Nitrous Oxide Emissions in Corn (*Zea mays* L) as Affected by Timing, Method of Application and Source of Dairy Manure

by

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ABSTRACT

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Field trials were conducted during three years to evaluate the effect of timing, method of application and manure source on N_2O emissions and corn grain yield at Elora, ON, Canada. A randomized block design was set up every year, evaluating two timings (fall vs. spring), three methods of manure application (surface broadcasting, incorporation and injection) and two manure sources (raw, RM vs. anaerobically digested, AD), using non steady state chambers. Three and two years of data were used to evaluate the effect of manure application timing and manure source respectively on N_2O emission, considering also application methods in each experiment. A hybrid, decision tree-based flux calculation method (DTBM) was developed and chosen to calculate N_2O emissions, given that it advantaged to other methods due to its ability to match each data type with the best model. Nitrous oxide emissions did not respond to timing of manure application; however, as the interaction year by manure application timing as well as application method significantly affected N_2O emissions ($p< 0.01$ and $p< 0.05$, respectively). The effect of method on cumulative N_2O emissions depended on manure source($p<0.01$), since surface broadcast AD had the highest emission (6.4 kg N_2O-N ha^{-1}), and both injected AD and incorporated RM had the lowest values (2.6 kg and 2.8 N_2O-N ha^{-1}, respectively). Manure source tended to affect cumulative N_2O emissions ($F=4.67$, $p<0.1$), with the largest emissions for AD (4.8 kg N_2O-N ha^{-1}). Anaerobically digested manure was proven to reduce cumulative N_2O emissions when it was fall injected to corn in cold climates; however, if AD is broadcasted or

broadcasted and incorporated, it may result in greater N_2O emissions than those produced by RM. Short (2-3 yrs.) and long term (26 yrs.) trends for cumulative N_2O emissions were simulated with a process based model (DNDC-CAN). Even though no difference between predicted application timings was found at short term, spring application was detected to decrease N2O emissions in the long term. The inter-annual variability canceled the effects of method of application in the long term on predicted N_2O emissions. Injection of AD showed to be a good technique to mitigate predicted N_2O emissions in the long term.

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Ancha chiri, Guelph ! (from Argentinian quecha language: It's cold, Guelph!)

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CHAPTER 1: GENERAL INTRODUCTION

1.1Background

The biosphere has been naturally emitting trace concentrations of nitrous oxide (N_2O) to the atmosphere for thousands of years, with the atmospheric concentration of this gas never exceeding 270 ppb (Le Treut *et al*. 2007); however, N_2O emissions increased since the beginning of the Industrial Era (1750), so that the current value of N_2O atmospheric concentration has reached 324 ppb (Hartmann *et al*. 2013). The increased concentration of N_2O has caused concern worldwide, since its global warming potential is 298 times that of CO_2 and 12 times that of CH_4 in a 100-yr time horizon (IPCC 2007). Furthermore, N_2O emissions contribute to the depletion of stratospheric ozone (Ravishankara *et al*. 2009). This dual effect of N_2O in the atmosphere makes the evaluation of practices for N_2O emissions mitigation a relevant topic in environmental science.

Approximately one third of the biosphere's N_2O emissions come from agricultural soils (Bouwman *et al*. 2010). The production of N_2O in the soil depends on microorganisms that use ammonium (NH_4^+) or nitrate (NO_3^-) as substrates in processes such as nitrate ammonification, nitrification, denitrification or nitrifier denitrification (Saggar *et al*. 2004, Baggs and Philippot 2010, Venterea *et al*. 2012, Butterbach-Bahl *et al*. 2013). The main drivers controlling soil N_2O production are physical factors such as temperature, rainfall, soil moisture and snow layer thickness (Granli and Bøckman 1994, Butterbach-Bahl *et al*. 2013 Burchill *et al*. 2014, Abalos *et al*. 2015), and biochemical parameters such as soil mineral nitrogen (N) availability, soil oxygen (O_2) concentration and easily metabolizable carbon (C) concentration (Granli and Bøckman 1994, Petersen 1999, Robertson and Groffman 2007, Baggs and Philippot 2010). In unfertilized soils, the dynamics of daily N_2O emissions respond mainly to the temporal variation of the physical factors, with the growth of N_2O-producing microorganisms limited by the availability of mineral N (Clark *et al.* 2009, Kariyaperumma *et al*. 2011a). This basal level of N_2O produced and emitted by the soil is referred to as *background emission* (IPCC 1996) and is strongly affected by the land use change when lands are used for agriculture.

The atmosphere receives about 15.8 Tg N_2O-N every year, with 5.6 - 6.5 Tg N_2O-N coming from anthropogenic sources, among which, agriculture represents 62 - 89 % of emitted N_2O (Crutzen *et al*. 2008, Bouwman *et al.* 2010). Within the agricultural sources, dairy farms significantly contribute to N_2O emissions through manure management and feed production (Jasayundara and Wagner-Riddle 2014). In Ontario, Canada, the farmers feed their dairy cows mainly corn, which implies the consumption of approximately 60 % of the planted area in the Province (844,700 ha during the period 2010-2014, Statistics Canada 2015 a). It also implies the addition of substantial amounts of nitrogen (N) to the soil through either inorganic or organic sources to guarantee the feed production, since corn grain yield is strongly constrained by soil N availability (Drury and Tan 1995, Hay and Porter 2006). Almost half of Canadian farms use liquid dairy manure to supply N to corn (Statistics Canada 2011); however, field application of liquid dairy manure may enhance N_2O emissions. Manure application provides not only N to the soil microorganisms, but also C and water, leading to conditions suitable for denitrification such as high soil water content and low O_2 diffusion (Petersen and Andersen 1996, Petersen et al. 1996, Petersen *et al*. 2008).

Practices for field application of liquid dairy manure vary from farm to farm, with each practice having a different effect on N_2O emissions. Variations in timing of manure application, application method and the type of manure used have been reported to affect N_2O emissions

(Dosch and Gutser 1996, Petersen 1999, Flessa and Beese 2000, Velthof *et al*. 2003, Rochette *et al*. 2004, Hernández-Ramírez *et al.*2009, Lemke *et al*. 2012). In Ontario, a gross two-thirds of the dairy farms apply manure during spring, while one-third of the farms perform this in fall. (Beaulieau 2004). Both fall and spring-applied liquid manure can increase N_2O emissions. Fall application increases emissions during spring-thaw, while spring application increases emissions when is followed by rainfall events, which added to higher soil temperatures, lead to denitrification during the growing season (Wagner-Riddle and Thurtell 1998, Wagner-Riddle *et al.*1998, Rochette *et al*. 2000, Rochette *et al.* 2004, Saggar *et al.* 2004, Wagner-Riddle *et al.* 2007, Kariyapperuma *et al.* 2011a). Few studies have been conducted comparing the effect of manure application timing on N_2O emissions for soils in cold climates, and those that have been published did not include a possible interaction with application methods and were short term (Rochette *et al.* 2004, Hernández-Ramírez *et al.*2009). There is a variety of methods to apply liquid dairy manure such as surface broadcasting, surface broadcasting followed by incorporation into soil, and injection (Meisinger and Jokela 2000). In Ontario, 56% of the farms using liquid manure inject manure into soil (Statistics Canada 2011), which is considered a best management practice to avoid ammonia volatilization losses (Dosch and Gutser 1996, Meisinger and Jokela 2000); however, it has been reported that manure injection promotes N_2O emissions compared to other practices such as manure incorporation (Flessa and Beese 2000, Velthof *et al*. 2003, (Dosch and Gutser 1996). Therefore, studies that investigate the interaction of manure application timing and application methods on N_2O loss are needed, especially in cold climates where N_2O emissions due to spring thaw are significant.

Dairy manure can be treated with anaerobic digestion to reduce methane emissions relative to raw manure (Amon *et al*. 2006). During the process of anaerobic digestion, conducted in large tanks called digesters, carbon (C) and N forms present in raw manure undergo numerous changes from complex organic compounds to simpler molecules (CO_2 and NH_4^+), resulting in a substrate with a lower C:N ratio than raw manure (Möeller and Müller 2012). Organic C contained in AD manure is less metabolizable, which may limit soil microbial growth and oxygen demand, leading to an aerobic soil environment and limiting denitrification after land application (Petersen 1999). Even though AD manure has potential for mitigation of N_2O emissions, few field studies compared the effect of AD manure and raw manure on N_2O emissions and grain yield. For example, Clemens *et al*. (2006) found that anaerobically digested cattle manure produced lower N_2O emissions after field application compared with untreated manure and calcium ammonium nitrate in pastures. Similarly, in a Saskatchewan (Canada), Lemke *et al.* (2012) reported a reduction in N_2O emissions after fall application of AD swine manure in corn compared to raw swine manure. Conversely, Amon *et al.* (2006) found no significant differences in N_2O emissions between AD cattle slurry and untreated cattle slurry after applying approximately 100 kg N ha^{-1} in late summer. Clemens *et al*. (2006) and Amon *et al*. (2006) applied AD manure directly on the surface or banded on the surface while Lemke *et al*. (2012) used an experimental applicator that injected AD manure at 10 cm depth. Therefore, to verify the capacity of AD manure for N_2O emissions mitigation, more studies are needed that also consider the interaction with manure application method.

To accurately estimate the effect of timing, method and source of manure on N_2O emissions, a reliable method for N_2O flux calculation should be considered (Parkin *et al.* 2012). Most N_2O emissions calculations are based on N_2O concentrations measured with non-steady state (NSS) chambers, which requires the adjustment of data with linear, quadratic regression or other models to calculate fluxes (Hutchinson and Mosier 1981, Livingston *et al*. 2006, Levy *et* *al*. 2011, Chadwick *et al*. 2014). However, using such models independently may lead to underestimations of the N_2O flux, since the variability of field measurements may modify the pattern followed by N_2O concentrations over time, making the method chosen not suitable for all the cases (e.g. high or low fluxes). Therefore, a hybrid model will be developed and used for our studies, in order to improve the stability of the mean flux due to field measurement variability.

