Sci.,* 25(4), 303.

Team, R. C. (2015). "R: A Language and Environment for Statistical Computing." *R Foundation for Statistical Computing,Vienna, Austria,* URL http://www.R-project.org/.

Thompson, R. B., Ryden, J. C., and Lockyer, D. R. (1987). "Fate of nitrogen in cattle slurry following surface application or injection to grassland." *J.Soil Sci.,* (4),.

Thorman, R., Sagoo, E., Williams, J., Chambers, B., Chadwick, D., Laws, J., Yamulki, S., Bosch, A., Teira, M., and Villar, J. E. M. (2007). "The effect of slurry application timings on direct and indirect N_2O emissions from free draining grassland." *Proceedings of the 15th Nitrogen Workshop: Towards a Better Efficiency in N Use. Editorial Milenio, Lleida (Spain),* 297-299.

U.S. Environmental Protection Agency. (2012). "Summary Report: Global Anthropogenic Non-CO_2 Greenhouse Gas Emissions: 1990 - 2030." Office of Atmospheric Programs, Climate Change Division, U.S. Environmental Protection Agency, Washington DC.

Uchida, Y., Clough, T. J., Kelliher, F. M., and Sherlock, R. R. (2008). "Effects of aggregate size, soil compaction, and bovine urine on N_2O emissions from a pasture soil." *Soil Biology & Biochemistry,* .

van Es, H. M., Sogbedji, J. M., and Schindelbeck, R. R. (2006). "Effect of manure application timing, crop, and soil type on nitrate leaching." *J.Environ.Qual.,* 35(2), 670-679.

Velthof, G. L., and Mosquera, J. (2011). "The impact of slurry application technique on nitrous oxide emission from agricultural soils." *Agric., Ecosyst.Environ.,* 140(1), 298-308.

Velusamy, K., and Krishnani, K. K. (2013). "Heterotrophic nitrifying and oxygen tolerant denitrifying bacteria from greenwater system of coastal aquaculture." *Appl.Biochem.Biotechnol.,* 169(6), 1978-1992.

Webb, J., Pain, B., Bittman, S., and Morgan, J. (2010). "The impacts of manure application methods on emissions of ammonia, nitrous oxide and on crop response—A review." *Agric., Ecosyst.Environ.,* 137(1), 39-46.

Weslien, P., Klemedtsson, L., Svensson, L., Galle, B., Kasimir-Klemedtsson, Å, and Gustafsson, A. (1998). "Nitrogen losses following application of pig slurry to arable land." *Soil use Manage.,* 14(4), 200-208.

Wolt, J. D. (2004). "A meta-evaluation of nitrapyrin agronomic and environmental effectiveness with emphasis on corn production in the Midwestern USA." *Nutr.Cycling Agroecosyst.,* 69(1), 23-41.

Yates, T. T., Si, B. C., Farrell, R. E., and Pennock, D. J. (2006). "Wavelet Spectra of Nitrous Oxide Emission from Hummocky Terrain during Spring Snowmelt." *Soil Sci.Soc.Am.J.,* (4),.

Zerulla, W., Barth, T., Dressel, J., Erhardt, K., von Locquenghien, K. H., Pasda, G., Rädle, M., and Wissemeier, A. (2001). "3, 4-Dimethylpyrazole phosphate (DMPP)–a new nitrification inhibitor for agriculture and horticulture." *Biol.Fertility Soils,* 34(2), 79-84.

Zheng, X., Wang, M., Wang, Y., Shen, R., Gou, J., Li, J., Jin, J., and Li, L. (2000). "Impacts of soil moisture on nitrous oxide emission from croplands: a case study on the rice-based agro-ecosystem in Southeast China." *Chemosphere-Global Change Science,* 2(2), 207-224.

Zhu, X., Burger, M., Doane, T. A., and Horwath, W. R. (2013). "Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability." *Proc.Natl.Acad.Sci.U.S.A.,* 110(16), 6328-6333.

6 Appendices

6.1 Protocols

Project Design,
Methodology and
Experimental Approach

With the aim of assessing nitrous oxide emission reductions from croplands receiving manure additions, we will carry out field experiments over three years (Summer/early Fall 2014 throughout
February 2017). We will plant barley for silage in every growing season. We will focus on nitrous oxide fluxes as a response to time of manure addition and in combination with two different nitrification inhibitors.

Method: Experimental Design

Two replicated experiments will be established in Central Alberta (Edmonton and Lacombe) in fields with a history of manure additions. Liquid dairy manure will be used at the Edmonton site, while liquid swine manure will be applied in the Lacombe site. By having these two different manure sources in the two locations, we can assess these two important liquid manure types in our research project while keeping the size of the field experiments (number of plots) to a reasonably manageable size. Manure will be injected in the fall (2014 and 2015) and spring (2015 and 2016) in split randomized sets of experimental plots to represent the different levels of nitrification inhibitor additives. Barley for silage will be seeded. Manure will be injected at a single volume rate of about 75 cubic meters per hectare (**Note:** AARD Trevor will look after these injections; also note that the injector tank will need to be flush clean between one additive and the second additive; the application of treatments will include control disturbance, spring/fall manure, additive1 manure, additive2 manure). Available liquid manure has a typical nitrogen concentration of 3.54 g N L-1 (10.7% coefficient of variation) and with 60% of N in ammonium form. Nutrient analyses will be conducted in each separate liquid manure batch. These manure nutrient
contents and volume of application will be used to back estimate the actual manure-nitrogen rates applied in each treatment every season. Weather stations are available near the experimental sites.

Note: Please see spreadsheet file entitled “Manure Plot Plan split plot design” for further details in experimental setting and design structure.

Treatments will be accommodated in a balanced incomplete split plot design with four replicates (if available field dimensions don’t allow for four replicates, three replicates can be establish).

- Control (including the traffic of equipment with no manure addition)

- Control (including the traffic of equipment with no manure addition)

- Fall injection (control, no inhibitor addition) (October 2014 & 2015)

- Nitrapyrin with fall injection (October 2014 & 2015)

- DMPP with fall injection (October 2014 & 2015)

- Spring injection (control, no inhibitor addition) (May 2015 & 2016)

- Nitrapyrin with spring injection (May 2015 & 2016)

- DMPP with spring injection (May 2015 & 2016)

The resulting flux data and findings from these manure treatments in spring 2014 will be comparable to fluxes
and responses in spring 2015 and 2016.

To conduct this field study for three years will enable contrasting the experimental effects of Fall 2014 versus Spring 2015, and Fall 2015 versus Spring 2016. This will allow accommodating and capturing flux measurements during two full barley growing seasons in 2015 and 2016.