The inter-annual variability of weather over the long term should also be taken into account, since it could modify the effects of mitigation techniques such as AD manure application. Experimental studies on application timing, method and manure source are usually conducted over the short term (i.e. 2-3 yrs.), but a long-term assessment is needed to better evaluate the effects of these practices on N_2O emissions. Long-term field studies are costly, but the use of properly calibrated and validated process-based models can be an alternative to obtain estimates of N_2O emissions over periods greater than 3 years. Process-based models simulate the interaction among climate, soil and agronomic variables, being able to predict grain yield as well as the dynamics of soil N and greenhouse gases emissions from agricultural soils (Li *et al*. 1992, Giltrap *et al*. 2009). The DeNitrification - DeComposition model (DNDC) is a process-based model and was originally developed for simulating and predicting N_2O emissions from cropped soils in the US (Li *et al*. 1992). It has since been used by many research groups, covering a wide range of countries and production systems, being in a continuous evaluation-improvement process. Currently, DNDC has evolved towards a ‘family of models’ (Global Research Alliance Modelling Platform, http://gramp.org.uk/models/), including a version for Canadian environments (DNDC-CAN). This version was calibrated and validated for Elora (Southwestern Ontario), to model the response of N_2O emissions and corn yields to inorganic fertilization strategies such as timing and anhydrous ammonia injection, showing a good performance to simulate N_2O emissions after inorganic fertilizer application (Abalos *et al*. 2016). To date, no study has evaluated DNDC performance for simulating N_2O emissions after raw manure injection using AD manure and/or other application methods.

1.2 Hypotheses and objectives

The general objectives of this study were to investigate how a set of manure management practices such as application timing, application method and source affected N_2O emissions and corn yield, and whether the short-term trends for N_2O emissions and grain yield were similar to the long-term trends. The general hypotheses of this research were: (i) that manure management practices (timing, method and source) influence N_2O emissions and corn grain yield, and (ii) that the short-term trends for N_2O emissions and grain yield are similar to the long-term trends.

The specific objectives of the study were (1) to evaluate an hybrid method of N_2O flux calculation; (2) to determine whether the effect of manure application method (injection, broadcasting or incorporation to soil) on N_2O emissions and grain yield in corn varies with timing of manure application (fall or spring); (3) to determine whether the effect of manure application on N_2O emissions method changes according to manure source (raw or anaerobically digested manure), and (4) to evaluate the ability of DNDC-CAN to predict N_2O emissions according to manure application timing, method and source for short and long term scenarios.

1.3 Format of the thesis

This thesis has been written and structured in a series of manuscripts, with one methods chapter (chapter 2) and three separate experimental chapters (chapters 3, 4 and 5). Each of these four chapters consists of introduction, materials and methods, results, discussion and conclusions sections. Each chapter is designed to be an “independent unit”, so that site description and the details of treatments may be somewhat repeated among chapters. Chapter 2 covers objective (1) by evaluating a new approach for N_2O flux calculation. Chapter 3 addresses objective (2) by studying N_2O emissions and grain yield as affected by timing and method of manure application in corn. Chapter 4 addresses objective (3) by investigating the effect of manure source and method of application on N_2O emissions and corn yield. Finally, Chapter 5 reports on a modelling approach using DNDC, to address objective (4) regarding the ability of DNDC-CAN to predict N_2O emissions in the short-term by comparing predicted values with observed emissions from Chapter 3 and 4, and compare short term results with simulations over long-term scenarios. A summary and the general conclusions of this study are presented in Chapter 6.

CHAPTER 2: A DECISION TREE-BASED APPROACH TO CALCULATE NITROUS OXIDE FLUXES FROM CHAMBER MEASUREMENTS

The following Chapter was submitted to the Canadian Journal of Soil Science on August 30, 2016 and is currently being revised. I am the primary author of the paper, with Dr. Claudia Wagner-Riddle, Dr. Craig Drury, Dr. John Lauzon and Dr. William Salas acting as supervisors, and appearing as coauthors on the journal article.

2.1 Introduction

Non steady state (NSS) chambers are widely used to measure nitrous oxide (N_2O) fluxes due to their simplicity, low cost and adaptability of the design to different situations (Clough *et al.* 2012, Rochette and Bertrand 2008). When this technique is used, N_2O flux calculations are based on the estimation of N_2O concentration over time (*dC/dt*). There is a variety of methods to estimate *dC/dt*, however their reliability may vary according to the situation. For example, the most used method to calculate *dC/dt* is linear regression (LR), but it is also the most criticised since it may underestimate fluxes under high flux situations (Hutchinson and Mosier 1981; Nakano *et al*. 2003). However, frequently used alternatives to LR such as quadratic regression models (QR) or the Hutchinson-Mosier equation (H/M) (1981) may also underestimate dC/dt (Livingston *et al*. 2006, Parkin *et al*. 2012). The reliability of flux calculation methods has been evaluated in several studies using meta-analysis (Rochette and Eriksen-Hammel 2008), or through scenario simulations (Parkin *et al*. 2012, Venterea *et al*. 2013), revealing the need for an improvement of flux calculation methods. This improvement can be achieved by reducing the uncertainty in flux estimations by selecting the right method for each field situation (Levy *et al*. 2011, Chadwick *et al*. 2014). Therefore, the choice of the appropriate model to calculate N_2O fluxes is critical because a non-biased estimate of dC/dt will improve the accuracy of both yearly cumulative N_2O emissions and the emission factor (cumulative N_2O emissions / kg of N applied), both of which are required for developing N_2O emission inventories (IPCC 1996).

Classifying the pattern of N_2O data points according to their curve shape across time is a potentially useful approach to selecting the flux calculation method but it has not been widely evaluated. Anthony *et al*. (1995) defined 6 curve shapes using 3 data points. They then compared the adjustment of non-linear vs linear models to each curve shape and found that the linear model underestimated the flux in 53% of the cases. Another possible approach is a decision rule based on an index. For example, Dyer *et al*. (2012), working in corn, supported their decision of using either linear or quadratic regression for estimating N_2O fluxes if the result of the equation $(C_m-C_0) / (C_f-C_m)$ was lower or larger than 1 (Hutchinson and Mosier, 1981), where C is N_2O concentration at initial (0), middle (m) and final (f) time points. The reciprocal of this equation was taken by Venterea (2013) to develop a curvature index to estimate the extent to which theoretical assumptions such as absence of lateral diffusion, biological uptake and chamber leakage were violated by simulated data in a model comparison study. A procedure combining curve shape classification (Anthony *et al*. 1995) with decision rules based in the curvature index (Venterea 2013) may guide and optimize the slope calculation under the framework of a decision tree model.

A decision tree is a model for classification or categorization used in sequential decision problems (Breiman *et al* 1984). In our study, we propose a decision tree-based model (DTBM) to combine linear and non-linear models, generating a hybrid scheme as suggested by Parkin *et al*. (2012) to properly match each curve shape with its corresponding model to estimate dC/dt. The objectives were to: (i) evaluate curve shape classification with DTBM and the dynamics of curve shapes in measured N_2O emissions; (ii) to evaluate dC/dt response to uncertainty when estimates are generated using the DTBM, LR, QR and H/M models with simulated data, and (iii) determine the effect of flux calculation method on yearly cumulative N_2O emissions and emission factor, using DTBM, LR, QR and H/M.

2.2. Material and methods

2.2.1 Model structure

The Decision Tree Based flux calculation Model (DTBM) is comprised of two parts: one procedure (henceforth procedure I) is used to classify data types and another procedure (henceforth procedure II) selects the model used to calculate dC/dt according to the data type classification (Fig 2.1). Our model uses N_2O samples which were collected at 4 sampling times: (e.g. 0, 12, 24 and 36 minutes after deployment).

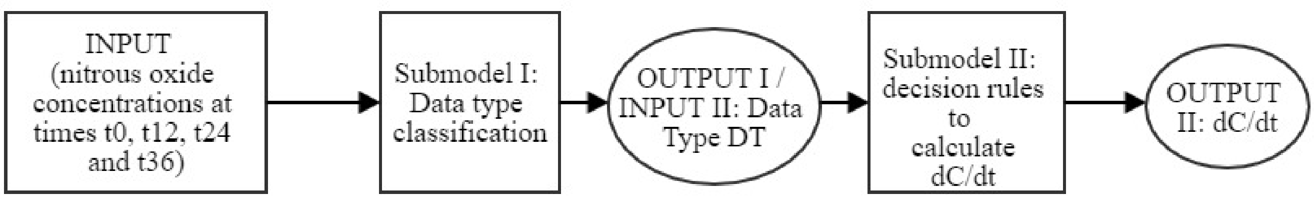

Figure 2.1: DTBM inputs, sub-models and outputs.

Our datatypes are an extension of the classification method described by Anthony *et al.* (1995), who determined 6 categories according to the curve shapes using 3 N_2O concentrations over 3 sampling times. We propose to use the DTBM to discriminate between 8 data types or curves shapes (Fig. 2.2).