We would like to offer a rationale for the selection of the two nitrification inhibitors (nitrapyrin and DMPP) included in the proposed field experiments. We have surveyed the existing literature on nitrification inhibitors to identify the two active ingredients with theoretically more likelihood for effective reduction of nitrous oxide emission from soil receiving liquid manure injections in the Canadian prairies. According to previous publications, these hypothetical biological effects are anticipated for nitrapyrin and DMPP when regarding continental temperate climates such as the Canadian prairies. Briefly, few publications have compiled world wide data on nitrification inhibitors. Akiyama et al. (2010) reported 40% effectiveness of nitrous oxide emission reduction for both nitrapyrin and DMPP when using synthetic fertilizer. Similarly, Wolt et al. (2004) indicated 51% greenhouse gas reduction by nitrapyrin for US Midwest. Likewise, data compiled by Liu et al. (2013) indicate that the use of DMPP with manure can result in up to 60% N2O emission reduction based on the few existing studies done in Europe.

The nitrification inhibitor products exist in liquid/emulsifiable formulations, and they are applied at a rate of 0.5 (or 1) kg active ingredient per Ha (**Note:** Guillermo's lab team will look after these additive rates for mixing with the liquid manure immediately prior to field injections; we will use 0.5 kg ha-1; also note that the injector tank will need to be flush clean between one additive and the second additive). The additive characteristics facilitate the mixing of the nitrification inhibitor products thoroughly with the liquid manure
batches by using agitators (e.g., propeller or recirculation) inside the tank of a manure applicator/spreader

for field plot injections. The manure batch is also mixed in a previous step by using pump and agitator
to recirculate and mix manure in conventional lagoon storage prior to the removal of manure batches. In sum, the mixing steps of liquid manure and addition of nitrification inhibitors can be easily adapted to our experimental settings as well as to commercial/custom manure operations in farms.

Method: Narrative for Measurements

Measurements will include nitrous oxide fluxes by both manual static chambers and automatic chambers, plant responses, and selected soil properties as a function of established treatment as well as manure characteristics such as nutrient content including total nitrogen and ammonium, density, and solids. Plant measurements of crop responses (barley for silage) will include aboveground biomass productivity (dry matter yield), plant nutrient uptake,
leaf area index, and population during the growth cycle. We will repeatedly quantify soil properties. A time series of mineral nitrogen (ammonium and nitrate
contents measured every two weeks), water content, oxygen concentration in soil air, and temperature before, within, and after the barley growing season. Pore-size distribution and water retention will be also measured. Measurements of organic and mineral nitrogen contents in soils will be
used to inform a partial nitrogen balance accounting for plant uptake, nitrogen input as manure, and nitrous oxide emissions.

A key aspect of this project is the ability to conduct accurate flux measurements at high frequency by using an automated chamber system as well as standard manual measurements. **Note:** A key aspect manure project in cold regions is the flux measurements before, within, and after the barley growing season; this *before* specifically includes during and following the spring-thaw events in 2015 & 2016. **Note:** For the autochambers, using 4 minutes flushing the system lines & analyzer cell followed by 14 minutes with chamber closed for flux measurement will result in measurement cycle of 18 minutes, and this will generate 80 flux measurements per day. Given that 10 chambers are available, this would result in 8 flux measurements per chamber per day (or every 3 hours). Because nitrous oxide fluxes from agricultural soils receiving N additions are very episodic in nature, fully convincing flux quantification cannot be achieved by means of infrequent manual chamber measurements due to high levels of uncertainty. In addition, compared to earlier research work, automated chamber equipment effectively allows us to offer more accurate, precise, and hence scientifically conclusive measurements of nitrous oxide fluxes and associated emission factors under varying N management practices. These automated frequent observations during the experimental periods will also enable us to identify at high temporal resolution which edaphic-climatic conditions are conducive to increased nitrous oxide fluxes (for example, following critical periods such as spring thawing and summer rainfall).

The automated chamber system includes a manifold, an air pump, a Fourier transform infrared sensor for online, nondestructive analyses (including N2O, CO2, CH4, NH3), an air compressor, and a computer for control. This system supports continuous flux quantification with instrument scanning at 10 Hertz and average concentration every minute. This general type of automated chamber system has been described in previous studies as closed, dynamic or as non-steady-state, flow-through chamber system.

Also flux method will be included regular manual static chamber measurements every week or twice a week following major rainfall or manure addition. See flux method for manual chamber below.

Research Design, Field Agronomy, Soil Sampling and Crop Measurement Protocol

Note: Please see spreadsheet file entitled "Manure Plot Plan split plot design" for further details in experimental setting and design structure.

Locations (2): Edmonton U of A South Campus; AAFC Lacombe. Same 8 treatments in the 2 locations, in the 2 years using same plots.

Manure Rate (1): 75 cubic meters per hectare (?); regardless of soil test

Manure Source (1): Liquid Dairy in Edmonton; Liquid swine in Lacombe

Manure Application Method (1): Injection

Research Design:
Aim to test two experimental factors: timing of manure (2 whole plot treatments) x additive (3 split plot treatments) for a split plot 2x3. Then add two controls to the experiment (for practicality, plots for controls would be the size of split-plot although in the statistical model they are at the level of whole plot factor).

Experiment List & possible layouts:

see spreadsheet file entitled "Manure Plot Plan split plot design" for generals and details

Stubble: Direct seed into standing stubble; record previous crop.

Pre-Seed Burnoff: If needed, burn-off with glyphosate at 900 g ae/ha prior to seeding

Seeder: Plot seeder

Manure Applicators: Injector

Crop Variety: to be determined

Seeding Rate: Barley at 300 seeds/m^2

Starter Fertilizer: Apply P_2O_5, K_2O and/or S if required based on AFFIRM recommendation

Herbicides: Apply herbicides as required for adequate weed control using labeled rates

Fungicide and Insecticide: As needed (blanket application over all plots); keep a close watch on potential disease pressure and spray appropriate pesticide if disease pressure is approaching the moderate level or if insect level is approaching critical level

Desiccation: NO PRE-HARVEST ROUNDUP

Experimental Design: Balanced Incomplete Split Plot Design; Specific Randomized and Blocking are provided; possible layouts are suggested (see spreadsheet file entitled “Manure Plot Plan split plot design” for generals and details)

Weather Data:

1. Automatic rain gauge must be installed on or before the date of soil moisture sampling in the spring
2. daily measurements from seeding to harvest
3. On site or nearest climate station data: temperature (hourly min and max), relative humidity (hourly average), wind speed (hourly average), solar radiation (hourly total)

Soil Sample Information for sending samples to laboratory for nutrient analyses (as before, samples collected and process by AARD to be sent to Exova lab for standard analyses):

1. Labeling – test #, name and year, location, time and year of sampling, rep or plot #,
   Len's address and Project ID e.g., "Additive Manure N2O Project"
2. When sending samples to Labs, send list of samples to laboratory and Len Kryzanowski and Guillermo.
3. Nutrient analyses should include soil ammonium, nitrate, phosphorus, potassium, sulfate, pH & EC (other analyses are welcomed if already included in the negotiated package). If texture analyses and data are available from the last 10 years, there is no need to submit for texture.