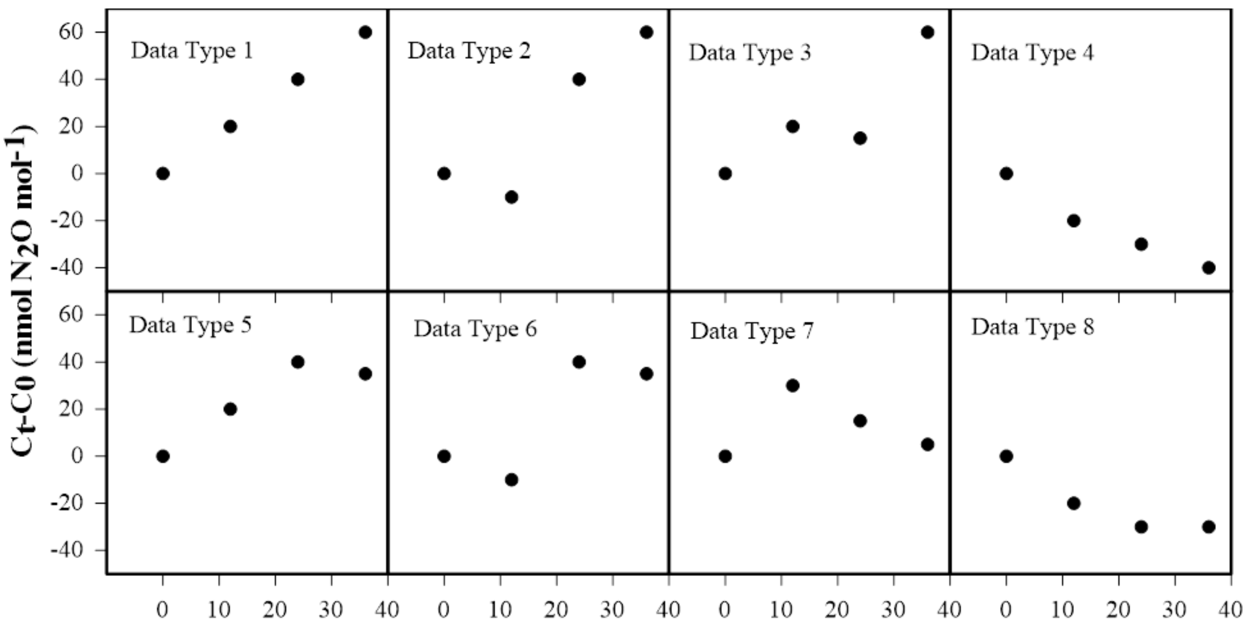

Figure 2.2: Data types classification obtained based on DTBM, expressed in difference of N_2O concentration between time t (C_t) and time zero (C_0).

The data types were determined and slopes were calculated as follows. The first decision tree considered data types 1 and 5 (DT1 and DT5) since both were easily fitted to either a linear or a quadratic regression. Each equation was calculated and the choice of using the slope of either regression was first based upon p-values and then, if both p-values were significant, the model with the greatest R^2 was selected. When both slopes for the two equations were not significant, then the N_2O flux was set to zero (Fig. 2.3). The DTBM was translated into logic formulas in Excel ® (2010) worksheets.

Data types 3 and 7 (DT3 and DT7) were considered for the second decision tree branch. The curvature index β ($\beta=(C_f-C_m)/(C_m-C_0)$), where C_0 is N_2O concentration in μmol N_2O mol $^{-1}$ at 0 min, C_m the average of N_2O concentration between 12 and 24 min and C_f the N_2O concentration at 36 min (f), (Venterea 2013), was then used as a parameter to decide which model to use: if $\beta > 0$, the data was assigned to the first decision tree and if $\beta<0$, then the flux was calculated through $F = (C_m\text{-}C_0)^2 / [18*(2*C_m\text{-}C_f\text{-}C_0)] * \ln [(C_m\text{-}C_0)/(C_f\text{-}C_m)]$ (Hutchinson and Mossier 1981) (Fig. 2.3). The reason for including H/M equation in the DTBM is that it allows us to decrease the uncertainty by averaging the two intermediate points, which can then address the situation when N_2O concentrations at 12 and 24 min do not conform to a consistently increasing pattern.

The third branch of the decision tree considers data types 2 and 6 (DT2 and DT6). This decision tree branch was similar to the previous one but in this case, if $\beta < 0$, the data was assigned to the first decision tree and if $\beta > 0$, then the N_2O flux was calculated with the Hutchinson and Mosier (1981) approach as previously described. Finally, the fourth decision tree branch accounts for data types 4 and 8, which depict situations with negative slopes. When such negatives slopes were significant, it was applied the same methodology as the first decision tree when the slopes were significant (Fig. 2.3).

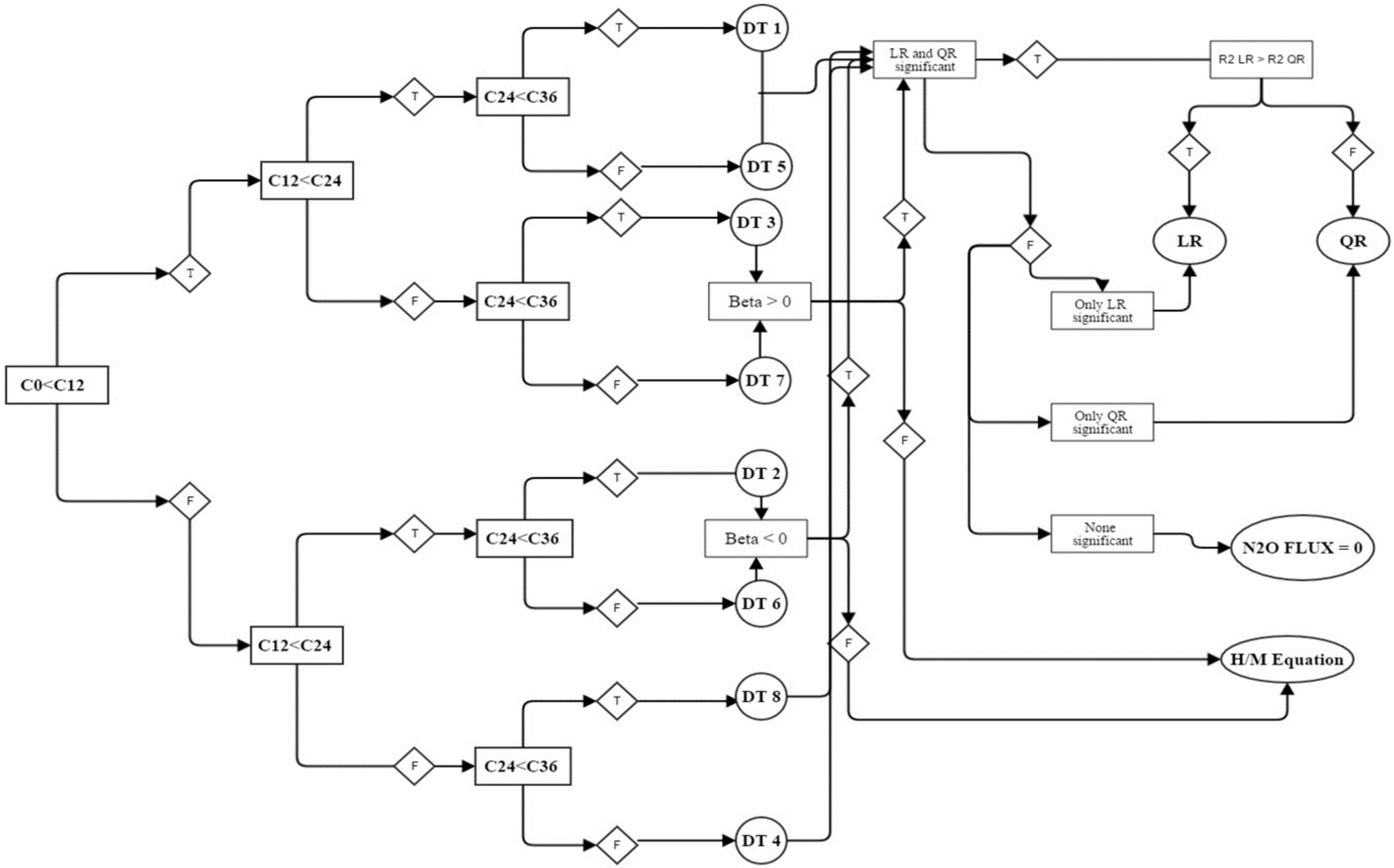

Figure 2.3: Model scheme. In boxes: conditions. C0, C12, C24 and C36: nitrous oxide concentrations at times 0, 12, 24 and 36 min, respectively. Diamonds: True or False statement. LR: linear regression. QR: quadratic regression. H/M: Hutchinson / Mossier equation.

2.2.2 Experimental background, data set and analysis

The NSS chambers used in our field study consisted of two units: a) the collar, which was an open cylinder made of PVC (44.2 cm inner diameter by 19 cm height), buried to 9 to 12 cm depth; b) the lid (8.3 cm high) was also made of the same material but it was covered with insulating material. The collars were removed prior to every field operation (manure application, corn planting), and then they were re-installed immediately after the operation was completed. Nitrous oxide samples were collected in 12 mL pre-evacuated sealed glass vials, and analyzed using a gas chromatograph (3800 GC, Varian, Mississauga, ON, Canada) fitted with a Combi-PAL auto-sampler as described by Drury *et al*. (2006). Further details of the chambers and the experiment can be found in Chapter 3.

We analyzed 4684 flux measurements which were collected over three years (2011-2014) at Elora, Ontario, Canada (43.85º N, -80.42º W) under a corn crop subjected to different manure and urea treatments (Chapter 3). There were 1248 flux measurements (set of 4 sub-samples) taken in 2012, 1772 measurements in 2013 and 1664 in 2014 (39, 34 and 34 sampling dates, respectively). We performed three analyses to evaluate DTBM performance. The first analysis consisted of running procedure I of DTBM with measured concentrations to evaluate data type proportions according to frequency of fluxes under high and low N_2O emissions events. In a second analysis, Monte Carlo simulations were applied to evaluate the performance of the model used (DTBM, LR, QR or H/M) according to the uncertainty of N_2O concentration measurements, following the methodology described in Parkin *et al.* (2012). A hypothetical case of positive flux was set, including the overall means of measured changes in N_2O concentrations at 12, 24 and 36 min ($\overline{C_{0-12}}$=39, $\overline{C_{0-24}}$=86, $\overline{C_{0-36}}$= 125 nmol N_2O mol^{-1}, respectively), using the data from 2012 (Fig. 2.4). Each N_2O concentration mean was used to create a normally-distributed population of n=10,000 data points per sampling time (40,000 data in total) as illustrated in Fig. 2.4. Data were generated in R-Studio (2015) using the script *rnorm* evaluating 4 levels of coefficient of variation (CV=5, 10, 20 and 40 %) as an indicator of uncertainty (the higher CV the higher the uncertainty). Each population was randomly sampled 1000 times per sampling time (4000 random samples in total) and data were used to obtain 1000 slopes (dC/dt). The slope calculations were performed with DTBM, LR, QR and H/M and statistical parameters such as mean, lower limit (5th percentile), upper limit (95th percentile); the significance of the slopes (dC/dt) was also tested.