Manure Sampling

1. **Sample Manure at the time of application**

a. Representative sampling of well agitated liquid manure in each batch (e.g., fall 2014 (first week of October), spring 2015, fall 2015, spring 2016)

2. **Submit manure sample for laboratory analysis**

a. NH_4-N, NO_3-N, Total N, Total P, Total K, Total S, pH, EC

Soil Sampling

1. **Soil Sampling prior to manure application – Fall 2014 (mid September)**

a. Sample unfertilized area in rep (4-5 cores/rep) at 0-15, 15-30, 30-60, 60-90 cm
b. Bulk by rep, mix thoroughly and subsample
c. 4 depths x 4 reps = total of 16 samples
d. Air Dry, Grind and send to Lab for analysis (see soil sampling info above)

2. **Soil Bulk Density Sampling prior to manure application – Fall 2014 (mid September) (all replicates for up to 32 plots)**

a. Sample unfertilized area in each rep (1 core/rep) at 0-15, 15-30, 30-60, 60-90 cm
b. Record diameter of soil core
c. 4 depths x 3 reps = total of 12 samples
d. Dry samples at 105° C for 24 hours to determine moisture content and calculate bulk density for each sample depth

3. **Soil Moisture just prior to seeding (soil sampling) – Fall 2014**

a. Just prior to seeding, sample at 0-15, 15-30, 30-60, 60-90 cm

b. Take wet weight immediately, and put in 105°C dryer until dry and take dry weight to get % moisture, discard sample

4. Fall 2015 (three replicates for three treatments CT, FW, SW for 9 plots)
Soil Sampling Immediately after Harvest
a. Sample all treatments 0-15, 15-30, 30-60, 60-90 cm
b. Sample 3 cores/plot and bulk by depth
c. 4 depths x 3 trts x 3 reps = total of 36 samples
d. Air Dry, Grind and send to Lab for analysis (see soil sampling info above)

5. Fall 2016 (all replicates for up to 32 plots)
Soil Sampling Immediately after Harvest
a. Sample all treatments 0-15, 15-30, 30-60, 60-90 cm
b. Sample 3 cores/plot and bulk by depth
c. 4 depths x 8 trts x 4 reps = total of 128 samples
d. Air Dry, Grind and send to Lab for analysis (see soil sampling info above)

6. Soil Moisture Immediately after Harvest
a. Immediately after harvest, soil moisture sample 2X treatments at 0-15, 15-30, 30-60, 60-90 cm
b. Take wet weight immediately, and put in 105^{o}C dryer until dry and take dry weight to get % moisture, discard sample

Crop Data Collection

1. **Emergence Date (row is visible):** every Mon, Wed & Fri – record Julian date of emergence (per plot)

2. **Photos:** Take pictures of treatments that show or do not show crop growth and/or injury – take pictures at 3-5 leaf stage of barley silage crop to show seedling growth/injury of various manure application. If time permits also take pictures at the stem elongation and fully headed for barley that show differences in growth, colour, etc and compare check treatments with manure treatments

7. **Heading Date (>50% of heads have emerged):** Check every Mon, Wed & Fri and record in Julian date per plot

8. **Lodging Notes:** Scale used is 1-9 (1=Standing, 9=Lodged)

Silage Yield: Square meter samples or forage harvester of the center of the plot if there is a bad edge effect (trim off the front & back). Dry sample to uniform moisture, weigh sample and record plot yield and average % moisture from samples, keep a subsample for further testing.
- If sample is dirty, you may need to clean a subsample to get a true yield.

Silage nutrient analysis: NIR analysis

N_2O Sampling Protocols

December, 2012

Craig Sprout, Tom Goddard
Alberta Agriculture and Rural Development, Edmonton

Gas Chamber Placement

1. **When (fall to fall, spring to spring)**: Install chambers on the fall fertilizer treatments and check immediately after application of the fall fertilizer. These chambers remain in place until just before seeding and fertilizing next spring. All the chambers (fall and spring treatments) will be put in immediately after seeding. The chambers in the fall fertilizer treatments and check will be taken out of this field site and put into next years field site immediately after fall fertilization is completed on the new site. The chambers in the spring treatments and check will remain in the first site until after spring thaw the next year when they will be moved to the new site for placement into the new spring treatment plots after seeding.
2. **Where**: Place the chambers about 2 m in from the front edge of the plot and straddling three fertilizer or seed rows. This assumes 8 inch spacing for fertilizer and seed placement.
3. **How**: Place the frame on the ground and mark or cut (using knife, trowel, or small shovel) the soil around the frame and then remove the frame. Dig a narrow trench on the outside of the marked soil to allow the frame to be buried into the soil. Try not to disturb the soil on the inside of the trench (inside the chamber area). The final soil level should be at the base of the corner reinforcements (5 cm) on the inside of the rectangular chamber, allowing for 10 cm headspace. Be careful installing the frame. Use a rubber mallet only to lightly knock the frame into place. Try not to disturb the soil within the chamber. Place the loose soil back around the frame. Also replace any residue that may have been displaced while installing the chamber.

Gas Sampling

1. Take the gas samples twice a week during peak times, which occur during spring thaw in March or April and after high rainfall events from May to July. Otherwise take samples once per week until end of August and then once every two weeks until freeze up. Winter sampling will depend on the amount of snowfall. If the chambers become exposed or snowfall is minimal, samples should be taken once a month during the winter months. Taking samples when the ground is frozen will ensure a zero flux and give another point to interpolate an annual cumulative number.
2. Gas samples should always be taken at the same time of day, preferably between 11am and 3 pm.
3. *Label evacuated exetainers and arrange in test tube rack in a small cooler (arrange tubes geometrically according to plot layout). The rack should contain extra evacuated tubes in case a tube is found not to have a vacuum.
4. At plot site remove chamber covers from storage and place next to the chambers along with the properly labeled exetainers for each chamber location before starting to sample. Cut or pinch back crop growth to the height of the chamber.
5. Prepare 20 ml syringe and # 20 needle. One syringe and needle is to be used for each sample event then discarded because of wear on plunger. The cooler should contain a spare syringe and needle. The syringe should be unwrapped and have the needle mounted but with the protective cap on. You may need to grab this quickly if you bend a needle in the sampling process.

6. Five ambient air samples will also need to be collected and labeled. One for each rep and one extra in case of extreme values, loss of sample, etc. The ambient samples can be taken before, during and after the sampling campaign. Syringe samples should be taken at chamber height level randomly in the plot area.
7. Each chamber will be sampled three times (use a stopwatch to time the sampling) at a 15, 30, and 45 minute time step sampling. Sampling with a one minute interval between chambers will allow for a maximum of 15 chambers that can be sampled at a time. Complete all three time step samplings before starting the next series of chambers.
8. Place cover on frame at time 0 minutes. Use elastic bands to latch down cover and place extra weights or rocks on cover if necessary. Start stopwatch and then move to next chamber.
9. Place cover on next frame at 1 minute later. (The time interval depends on how much time the person who is sampling will need to move from one frame to the next and take a gas sample). After 15 minutes sample gas from first chamber, then sample in the same time increments that the lids were placed on the frames (i.e.):

Treatment #	Cover On (minutes)	Gas Sample (minutes)
1	0	15
2	1	16
3	2	17
4	3	18
5	4	19

10. Push the plunger all the way down and insert the needle into the center of the stopper of the chamber. With the needle in the chamber flush the syringe twice before extracting a **full** syringe of gas sample (~ 23 ml).
11. Push needle into 10 ml evacuated exetainer. Allow sample to draw down into exetainer; if it does not draw down, remove and inject sample into properly evacuated exetainer (extra in cooler) and then push the rest of gas from syringe into exetainer. If you feel that the sample has been jeopardized, take another sample from the chamber immediately.
12. Keep pressure on the plunger while removing the needle from the exetainer.
13. Place exetainer containing the gas sample back into test tube rack in a logical sequence.
14. Before taking the next gas sample in sequence, flush the syringe twice with ambient air.
15. At the completion of sampling remove all the chamber covers and store them bottom to bottom (foam to foam with one of the lids vent tube on one side and the other lids vent tube on the other side).
16. Record all relevant information on the field sheet such as the sampling time, weather conditions, soil and crop conditions, relative humidity, average wind speed, and air temperature. **Sheltered air temperature near ground level during time of sampling is important for mass calculations of gas flux.**
17. Upon the completion of sampling, mark (using permanent sharpie) the exetainer cap with a line to indicate the number of uses. Marking the cap is important because the caps of the exetainer should be replaced after three uses. The exetainers will be reused as long as they are properly evacuated and the caps are replaced after their third use.
18. Transport the gas samples in a cooler to and from the field and store samples in a fridge until the samples can be sent to Leigh-Anne Powers in Edmonton for GC analysis. The samples can be grouped by rep with an elastic band placed around them and packed securely for transporting along with a copy of the field sheet.

19. We will send more evacuated tubes and other supplies periodically and/or as needed to the people who are doing the sampling.

20. **S**upply kits will include field sheets, air temperature/relative humidity/wind speed sensor, Theta moisture probe, Hobo data logger and soil temperature sensors, cooler, evacuated exetainers, test tube racks, syringes, needles, stop watches, elastic bands, labels, extra septums (septums should be replaced each season or sooner if needed), silicone, extra closed cell foam and hooks for repairs, small shovel and trowel.

Snow-cover conditions:

Prior to putting on the cover take out enough snow to expose the chamber and avoid needle contact with snow when taking your sample. After sampling place snow back in and around the chamber, similar to way you found it. This ensures that the conditions in and around the chamber will remain relatively the same.

***Labeling**:

Label exetainers with site (location and plot-year), treatment number, time step, and date.
Example: *ED08-1-1*
15/10/07
ED = Edmonton (**ED**monton, **LA**combe) and 08 = 2008 plot (08, 09, 10, 11)
1 = Treatment 1 (1-40)
1 = Time Step 1 (1-3)
15/10/07 = Day/Month/Year

Other Sampling Protocols

Soil Moisture Sampling (Yes, please, this will be helpful to data interpretation)

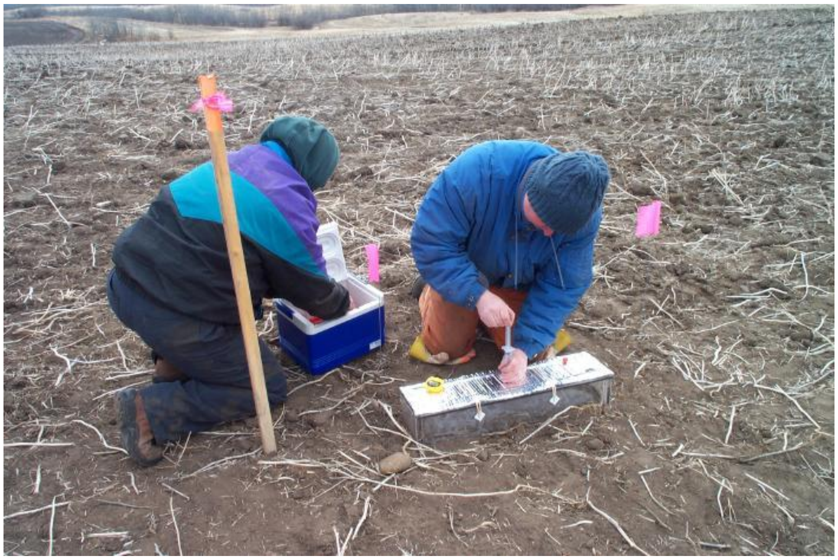
Theta soil moisture probe (6 cm depth)

Three readings on control and high N treatment for each rep.

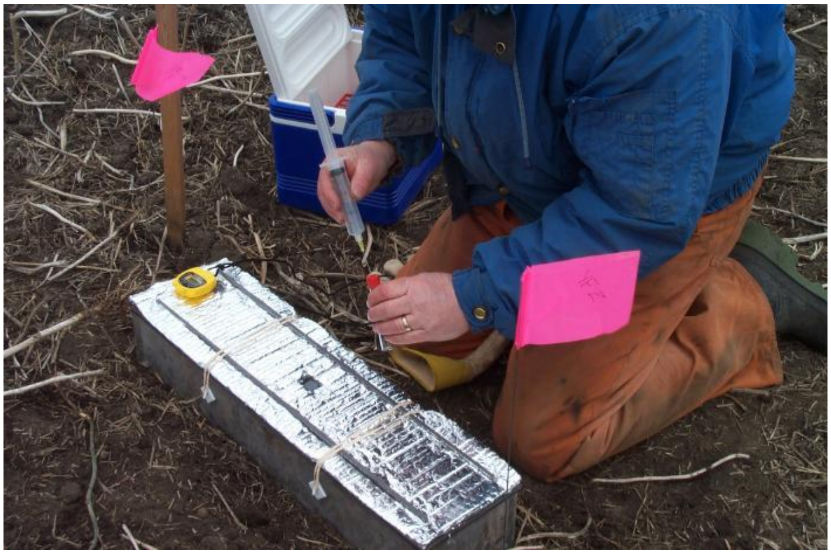
- At each greenhouse gas sampling event
- Down load to computer and name file with site and date
  http://www.delta-t.co.uk/products.html?product2005092818876

Soil Temperature (Yes, please, this will be helpful to data interpretation)

- Onset Hobo H8 Industrial outdoor logger (or U12-008) and 4 temperature sensors
- Installed on selected treatments (control and adjacent treatment) – At 5 and 10 cm depths
- Continuous one hour readings
- Download once a month to laptop computer and name file with site and date
  http://www.onsetcomp.com/



Taking an air sample from the chamber.