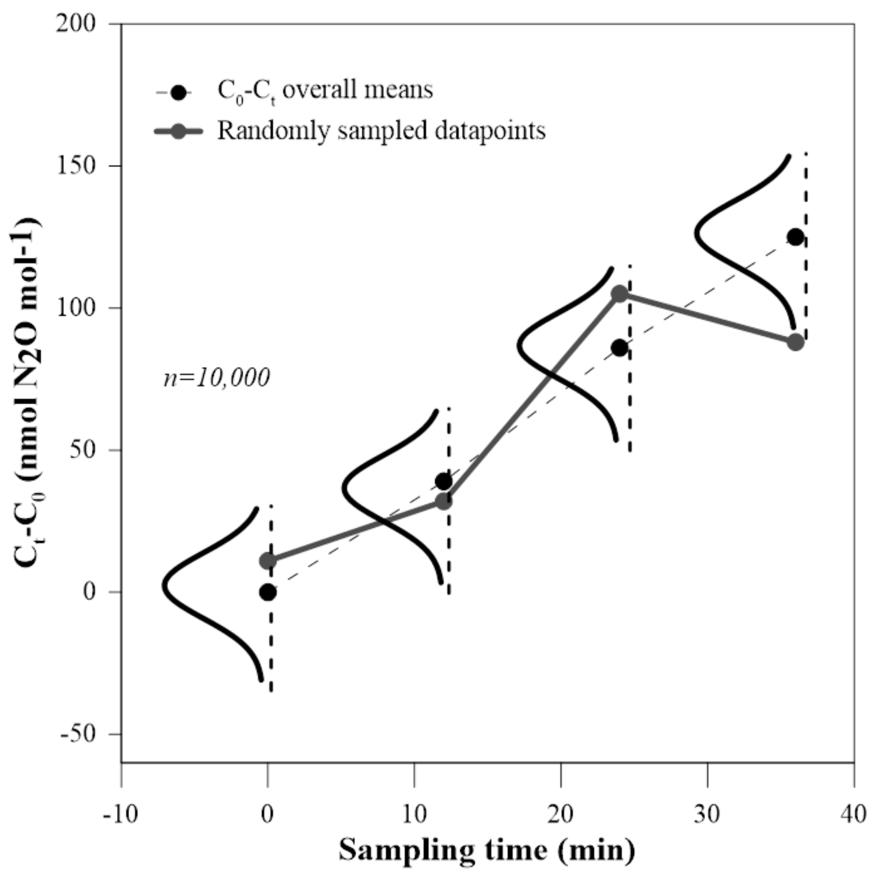

Figure 2.4**:** Illustration of the hypothetical situation created to evaluate the models, considering a normal distribution for each N_2O concentration mean, with a populational size of n=10,000

In the third analysis, part of the Elora dataset mentioned previously (2012 and 2013) was used to determine the effect of flux calculation method on yearly cumulative N_2O emissions and emission factor. Two treatments were considered: one with no N applied (check plots, 4 replicates) which should result in low N_2O emissions (low flux situation) and one with added urea (plots urea-applied, 4 replicates), which should result in comparatively higher N_2O emissions (high flux situation) and dC/dt was estimated with DTBM, QR, LR and H/M. Nitrous oxide flux was calculated as $F = (dC/dt) * (V/A)* [(p M) / (R T)] * k$, where V is the headspace volume inside the chamber (L), A is the surface area inside the collar (m^2), p is the barometric pressure (Pa), M is the molar mass (N_2O-N = 28 g mol^{-1}), R is the Universal Gas Constant (8.314 Joule mol^{-1} K^{-1}), T is the absolute temperature (K) and k is a conversion factor (14.4 g μg^{-1} m^2 ha^{-1} min d^{-1}) used to obtain the flux in g N_2O-N ha^{-1} d^{-1}. Linear interpolation between sampling dates was used to calculate daily N_2O emissions and yearly cumulative N_2O emissions were calculated as the summation of the daily emissions. Emission factor (EF) was calculated as N_2O emissions in urea-applied plots minus N_2O emissions in no N added plots as a percentage of the amount of fertilizer N applied (i.e. 150 kg N). The effect of the method of flux calculation on yearly cumulative N_2O emissions and on EF was tested with an ANOVA in each situation, using the model V= Y + M+ Y x M, where V is the response variable, either yearly cumulative N_2O emissions or EF, Y is year and M is flux calculation method. The least significant difference (LSD) test was used to compare means.

2.3 Results and Discussion

Overall, among all data types evaluated, datatypes 1 + 5 were consistently found to have the highest proportion of N_2O flux data over the 3 years (52-55 %, Table 2.1). The proportion of data types 1+5 was also coupled to the dynamics of daily N_2O emissions, since during peak events the proportion of DT1 + DT5 was > 70 % (Fig. 2.5). This is due to the generation of a strong N_2O concentration gradient between soil and chamber headspace during peak events, giving place to increasing N_2O concentrations (Markfoged *et al.* 2011) which follows a linear or quadratic pattern (i.e. LR or QR). The other data types were more abundant during winter as well as after July, where a pattern of overall decrease in DT1 + DT5 was found consistently across years (Fig. 2.5). The increase of data types other than DT1 + DT5 during low flux periods may be linked to periods in which soil acts as a sink of N_2O, with a net rate of N_2O consumption (Chapuis-Lardy *et al*. 2007). The condition conducive to soils acting as a sink may be masked in DT2 + DT6 or DT3 + DT7, but is clearly evident when DT4 + DT8 (negative slope) is present in high proportions. Overall, our results suggest that, for a four-points gas sampling, there is a close to 50 % of the N_2O concentration data which is considered to have a DT1 or DT5 pattern; however, this varied across the season with > 70% with this pattern during peak emission events and the and the occurrence of other data types increases (48-66% of measurements) in periods with low N_2O fluxes (after corn silking). Therefore, data type classification by DTBM is an alternative tool which can be used to accurately depict the temporal variability of the N_2O emission data.

Table 2.1:.Nitrous oxide accumulation patterns classified with DTBM. Number of cases in absolute and relative values for 2012, 2013 and 2014 experiments with manure treatment according to overall values, low emission events and high emission events.

Data types	2012		2013		2014	
	Observations†	Proportion (%)	Observations	Proportion (%)	Observations	Proportion (%)
Overall values						
DT1+DT5	682	54.6	961	54.2	926	52.3
DT2+DT6	230	18.4	364	20.5	326	18.4
DT3+DT7	220	17.6	331	18.7	317	17.9
DT4+DT8	116	9.3	116	6.5	95	5.4
Low emission events						
DT1+DT5	562	51.7	512	43.5	685	49.6
DT2+DT6	225	20.7	296	25.2	312	22.6
DT3+DT7	218	20.0	270	23.0	289	20.9
DT4+DT8	115	10.6	98	8.3	94	6.8
High emission events						
DT1+DT5	120	93.8	449	75.3	241	84.9
DT2+DT6	5	3.9	68	11.4	14	4.9
DT3+DT7	2	1.6	61	10.2	28	9.9
DT4+DT8	1	0.8	18	3.0	1	0.4

†: 1248, 1772 and 1664 observations for 2012, 2013 and 2014, respectively.