Transferring the air sample to an Exetainer.

Calculation for mass of N_2O-N (ug m^{-2} hr^{-1}) from PPM using Rectangular Gas Chamber

PV=nRT (Equation of State for Ideal Gas)
Where:
P = 1 atm at standard pressure
V = volume of gas (L)
n = # moles of gas
R = Universal Gas Constant (0.08206 L atm / mole degree K)
T = temperature (K) - 273 K at standard temperature

This equation is the basis to convert a change in [N_2O-N] within the chamber over the sampling times into a flux for a period (g N_2O -N m^{-2} h^{-1} or, ha^{-1} d^{-1} or, ha^{-1} yr^{-1}, etc). The flux is adjusted for average ambient air pressure at the site due to elevation (barometer effect) and fluxes are only considered real if they are larger than the ambient variance of [N_2O]

The mass of N_2O-N in g N/ha/day are calculated from the Excel GHG Calculator version 6.45 developed by Dr. Rich Farrell and others with the University of Saskatchewan, Dept. of Soil Science. This calculator tests both linear and quadratic models for fit of the time step concentration data.

Graphs and tables can then be constructed from the data.

6.2 Work Plan

I. Moisture & Temperature Sensor Installation

Objectives

To identify the relationship between the nitrous oxide emissions and the soil moisture & temperature, 16 '5TM Water content and temperature' sensors will be installed in plot 201-208 at the Edmonton site (8 plots in total).

Hypotheses

1) There would be a positive relationship between soil moisture content and N_2O emissions;
2) There would be a positive relationship between soil temperature and N_2O emissions;
3) The soil moisture in the plots with the manure injection would have higher moisture contents;
4) The average soil moisture content in spring season would be higher than the average soil moisture content in fall season;
5) The soil moisture content would be uniform but with relatively small fluctuations with time except an increase after the spring season snow melting and rainfalls;
6) The soil temperature would be related with the atmospheric temperature.

Time duration

2014 Fall, 2015 Spring, 2015 Fall, 2016 Spring

Key processes

1. **Removal of sensors.** Remove all 5TM sensors from the soil before each manure application (June 2015, October 2015, June 2016 and October 2016).
2. **Preparation.** About one week before each installation, all 5TM sensors and data loggers should be tested to ensure the working status. After checking status and before each installation, all 5TM sensors should be soaked into the water for at least three days.
3. **Installation.** After the manure application, all 16 5TM sensors should be installed immediately into the plot 201-208 at the Edmonton site. Each 5TM sensor should be installed horizontally and the middle prong of the sensor would be at established soil depths (Fig. 1). Two 5TM sensors would be installed in one plot at 10cm and 20cm below top soils respectively. Each 5TM sensor has its corresponding O_2 sensor. Four 5TM sensors would share one data logger (4 Em50 dataloggers in total).

4. **Data loggers working in winter.** Keep all data loggers working during the winter and ensure the power of the lithium batteries.

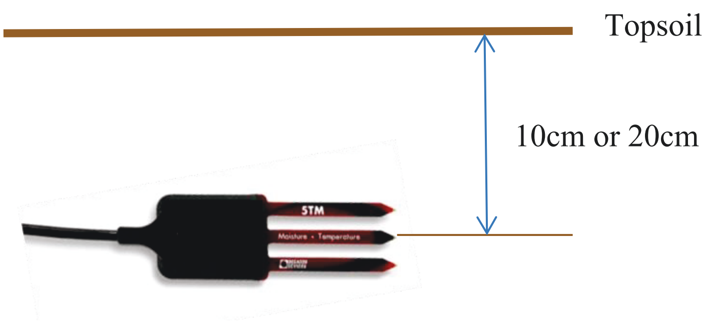


Fig. 1. 5TM sensor installation in the soil profile

Data collection

The data is collected frequently by ‘ECH2O utility’ software after the first two weeks of installation to make sure all sensors are working well. After this, data can be collected once two weeks.

Note: Check the battery status every time when collecting data. If the battery power is low, change with the standby batteries which should be always taken with the data collector.

Materials

Rulers, shovels, gloves, 16 5TM sensors, dataloggers, stakes, a hammer, colored tapes, PVC wrappers, cable ties

II. O_2 Sensor Installation

Objectives

To identify the relationship between the nitrous oxide emissions and the soil oxygen content, 16 'Apogee SO-110 oxygen' sensors would be installed in plot 201-208 at the Edmonton site (8 plots in total) with corresponding 5TM sensors.

Hypotheses

1) There would be an inverse relationship between soil oxygen content and N_2O emissions;
2) There would be an inverse relationship between soil oxygen content and soil temperature;
3) There would be an inverse relationship between soil oxygen content and soil water content;
4) The average soil oxygen content in spring season would be lower than the average soil oxygen content in fall;
5) The soil oxygen content would decrease after the spring season snow melting and rainfalls.

Time duration

2014 Fall, 2015 Spring, 2015 Fall, 2016 Spring

Key processes

1. **Preparation.** The detection side of each O_2 sensor would be connected with an 'elbow' and two layers of teflon would be covered at the end of the elbow.
2. **Calibration.** About one week before each installation, all oxygen sensors and the working system should be tested in order to collect data for calibrations and to ensure their working status.
3. **Minimum detection limit.** All O_2 sensors should be tested for the minimum oxygen detection before installations. A dinitrogen gas bottle and hermetic plastic bag would be used to generate anoxic conditions to calibrate the O_2 sensors, which allows to learn what zero is in ## mV.
4. **Installation.** After the manure application, all 16 O_2 sensors are installed immediately into the plot 201-208 at the Edmonton site on the same day of 5TM sensors installation. Each O_2 sensor is installed vertically and the open side of the elbow with the teflon layers is the level of the depth standard (See Fig. 2). Two O_2 sensors are installed in one plot at 10cm and 20cm below top soils respectively with its corresponding 5TM sensors. All O2 sensors are connected with the AM16/32B Relay multiplexer with a CR1000 control system and a battery in a container inside of the trailer at the Edmonton site.

5. **Disenabling the system.** After each fall season data collection, the CR1000 control system should be disconnected with the battery in the container.

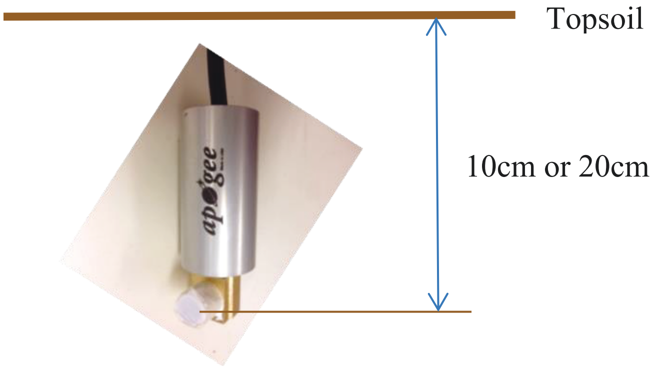


Figure 2. O2 sensor installation in the soil profile

Data collection

The data would be collected frequently by ‘PC200W’ software after the first two weeks of installation to ensure all sensor working conditions. Otherwise, data could be collected once two weeks.