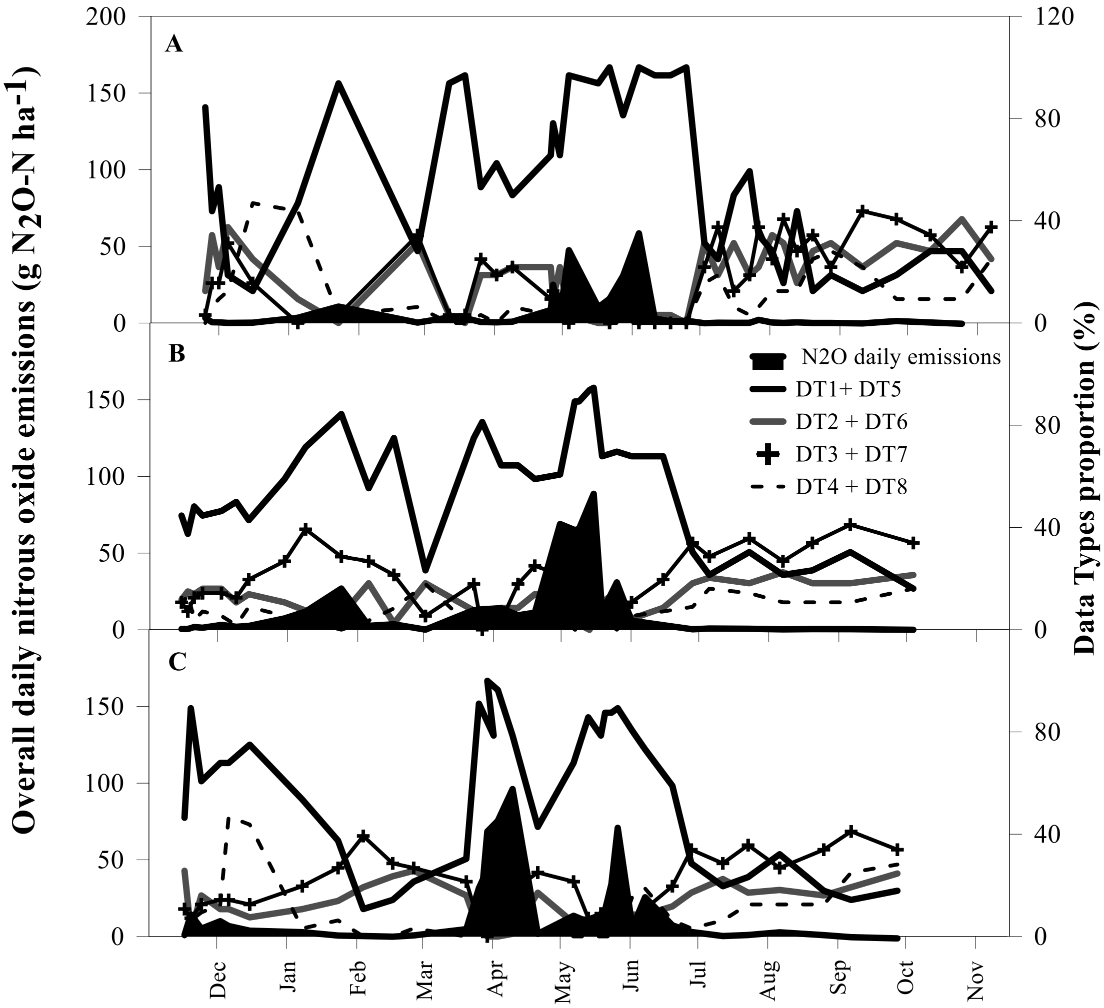

Figure 2.5: Overall average of daily N_2O emissions and data types proportion according to date and year. A: 2012. B: 2013. C: 2014.

The overall mean flux (dC/dt) for 2012 was 3.52 nmol N_2O mol^{-1} min^{-1} estimated with LR as well as with QR and using mean N_2O concentration values mentioned above. Monte Carlo simulations showed that with CV = 5 %, values of dC/dt obtained with DTBM, LR and H/M were closer to overall mean than that of QR; however, with CV = 40 %, values of dC/dt obtained with LR and QR decreased by 82 and 73 % respectively compared with the values at CV= 5% while the value obtained with DTBM decreased by only 36 % (Table 2.2). This is likely because the proportion of dC/dt = 0 with either LR or QR used alone was larger than that of LR and QR embedded in DTBM and it increased with larger CV (Table 2.2). The H/M model was also unstable since with CV=20 % it produced a mean value of dC/dt that was 36 % higher than that calculated at CV=5%, but with CV = 40% it produced a negative slope. Also, when CV=40%, the equation $(C_m-C_0)/(C_f-C_m)$, resulted in values smaller than 1 for 57% of the time, which prevented the slope to be estimated by using the H/M model. These results are in agreement with those of Parkin *et al.* (2012) and Levy *et al*. (2011), suggesting that a hybrid model such as DTBM can improve the estimation of dC/dt values through a better mean stability when precision in the measurements is affected.

Table 2.2: Monte Carlo simulations for dC/dt estimated according to models DTBM, LR, QR and H/M at five levels of CV. Mean, lower limit (5th percentile), upper limit (95th percentile) and failures ((Cm-C0)/(Cf-Cm))<1)) to estimate dC/dt.

Model	CV	dC/ dt				
		Mean	5th perc.	95th perc	Diff. from zero	Failures
	-----%------	---------------------------nmol N_2O-N mol^{-1} min^{-1}---------------------				
DTBM	5	3.25	0.00	6.05	922	N/A
	10	3.07	0.00	7.17	835	
	20	2.44	0.00	11.4	568	
	40	2.07	-4.77	16.5	456	
LR	5	3.13	0.00	4.31	885	N/A
	10	2.47	0.00	4.70	672	
	20	0.86	0.00	6.05	172	
	40	0.55	0.00	6.48	73	
QR	5	2.51	0.00	6.05	709	N/A
	10	2.00	-0.12	7.09	552	
	20	0.85	-2.14	9.16	239	
	40	0.68	-5.32	13.9	179	
H/M	5	3.76	1.53	6.79	998	2
	10	4.09	0.62	9.61	988	12
	20	5.14	0.03	15.5	666	334
	40	-8.38	-33.0	-0.11	434	566

The models' performance for calculating cumulative N_2O emissions varied according to the situation considered. For the low flux situation, a significant effect ($p < 0.05$) of flux calculation method was found on the yearly cumulative N_2O emissions. Our DTBM model provided a value of cumulative emissions slightly larger than that of LR and QR (521 ± 97 vs. 489 ± 87 and 276 ± 64 g N_2O-N ha^{-1}, respectively) (Fig. 2.6 A). The LR and QR models were expected to produce values of cumulative N_2O emission lower than DTBM, given their trend to underestimate fluxes (Livingston *et al*. 2006). Besides, the DT1+ DT5 pattern was present in 56 and 37% of the cases for 2012 and 2013, respectively (data not shown), so that applying LR or QR without classifying data produced cases with 'apparent zero flux', resulting in a decrease of cumulative N_2O emissions. Although DTBM and H/M produced similar values of cumulative N_2O emissions (521 ± 97 vs. 698 ± 111 g N_2O-N ha^{-1}, respectively) (Fig. 2.6 A), the values of mean flux estimated using DTBM were less sensitive to the increase of variability in the measurements of N_2O concentration compared to those of H/M, since DTBM-based mean fluxes went from 3.25 to 2.07 nmol N_2O-N mol^{-1} min^{-1} as CV increased from 5 to 40 %, while H/M values went from 3.76 to -8.78 nmol N_2O-N mol^{-1} min^{-1} (Table 2.2). Therefore, our results suggest that for low flux situations in corn, data classification included in DTBM may offer a more robust method to calculate cumulative N_2O emissions, given its greater stability for periods when data types 1 and 5 are less predominant.

Flux calculation method did not affect cumulative N_2O emissions for the high flux situation; additionally, the calculation method did not affect EF as well (Fig. 2.6 B). The relatively short chamber deployment time used in our study (36 min) likely promoted conditions of linearity during the high flux conditions. These conditions were mirrored in the proportion of data types 1 and 5 for high flux situation (57 and 54 % for 2012 and 2013, respectively). Duran and Kucharik (2013), compared N_2O fluxes estimated with LR and H/M collected from corn, switchgrass and hybrid poplar grown in a silt loam, and found that the LR method underestimated fluxes with a deployment time of 60 min. Conversely, Anthony *et al.* (1995) reached conditions of non-linearity at deployment times of 20 min and also obtained higher fluxes with H/M than with LR, although their measurements were performed on a growing pasture. Our results suggest that under a high flux situation in corn and with a chamber deployment time of 36 min, all methods tested were acceptable to calculate N_2O emissions, but that DTBM and H/M performed better under low flux conditions.

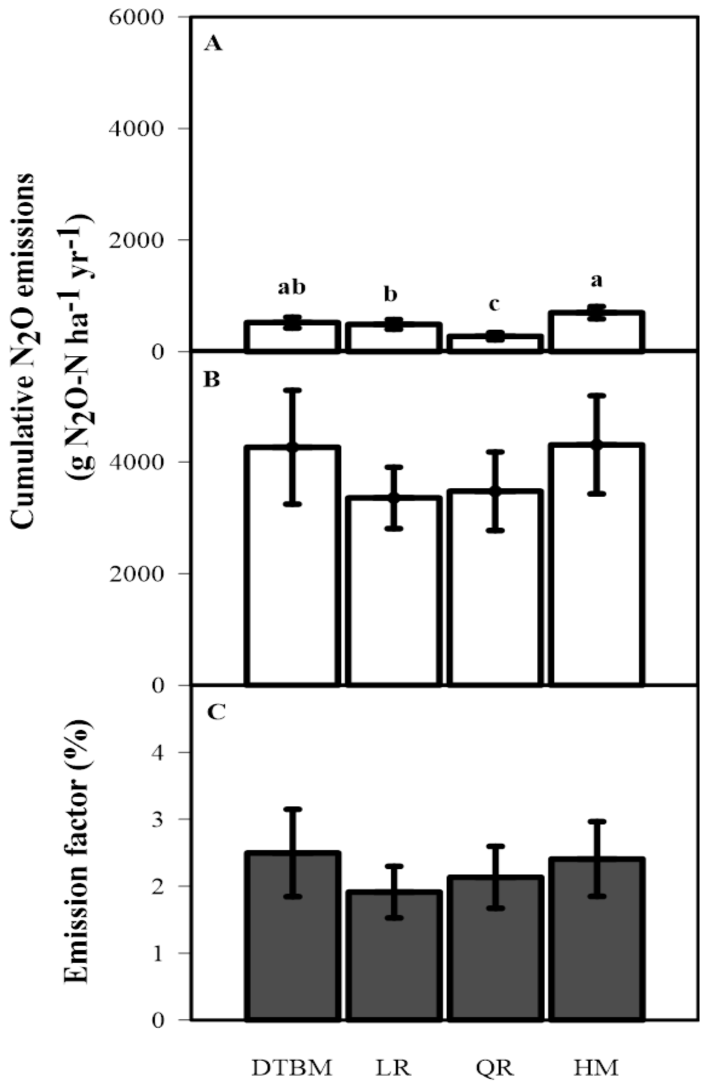

Figure 2.6: Cumulative N_2O emissions derived from fluxes estimated with DTBM, LR, QR and H/M. A: Control plots (no nitrogen added). B: Urea-applied plots. C: Emission factor

2.4 Conclusions

Summarizing, DTBM showed to be an alternative method to estimate dC/dt compared to LR, QR and H/M, due to its ability to match each data type with its best model. Among all data types, proportion of data types 1 and 5 was shown to be important during peak events and also consistent across years. Values of dC/dt estimated with DTBM were the least sensitive to the increase in measurement variability (increase of CV) and therefore more robust. Under low flux situations such as in the absence of N input, non linear data types may dominate, so that LR and QR are not recommended to calculate cumulative N_2O emissions. Under high flux situations, either method was acceptable to calculate cumulative N2O emissions; however, estimates obtained with DTBM were more robust in situations with high variability. Since our study considers only a deployment time of 36 min, further tests should be performed below and above this time to keep evaluating DTBM performance.

CHAPTER 3: YEAR-ROUND NITROUS OXIDE EMISSIONS AS AFFECTED BY TIMING AND METHOD OF DAIRY MANURE APPLICATION TO CORN

The following Chapter was accepted for publication by the Soil Science Society of America Journal on November 18, 2016. I am the primary author of the paper, with Dr. Claudia Wagner-Riddle, Dr. Craig Drury, Dr. John Lauzon and Dr. William Salas acting as supervisors, and appearing as coauthors on the journal article.

3.1 Introduction

Nitrous oxide (N_2O) is a greenhouse gas (GHG) whose concentration in the atmosphere has increased from 270 ppb in the late eighteenth century to 324 ppb in 2011 (Hartmann *et al*. 2013). This trend is of concern because N_2O has a high atmospheric warming potential (298 times greater than CO_2, considering a time horizon of 100 years) and also high ozone depletion potential (Ravishankara *et al.* 2009). Mitigating agricultural N_2O emissions is important since agricultural soils are responsible for 62% to 72% of the total anthropogenic emissions (Bouwman *et al*. 2010). Soil microorganisms use ammonium (NH_4^+) and nitrate (NO_3^-) as substrates to produce N_2O through processes such as nitrate ammonification, nitrification, denitrification or nitrifier denitrification (Saggar *et al*. 2004, Baggs and Philippot 2010, Venterea *et al*. 2012). The dominant process contributing to N_2O emissions varies according to soil temperature (Butterbach-Bahl *et al*. 2013), soil water content and aeration (Granli and Bøckman 1994, Robertson and Groffman 2007, Baggs and Philippot 2010), with denitrification as the dominant process in N_2O production if anaerobic conditions prevail. Soil may become anaerobic either through elevated O_2 consumption or through limited O_2 diffusion; the former process is stimulated by the abundance of carbon (C) while the latter by high soil water content (Petersen and Andersen 1996, Petersen *et al*. 1996, Petersen *et al*. 2008). Land application of organic fertilizer such as liquid dairy manure supplies the soil with C and water, as well as nitrogen (N), leading to suitable conditions for N_2O production through denitrification. However, it has been suggested that N_2O emissions induced by manure application can be controlled through manure management techniques such as adjusting the timing of manure application to match N supply with crop demand (Mosier *et al*. 1996), and by using application methods to place manure at a soil depth deep enough to avoid losses via ammonia (NH_3) volatilization (Meisinger and Jokela 2000) and shallow enough to mitigate N_2O emissions (Drury *et al.* 2006).

Corn represents a large proportion of feed on Canadian dairy farms (Statistics Canada 2011). Dairy manure is an inexpensive source of N and widely used in corn (Xue *et al*. 2014, Statistics Canada 2015). Around 40% of the total N present in dairy manure is NH_4^+ (OMAFRA 1994), which can be nitrified to NO_3^- and promote denitrification, depending on soil water and temperature conditions following manure application (Granli and Bøckman 1994, Robertson and Groffman 2007, Baggs and Philippot 2010). Nearly 30% of Ontario dairy farms apply manure during fall (Beaulieau 2004) due to practical reasons such as limited manure storage, time constraints in spring and /or field conditions.

Application prior to corn planting in spring is considered a best management practice to ensure N uptake by the crop (OMAFRA 1994, Meisinger and Jokela 2000), but few studies have been conducted comparing the effect of application timing on soil N_2O emissions in cold climates. Fall-applied manure can result in larger N_2O emission events during spring thaw (Wagner-Riddle and Thurtell 1998, Rochette *et al*. 2000, Kariyapperuma *et al.* 2012). Spring-applied manure can also increase N_2O emissions, particularly if a large rainfall event results in low O_2 levels coinciding with the high NO_3^- concentrations that follow manure application

(Wagner-Riddle *et al.* 1997, Saggar *et al.* 2004, Rochette *et al.* 2004). Direct comparison of N_2O emissions resulting from fall and spring application showed contrasting results. For example, Hernández-Ramírez *et al.* (2009) evaluated N_2O emissions induced by swine manure application and found lower N_2O emissions when manure was applied in fall compared to spring for corn in Indiana. Rochette *et al*. (2004) found that spring application produced larger cumulative N_2O emissions than fall application of pig slurry for a corn crop in Quebec. However, winter N_2O emissions were not measured in either study. In recent year-round studies performed in Ontario, there was no effect of dairy manure application timing on N_2O emissions from corn (Abalos *et al.* 2015; Schwager *et al.* 2016). Given these contradictory results, additional studies are needed to determine the effect of manure application timing on cumulative N_2O emissions from corn grown in cold climates especially considering the annual variability in precipitation.

The methods used to apply manure can also affect N_2O emissions, so that the interaction between application timing and method should be considered. There are a variety of methods and equipment used to apply manure to land, and they may be grouped into different categories, including (i) surface broadcasting, (ii) surface broadcasting followed by incorporation into soil, and (iii) injection (Meisinger and Jokela 2000). In Ontario, 56% of the farms applying liquid manure use injection (Statistics Canada 2011). Injection is considered to be a best management practice for avoiding ammonia volatilization losses (Dosch and Gutser 1996, Meisinger and Jokela 2000), but its effect on N_2O emission should also be taken into account. Significant increases in N_2O emissions from injection compared with other methods have been observed in laboratory studies (Flessa and Beese 2000, Velthof *et al*. 2003) as well as in the field (Dosch and Gutser 1996), probably due to restricted aeration at the injection site (Flessa and Beese 2000). Comfort *et al*. (1990) found that the largest N_2O emissions occurred shortly after injection followed by a shift to N_2 production, with the maximum gaseous-N losses occurring 5 days after injection. The interaction of timing and application methods on N_2O loss has not been investigated in cold climates where N_2O emissions due to spring thaw are significant. It is hypothesized that the interaction between timing and method of application affects N_2O emissions and that manure injection results in larger N_2O emissions compared to surface broadcasting or broadcasting and soil incorporation.

To test these hypotheses, a 3-year study was conducted to evaluate the effects of timing and method of dairy manure application on N_2O emissions for corn grown in Ontario, Canada, in a cold climate subjected to freeze/thaw-induced emissions. The main objectives of this study were to evaluate: (i) the effect of the interaction between manure application timing (fall vs. spring) and application method (injection vs. incorporation vs. broadcasting) on year-round cumulative N_2O emissions; (ii) the response of daily N_2O emissions to manure application method according to time of year, and (iii) the response of grain yield to timing and method of manure application.

3.2 Material and methods

3.2.1 Experimental Site and Set up

The study was conducted from 2011 to 2014 at the Elora Research Station, Elora, ON, Canada (43.85º N,-80.42º W). Each fall, a new field was chosen to establish the experimental plots and measurements were conducted from November to October. Soybean (*Glycine max*) was grown in 2011 prior to the establishment of the plots in the fall of 2011 and barley (*Hordeum vulgare*) was the preceding crop in 2012 and 2013. The historical average precipitation of the site is 874 mm and the historical average temperature 6.7 ºC (period 1980-

2010). The soils in each of the three fields used in this study belonged to the Guelph loam series, which is classified as Grey-Brown Luvisol according to the Canadian System of Soil Classification (Hoffman 1963, OMAFRA 1999) or as a Haplic Glossudalf under the US classification system (USDA-NCSS 2012). Soil was sampled for site characterization prior to experiment initialization and key attributes for each site-year are shown in Table 3.1.

Table 3.1.Soil total carbon, organic carbon, clay, sand and silt content, and pH for each experimental field used during the three study years in the top 15 cm of soil

Period	Total Carbon (%)	Organic Carbon (%)	Soil Clay (%)	Soil Sand (%)	Soil Silt (%)	Soil pH
Nov 2011-Oct 2012	2.52 ± 0.13	1.85 ± 0.17	18.5 ± 0.47	30.3 ±1.04	51.2 ± 0.66	7.67 ± 0.05
Nov 2012-Oct 2013	2.26 ± 0.16	1.25 ± 0.09	16.2 ± 0.33	34.7 ± 1.15	49.1 ± 0.90	7.81 ± 0.03
Nov 2013-Oct 2014	2.47 ± 0.10	2.14 ± 0.12	19.6 ± 0.34	28.3 ± 0.60	52.0 ± 0.66	7.81 ± 0.02

The experimental design consisted of a 2 by 3 factorial randomized complete block design with 4 replications. Factors studied were the timing of manure application (fall vs. spring) and three application methods (broadcasted, broadcasted and immediately incorporated, injected). Control plots receiving no manure were included in each replicate. Fall manure was applied on November 24, 2011, November 15, 2012 and November 13, 2013 whilst spring manure was applied on April 24, 2012, May 16, 2013 and May 26, 2014. Corn (*Zea mays*) was planted on May 10, 2012, May 17, 2013 and May 27, 2014. The earlier manure application in spring 2012 was related to the warm and dry conditions which prevailed before April. Dairy manure was applied with a research applicator unit with an 11.4 m^3 tank and four 75 cm spaced fan outlets. For surface broadcast (SB) treatment, manure was spread and left on the surface of the bare soil. For the surface broadcast followed by incorporation (SBI) treatment, manure was spread and then incorporated with a C-tined swept tooth cultivator to approximately 12 cm depth within 2 h after application. For the injected treatment (INJ), a vertical injection system was used, consisting of a traveling shoe and disk opener, which resulted in a 20 cm depth of injection with 75 cm space between injection lines. Throughout the experiments, the target manure application rate of 150 kg N ha^{-1} was based on the total nitrogen analysis of manure samples (TN, %) (performed by Agri-Food Laboratories Inc., Guelph, ON) taken from the dairy facility storage tank approximately 30 days prior to application. The application rate was determined by multiplying the volume of applied manure by TN. An average volume of 50.2 m^3 of manure was applied each time. Three manure samples for TN analysis were obtained during each field application to compare with TN of stored manure. Due to changes in TN during the 30 days prior to application and heterogeneous nature of manure, actual application rate varied from the target rate (Table3. 2); however, the average N rate across years and application times (163 ± 21 kg N ha^{-1}) was not different than the target rate. Nevertheless, given the response of N_2O to N rate (Halvorson *et al.* 2014, Burzaco *et al*. 2013), the manure N application rate was included as a covariate in the statistical analyses to avoid biases as explained below.