Note: Check the internal battery status in CR1000 multiplexer and the power voltage every time when collecting data. If the internal battery power is less than 2.7Vdc (Datalogger>>Station Status>>Battery Information>>Lithium Battery), change it immediately. The normal voltage for the power in the container varies from 12.5V to 13V.

Materials

Rulers, shovels, gloves, 16 O_2 sensors, stakes, a hammer, colored tapes, PVC wrappers, cable ties

III. Topsoil samples

Objectives

To understand how N would be transformed in the soil through mineralization (ammonification) and nitrification. Also, to understand how the nitrification inhibitors would affect the speed of these transformations.

Hypotheses

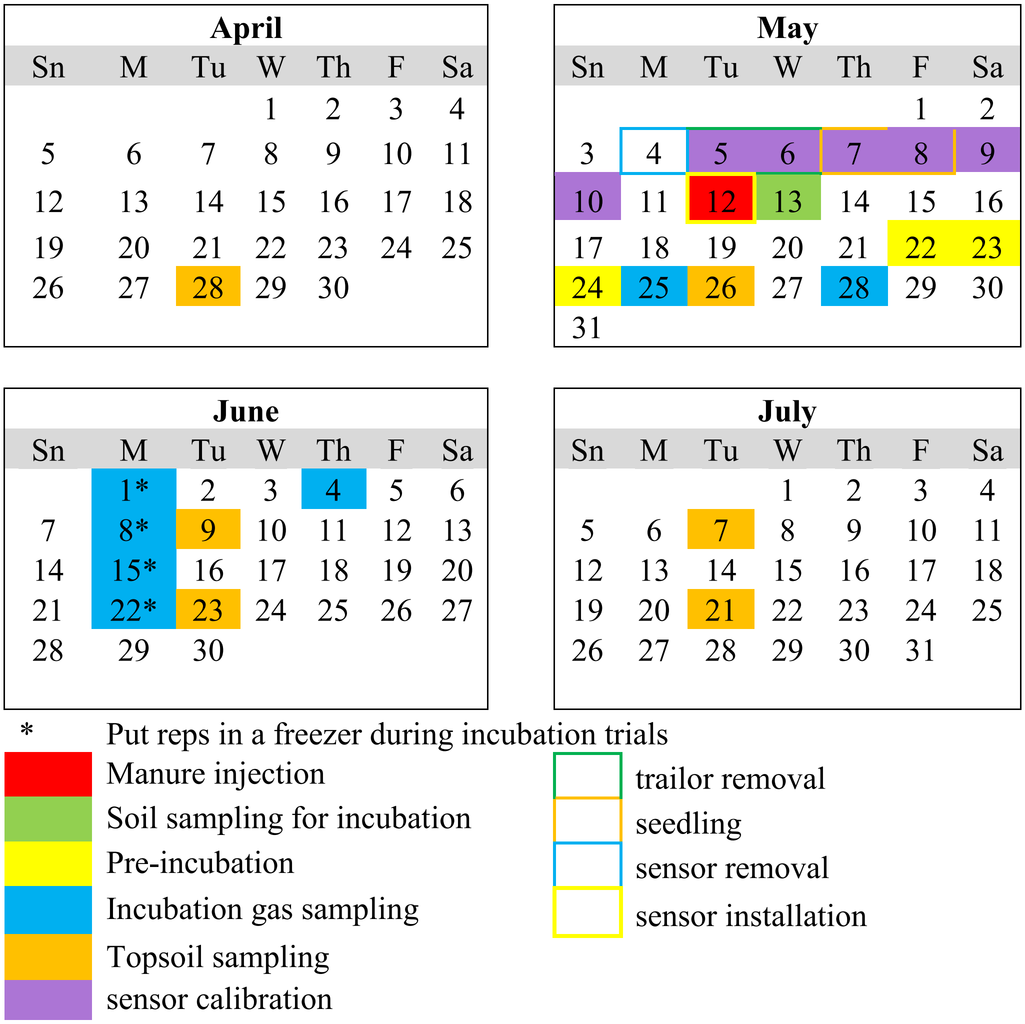
1) The amount of mineral N(NH_4^++NO_3^-) in the plots with manure injections would be higher than the plots without manure injections;
2) The amount of NH_4^+ in the plots with manure injections would be higher than the plots without manure injections;
3) The amount of NO_3^- in the plots with manure injections would be higher than the plots without manure injections.
4) The amount of NH_4^+ in the plots with nitrification inhibitors would be higher than the plots without nitrification inhibitors;
5) The amount of NO_3^- in the plots with nitrification inhibitors would be lower than the plots without nitrification inhibitors.

Time duration

2015 Spring

Key processes

a. Take topsoil samples every two weeks for mineral nitrogen contents analyses in the specified field. The samples would be collected before and after the manure injection. The specific sampling dates are shown in Fig 3. (***Note:*** The samples collected after manure injection would include band area and interband area samples. The samples collected before manure injection wouldn’t account for this.)



April

Sn	M	Tu	W	Th	F	Sa
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

May

Sn	M	Tu	W	Th	F	Sa
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30
31						

June

Sn	M	Tu	W	Th	F	Sa
	1*	2	3	4	5	6
7	8*	9	10	11	12	13
14	15*	16	17	18	19	20
21	22*	23	24	25	26	27
28	29	30				

July

Sn	M	Tu	W	Th	F	Sa
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30	31	

* Put reps in a freezer during incubation trials
🟥 Manure injection
🟩 Soil sampling for incubation
🟨 Pre-incubation
🟦 Incubation gas sampling
🟧 Topsoil sampling
🟪 sensor calibration
☐ trailor removal
☐ seedling
☐ sensor removal
☐ sensor installation

Figure 3. Specific working dates for spring 2015.

b. Sample all 8 plots in each block (3 cores /plot, 8 plots /block) at 0-15cm (sampling on June and July has band area and interband area in some plots: spring manure treatments);

c. Mix the samples from the same plot thoroughly in a container and then transfer into a labelled bag.

d. Samples after manure injection: (2 areas * 3 plots * 3 blocks) + (5 plots * 3 blocks) = 33 samples. Samples before manure injection: 8 plots * 3 blocks = 24 samples

e. Collected soils will be stored in the refrigerator at 5°C.

f. Air dry, grind to 2mm, and then send to Lab for ammonium and nitrate.

Materials

2 augers, 250 bags, 2 containers, a sharpie pen, gloves, a ruler.