Table 3.2. Chemical properties of dairy manure at application time according to year and timing.

Timing	Year	DM† (%)	Total N (%)	NH_4^+-N (%)	N Rate (kg N ha^{-1})
Fall	2011	6.7 ± 0.05	0.29 ± 0.01	45.6 ± 5.90	116
	2012	7.5 ± 0.20	0.36 ± 0.03	37.2 ± 3.65	182
	2013	6.4 ± 0.10	0.38 ± 0.08	36.9 ± 8.77	209
Spring	2012	6.4 ± 1.00	0.31 ± 0.08	85.9 ± 21.1	160
	2013	4.0 ± 0.20	0.18 ± 0.01	42.9 ± 5.74	90
	2014	9.3 ± 0.20	0.44 ± 0.05	44.2 ± 5.90	218

†DM, dry matter.

The corn hybrids used were Pioneer 39D85 (2625 Corn Heat Units, CHU, glyphosate tolerant) in 2012, and Dekalb DKC39-97RIB (2700 CHU, glyphosate tolerant) in 2013 and 2014. The plots were tilled with a C-tined swept tooth cultivator to an approximate depth of 12 cm prior planting. Corn was planted at 80,000 seeds ha^{-1} with rows spacing of 0.75 m. Every year, phosphorus (triple super phosphate 0–46–0), potassium (potassium sulphate 0–0–50) and sulphur (potassium sulphate, 0-0-50-18) were applied at 80 kg P_2O_5 ha^{-1}, 110 kg K_2SO_4 ha^{-1} and 25 kg SO_4^- ha^{-1}, respectively, prior to planting. Weed control was performed using labelled rates of glyphosate post-emergence. Corn was harvested on Oct 25, 2012, Oct 27, 2013 and Nov 17, 2014.

3.2.2 N_2O Flux Measurements

Non steady state circular chambers were used to measure N_2O fluxes. Chambers were installed near the center of each plot before planting, removed during planting, and re-installed between corn rows after planting. Fluxes were measured weekly from November to December, twice per month from January to February, weekly from March to mid-May, biweekly from mid-May to July and weekly until harvest. Gas sampling was performed on 39, 34 and 34 events for the first, second and third year of study, respectively.

The chambers were designed following protocols suggested by Rochette and Bertrand (2008) and Parkin and Venterea (2010) as described in Snider *et al.* (2015). A 45.7 cm PVC pipe (IPEX Ring-Tite® SDR35, Marks Supply Inc.) was used to construct the chambers. Each chamber consisted of two units: a) a collar, which was an open cylinder (44.2 cm inner diameter by 19 cm height), buried to leave 6-10 cm aboveground; b) a lid (8.3 cm high), which was also made of the same PVC pipe where the top was covered using a circular disk made of a 1.27 cm thick PVC sheet, fixed into position using PVC cement. Lids were insulated on the outside with double-layered reflective bubble wrap. All the collars were inserted one day after every fall manure application, removed a day prior to spring manure application and re-inserted the following day until corn harvest. Sampling was resumed on the day of collar re-insertion. Each lid had a sampling port which was connected to an internal 4-port manifold, made of polypropylene union tees and four 15 cm long x 0.16 cm i.d. tubing (Chemfluor FEP, Cole-Parmer, Inc.), to have four sampling points within the headspace and ensure a representative sample (Venterea and Parkin 2012). A vent tube (0.48 cm i.d. X 10 cm long) was connected to the lid to compensate inner and outside air pressure (Xu *et al.* 2006). At every sampling date, lids were deployed for 36 min, and then removed after sampling. Syringes (20 ml polypropylene; Becton Dickinson, Rutherford, NJ) were used to extract a gas sample at 0, 12, 24 and 36 min after installing lids, and these were injected into 12 mL evacuated (-10 mbar), sealed vials (Labco, High Wycombe, UK). The concentration of N_2O in the vials was analyzed using a gas chromatograph (3800 GC, Varian, Mississauga, ON, Canada) fitted with a Combi-PAL auto-sampler as described by Drury *et al*. (2006) and Guo *et al*. (2013).

3.2.3 N_2O Flux Calculation

Nitrous oxide flux (F, g N_2O-N ha^{-1} d^{-1}) was calculated based on the change of N_2O concentration over time as shown in the following equation:

$$F = \frac{dC}{dt} * \frac{V}{A} * \frac{pM}{RT} * k$$

Where $\frac{dC}{dt}$ is the slope or change of concentration of N_2O over time (μg N_2O g air^{-1} min^{-1})

calculated by linear or quadratic adjustment as explained below, V is the headspace volume inside the chamber (m^3), A is the surface area inside the collar (m^2), p is the barometric pressure (Pa = J m^{-3}), M is the molar mass (N_2O-N = 28 g mol^{-1}), R is the Universal Gas Constant (8.314 J mol^{-1} K^{-1}), T is the absolute temperature (K) and k is a conversion factor (14.4 g μg^{-1} m^2 ha^{-1} min d^{-1}) to obtain the flux in g N_2O-N ha^{-1} d^{-1}.

The slope $\frac{dC}{dt}$ may be calculated by using a linear regression, or a non-linear approach using either a quadratic regression or the Hutchinson-Mosier equation (1981). The recommendations of Parkin *et al.* (2012) were followed and a hybrid method for slope calculation was developed which combining these three slope calculation methods with a decision tree system (Chapter 2).

3.2.4 Supporting environmental measurements

Daily mean air temperature, precipitation, barometric pressure and snow layer thickness were collected from the Elora Research Station weather station, located less than 500 m from the experimental plots *(https://www.uoguelph.ca/ses/service/weather-records)*.

Soil samples were collected prior to and after every manure application during the three years of study to analyze the dynamics of soil ammonium (NH_4^+-N) and soil nitrate (NO_3^--N) in the 0 - 15 cm layer. The soil sampling events ranged from 9 to 13 per year of study and occurred when the soil was not frozen (during November and from April to October). In the plots with broadcast treatments, the sampling consisted of taking 8 subsamples (0 - 15 cm depth) randomly within a 1 m boundary around each chamber. Soil sub-samples were thoroughly mixed in a bucket to obtain 1 sample per plot. For injected plots, 4 subsamples from the injection zone and 4 subsamples outside the injection zone were taken and thoroughly mixed. All samples were stored frozen at -18 ^{o}C until analysis. The samples were analyzed under a protocol based on Maynard *et al.* (2008) for N extraction: (i) field moist samples were sieved passing through a stainless steel 4.75 mm mesh sieve; (ii) after sieving, a subsample of 10 g soil was mixed with 50 mL of 2.0 M of KCl; (iii) after shaking for 30 min, the samples were filtered with Whatman no. 42 filter paper. The concentrations of NH_4^+-N and NO_3^--N in the extracts were determined using an auto-analyzer (AACE 6.07 software, SEAL Analytical Inc., Wisconsin) and expressed on a dry weight basis.

Soil bulk density (BD) was determined at the beginning of the experiment (November) and around harvest each year. Stainless steel cylinders with a known volume (4.72 cm i.d. x 5 cm height) were used to collect soil samples. The samples were dried at 105 ºC for 72 h, weighed and then BD determined on a dry-weight basis.

Soil volumetric water content was measured on 8 subsamples per plot during every gas sampling date with a portable time domain reflectometer (TDR; model TDR 300, Spectrum Technologies, Inc., Plainfield, IL, USA) for the 0-12 cm depth layer, except during the cold months, due to the presence of snow and frozen soil conditions. One copper-constantan thermocouple (built in-house) was installed at 5 cm depth close to each chamber and a digital thermocouple reader (Type J-K-T thermocouple, Model HH23, OMEGA) was deployed to obtain soil temperature values on every gas sampling date.

A datalogger (CR23X, Campbell Scientific Inc.) was installed in the boundary between Blocks 3 and 4, during the growing season, to record half-hourly values of soil temperature and water content from thermocouples and TDR probes, respectively. These additional TDRs (CS605-L, Campbell Scientific Inc.) and thermocouples were installed in 8 random plots and connected to the datalogger. Thermocouples were inserted at 5 cm depth (one per plot, 8 in total) and TDR probes inserted at an angle of 22º, to cover the 0-12 cm depth (one per plot, 8 in total). Water filled pore space (WFPS) was calculated for each plot using $WFPS = \frac{VWC}{\left(1-\frac{BD}{PD}\right)} * 100$, where VWC is the Volumetric Water Content (%) measured manually and automatically and PD is the particle density (2.65 g cm^{-3}, Lynn and Doran 1984).

3.2.5 Corn Grain Yield

Corn grain yield (GY) was determined at harvest each year. An area of 7.5 m^2 (2 rows 5 m long x 0.75 m) was marked between the 4th and 5th row of each 8-row wide plot. Cobs were hand-harvested and stover was hand-clipped and weighed in field, thus obtaining fresh weights. A sub-sample of 10 cobs and 7 plants per plot was weighed and dried at 60ºC until reaching constant weight. After obtaining grain dry weight, GY was determined by correcting the data obtained to 15.5% moisture. Hence, GY (kg ha^{-1}) was calculated as $GY = \frac{FW}{0.866} * \frac{DWs}{FWs} * \frac{c}{A}$, where FW is the fresh weight of sample (g), FWs is the fresh weight of subsample (g), DWs is the dry mass of grain (g), c is a conversion factor (10 kg g^{-1} m^2 ha^{-1}) and A is the harvested area (m^2).