IV. Incubation Plan

Objectives

Under the controlled/ideal conditions, incubation experiments are conducted to understand the potential effects of manure injection on N_2O emissions, to evaluate the potential effects of injection timing on N_2O emissions and to quantify the potential amount of N_2O emissions reduced by nitrification inhibitors.

Hypotheses

1) The amount of N_2O emissions in the plots with manure injections would be higher than that in the plots without manure injections ➔ N_2Oe(manure)> N_2Oe(without manure);
2) The amount of N_2O emissions in the plots with nitrification inhibitors would be lower than that in the plots without nitrification inhibitors ➔ N_2Oe(inhibitors)< N_2Oe(without inhibitors) ;
3) The amount of N_2O emissions in the spring manure injection plots would be higher than that in the fall manure injection plots➔ N_2Oe (spring)> N_2Oe (fall).

Time duration

2015 Spring

Key processes

a. Soil sampling 3 days after manure injection in spring 2015 (in May) (Fig.3)

   a) Sample correlated plots in rep (8 cores/plot) at 0-15 cm;
   b) Sample for 7 plots: CT, SW,SD,SN,FW,FD and FN;
   c) For spring manure treatments, sample band and interband areas separately (SW,SD,SN only);
   d) Bulk by rep, mix thoroughly and sample;
   e) For spring manure treatments (CT,SW,SD,SN): 2 areas(band/interband)*4 treatments(plots)*3 reps(blocks) = 24 samples

      For other treatments without manure (FW,FD,FN): 3 treatments(plots)*3reps(blocks) = 9 samples

      Total samples = 24 + 9 = 33 samples;

f) Collected soils would be stored in refrigerator at 5 °C.

Note: The soil samples should be taken in the specific area.

b. Moisture content analyses

a) The soils used for moisture content analyses would be from CT(band area), CT(interband area), SW(band area), SW(interband area) treatments;
b) Each treatment would have 3 reps;
c) Label all moisture tins (3 reps * 4 trts = 12 tins in total) and record the weights;
d) Add around 15g of moist soil to a moisture tin and record the total weight;
e) Place the moisture tins into the drying oven (105°C) for 24-48 hours.

c. Pre-incubation

a) Take 90g soil from each soil sample above into a labeled container (6 replicates[1]) 33*6=198 containers;
b) Pre-incubate all containers for 2 days at room temperature and 60% WHC in order to stabilize soil microbe activities, otherwise collected soil samples would be stored in refrigerator at 5 °C;
c) Pre-incubate all the containers at room temperature and 60% WHC.

d. Incubation

a) The aerobic incubation will last 28 days (Fig. 3);
b) Incubate all the containers at room temperature and 60% WHC (the same conditions with pre-incubation);
c) All the containers would be adjusted at 60% WHC by adding deionized water and checked every day by weighing (***Note***: adding deionized water carefully to minimize soil disturbances and distributing the deionized water evenly);
d) Record the room temperature every day.

e. Gas samples

a) Put each container into a Mason jar with a rubber septum;
b) Take gas samples 2 times/week following the first two weeks of the incubation and then sample 1 time/week (after 0,3,7,10,14,21,28 days incubation)(Fig. 3);
c) 33 gas samples would be separately collected. (One group has 16 container/mason jar; another has 17 container/mason jar)*3 times (17min,34min,51min) for each rep;

d) Place a lid on the Mason jar at time 0 minutes. Start stopwatch and then move to next Mason jar;

e) Place another lid on next Mason jar at 1 minute later. After 17, 34 and 51 minutes sample gas from the first Mason jar, then sample in the same time intervals (Table 1).

Table 1. Timetable for gas sampling

Sampling #	Lid on(minutes)	1st Gas sample(minutes)	2nd Gas sample(minutes)	3rd Gas sample(minutes)
1	0	17	34	51
2	1	18	35	52
3	2	19	36	53
4	3	20	37	54
5	4	21	38	55
6	5	22	39	56
7	6	23	40	57
8	7	24	41	58
9	8	25	42	59
10	9	26	43	60
11	10	27	44	61
12	11	28	45	62
13	12	29	46	63
14	13	30	47	64
15	14	31	48	65
16	15	32	49	66
17	16	33	50	67

f) Push a plunger all the way down and insert the needle into the center of the Mason jar. With the needle in the Mason jar flush the syringe twice before extracting a full syringe of gas sample (~20ml);

g) Push needle into 10ml evacuated exetainer. Keep pressure on the plunger while removing the needle from the exetainer;

h) Place the exetainer in a tube rack;

i) Before next gas sample, flush the syringe twice with ambient air;

j) Take 3 ambient air samples before each round of gas sampling;

k) After gas sampling, remove the containers from the Mason Jars.

d. N analyses (ammonium, nitrate)

a) On the 7th, 14th, and 21st day of incubation (Fig. 3), randomly take one replicate for each sample (33 reps on each day) in a freezer below zero (to make sure no more N transformation taking place) (33 soil reps/week, 33*3=99 soil reps/3 weeks) for further N analyses;

b) At the end of 28 days incubation, store all the rest of soils reps (33*3 = 99 soil reps) in a freezer below zero (to make sure no more N transformation taking place) ;

c) Air-dry all soil reps and then send to Lab for mineral N analyses.

<u>*Materials*</u>

~3 Syringes, ~1600 exetainers (99*3+9=306;306*5=1530)/gas sampling (7 times gas sampling in one month), 35 Mason jars, 200 containers, 35 rubber septum, a drill, silicone, a stop watch, 12 moisture tins, a cooler, tube racks, a freezer below zero, a thermometer.

Note:

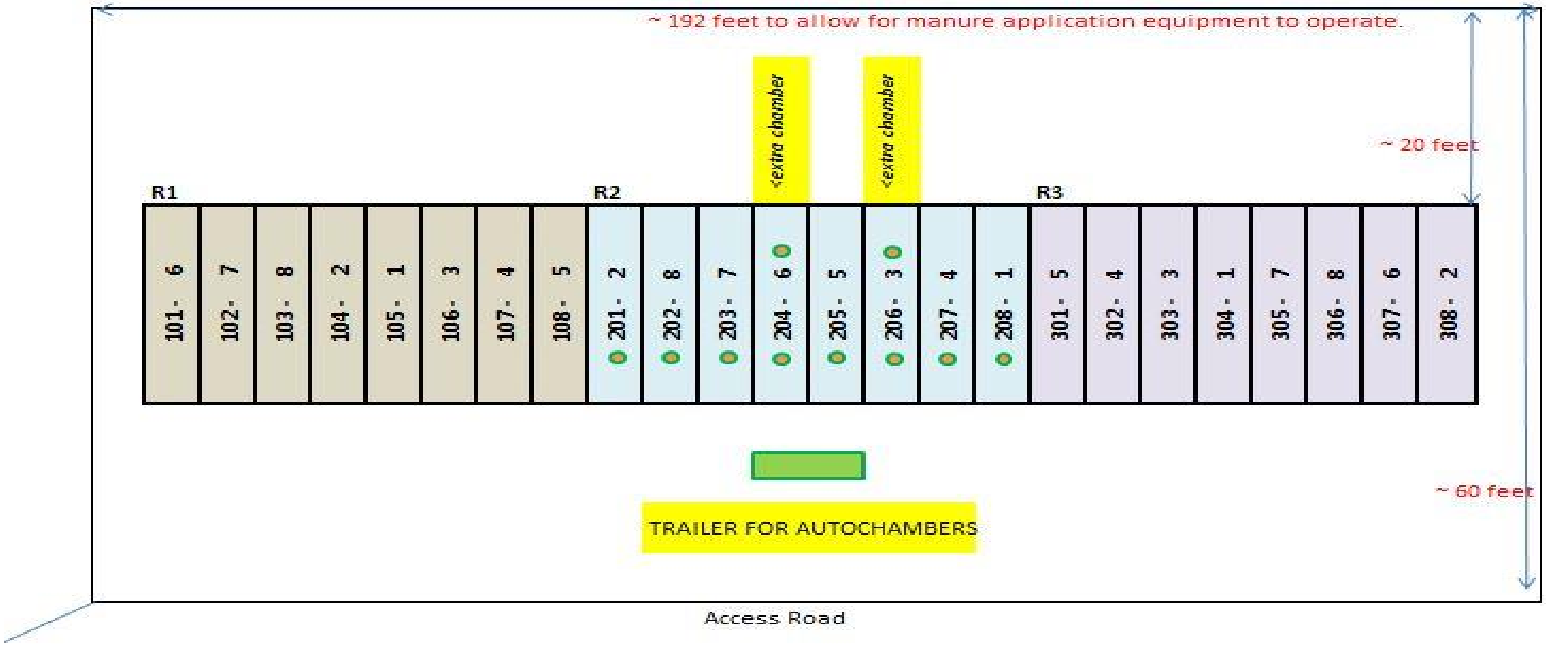
[1]. There are 6 replicates in total. Three of them would be incubated throughout 28 days incubation. The other three would be used for the first three weeks extraction (also call destructive sampling).

6.3 Field Experimental Designs

Edmonton South Campus Site of U of A 2014-2016 Manure Additive/GHG Project

Plot dimensions for All experimental units 8' x 20'

Block replicates dimensions 20 by 64 feet Experiment dimensions 20 by 192 feet



separate 220 volt/30 Amp circuit & outside weather proof twist lock connection

Main power available in Edmonton si Approx 300' of power cable

All 10 available autochamber to be deployed in R2 (replicate two); plots for treatment combinations (3 FW, 6 SW) will have two chambers.

Seed barley silage in the whole area Spring 2015

Apply manure N-S along plots in Rep 1, 2 & 3

Seeding to be done E-W!

All Treatment plots 8' wide and ~ 20' long

Rep 40' wide and ~ 20' long

Static chamber in middle of every plot placed across the direction of manure application

🟩 Trailer (8' x 18') - FTIR, Multiplexer, Computer

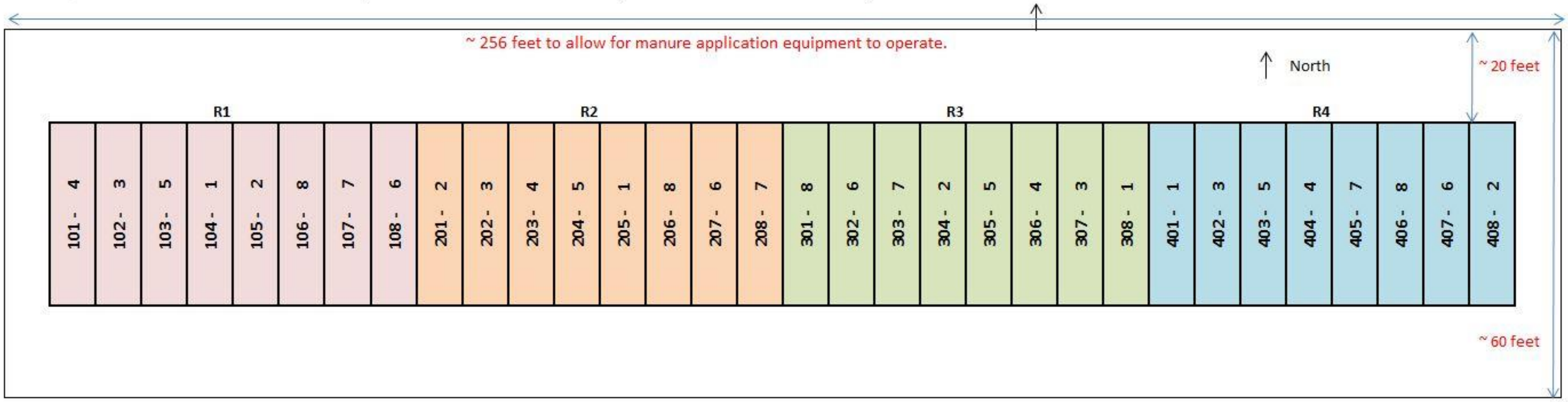
● Automated GHG Chamber 1-2 m into plot

Tubing from automated chamber to multiplexer ~ 50' or 15 m

1	CZ	Control Zero
2	CT	Control disTurbance
3	FW	Fall WithoutAdditive
4	FN	Fall Nitrapyrin
5	FD	Fall Dmpp
6	SW	Spring WithoutAdditive
7	SN	Spring Nitrapyrin
8	SD	Spring Dmpp

Lacombe Site of U of A 2014-2016 Manure Additive/GHG Project Fall 2014 Applied Manure-Additives; Cropped 2015

Plot dimensions for All experimental units	8 by 20 feet		
Block replicates dimensions	20 by 64 feet	Experiment dimensions	20 by 256 feet



R1	R2	R3	R4
101 - 4	201 - 2	301 - 8	401 - 1
102 - 3	202 - 3	302 - 6	402 - 3
103 - 5	203 - 4	303 - 7	403 - 5
104 - 1	204 - 5	304 - 2	404 - 4
105 - 2	205 - 1	305 - 5	405 - 7
106 - 8	206 - 8	306 - 4	406 - 8
107 - 7	207 - 6	307 - 3	407 - 6
108 - 6	208 - 7	308 - 1	408 - 2

Plot #s-treatments; 101-(1-8) to 108-(1-8), 201-(1-8) to 208-(1-8), 301-(1-8) to 308-(1-8) & 401-(1-8) to 408-(1-8)

1	CZ	**C**ontrol **Z**ero
2	CT	**C**ontrol dis**T**urbance
3	FW	**F**all **W**ithoutAdditive
4	FN	**F**all **N**itrapyrin
5	FD	**F**all **D**mpp
6	SW	**S**pring **W**ithoutAdditive
7	SN	**S**pring **N**itrapyrin
8	SD	**S**pring **D**mpp