3.2.6 Data and Statistical Analyses

Nitrous oxide flux calculations were performed using Excel ® (Microsoft 2010) and R-Studio (2015). It was assumed that N_2O flux measured between 1000 and 1400 h on a sampling date was representative of the average daily N_2O flux (Fassbinder *et al*. 2013). Emissions measured in the INJ plots were corrected to account for the placement of the chambers on top of the injection row. Extrapolating the value measured in the chamber to 1 m^2, would bias the estimation, since the area surrounding the chamber (0.54 m^2) has a lower level of emission compared with the 'hotspot' in the injection row (Markfoged *et al.* 2011). Hence, we used the average N_2O flux of all control plots as representative of the 0.54 m^2 surrounding the chamber, and estimated the flux for INJ plots as the sum of 54% of the control average and 46% of the N_2O flux measured in the INJ plots.

Nitrous oxide fluxes for days between sampling dates were calculated by linear interpolation (Dambreville *et al*. 2008), and annual cumulative N_2O emissions were calculated as the summation of daily estimates of N_2O fluxes over one year, from November to October of the following year. Two statistical analyses were performed: (i) a per-year basis ANOVA, and (ii) a combined ANOVA including all years, enabled due to homogeneous variances among years as confirmed with the Bartlett's test (homogeneity of variance). In the combined analysis, cumulative N_2O emissions, emission factor (EF; g N_2O per g^{-1} N applied expressed in %), grain yields and N_2O intensity (NOI; N_2O yield scaled emissions, g N_2O t^{-1} grain) were analyzed with an ANOVA in R-Studio (2015), using a sub-sub-plot model (Crawley 2015). In this model, the main-plot factor was the year, the sub-plot factor was the application time and the sub-sub-plot factor was the application method. Blocks were included as an additive factor and N rate was included as a covariate in order to decrease the residual sum of squares and the mean squared error. Emission factor was calculated by subtracting the average emission of control treatments across all blocks from emissions of manure-applied plots and dividing by the rate of N applied (Asgendom *et al*. 2014). All the analyzed variables were ln-transformed when non-normally distributed and a $p < 0.05$ significance level was used for the analysis of each variable.

To test the effect of manure application method on daily N_2O emissions depending on time of year, the year was divided into periods to correspond to different climatic conditions and field activities: Cold and Snow Season (CSS, from fall manure application date to March 31); Pre-Growing Season (PGS, from April 1 to planting date); Early Growing Season (EGS, from planting date to flowering date) and Late Growing Season (LGS, from flowering date to harvest). There was a short gap (~20 days) between harvest date and next year's fall application date, when no samplings were performed. For each period and annually, an average emission was calculated, in order to estimate the interaction between method and period for each year and timing combination. Analysis of interaction between period and method on N_2O emissions in each year and timing combination was performed with a multivariate analysis of variance (MANOVA) according to the methodology suggested by O'Brien and Kaiser (1985). The Henze - Zirkler's statistic was used to test multi-normality (package MVN, R Studio). The Pillai's statistic test was conducted to determine if N_2O emissions over each period as defined above presented an interaction with method. If the Pillai test was significant, orthogonal contrasts were used as a post-hoc test, comparing N_2O emissions among treatments. Daily emissions were ln-transformed when needed.

Ammonium and nitrate concentrations in soil were also analyzed with a MANOVA, to test the significance of the interaction between method and period, using the same methodology as mentioned previously. We also time-scaled these two variables to obtain intensities as measures of hypothetical NH_4^+ and NO_3^- accumulations in the soil over time (Burton *et al*. 2008). Soil ammonium intensity (SAI, g NH_4^+-N day kg soil^{-1}) and soil nitrate intensity (SNI, g NO_3^--N day kg soil^{-1}) were calculated by the daily summation of concentrations, obtained through linear interpolation, integrated between the beginning and the end of the experimental periods (Zebarth *et al.* 2008). Thus, the effect of the interaction between timing and method of application on SAI and SNI was also tested on an individual year basis ANOVA as well as on a pooled-year basis ANOVA, using the above mentioned model. The analyses were run using the *aov* (analysis of variance) function in R-Studio (2015), and the assumptions for the ANOVA were tested with Shapiro-Wilks test (normality of residuals) and Bartlett test (homogeneity of variance). A Least Significant Difference ($p < 0.05$) test was performed to compare means if any effect was found.

3.3 Results

3.3.1 Supporting environmental measurements

Fall 2011 (October-December) was wetter than 2012 and 2013 by 33 and 120 mm, respectively (Table 3). However, the growing season (May-October) in 2012 was drier (435 mm) than in 2013 and 2014 (786 mm and 561 mm, respectively). Accumulated precipitation between May and July was below the historical average by 131 mm during 2012, exceeded the historical average by 143 mm in 2013, and was similar to historical average in 2014 (Table 3.3).

Table 3.3. Monthly precipitation (mm; snowfall + rain) during the study period and 30-yr monthly average at Elora, Ontario.

Month	2011	2012	2013	2014	30-yr average†
January	48	56	83	51	63
February	58	32	75	58	48
March	86	31	41	47	58
April	101	30	124	102	72
May	113	28	143	54	82
June	87	64	123	69	88
July	32	30	131	134	84
August	159	63	74	52	84
September	76	106	177	178	77
October	129	143	139	74	77
November	91§	50	46	63¶	76
December	86	80	26	48	66
Annual Total	1066	714	1181	930	874

† Average calculated from monthly data obtained from Environment Canada for Elora Research Station.§ Beginning of the experimental period. ¶ End of the experimental period.

Snow layer thickness varied between years. In 2012, the average thickness of the snow layer covering the ground from November 24 to March 31 (CSS) was 6.4 cm while in 2013 and 2014 it was 12.9 cm and 31.9 cm, respectively, for the same period. Water-filled pore space also varied widely among years and among periods within a year; overall, the soil was drier in 2012 (WFPS<40 % in PGS) (Fig. 3.1 A) and wetter in both 2013 and 2014 (WFPS>40 % in PGS) (Fig. 3.1 B, C).

Air temperature varied among years, especially during the CSS period. The coldest CSS period was in 2014, with an average air temperature of -7.7 ºC vs -0.2 ºC for 2012 and -2.8 ºC for 2013, (Fig. 3.1 D, E, and F). The CSS period in 2014 also had the coldest soil, with an average soil temperature of -0.9 ºC at 5 cm (Fig 3.1 F).

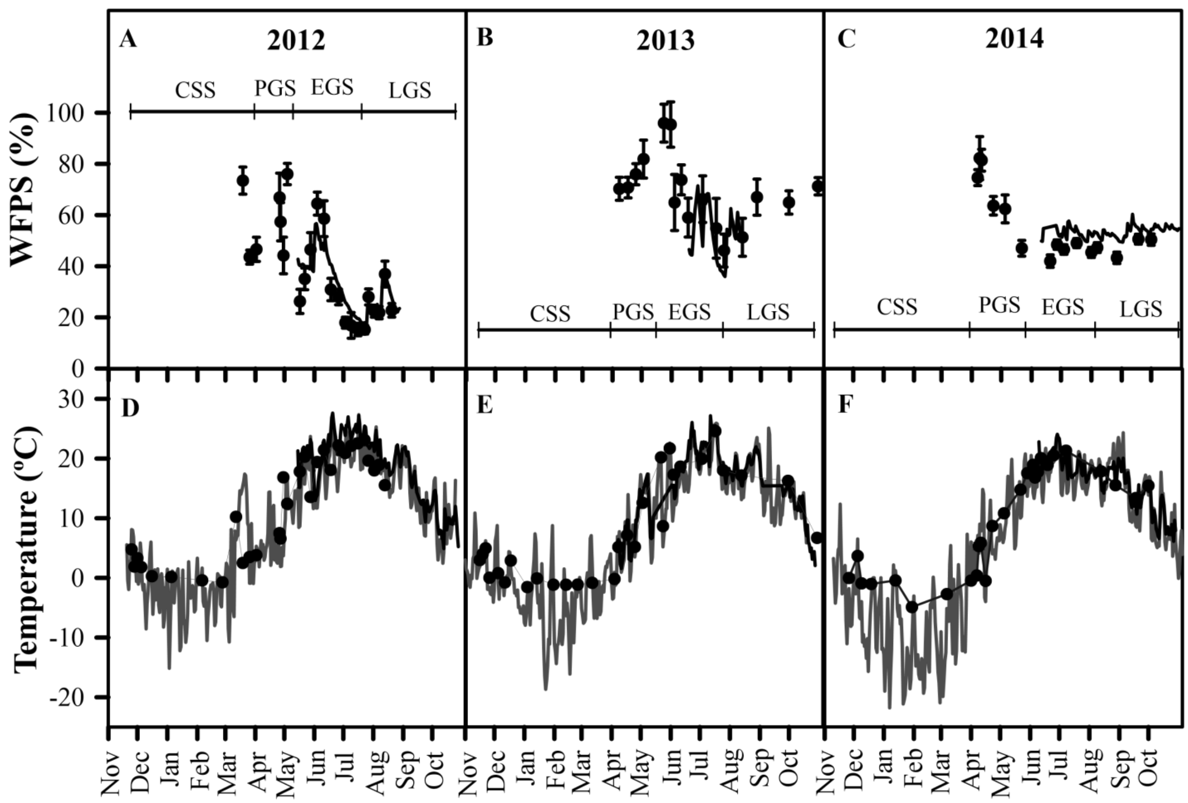

Figure 3.1. Levels of soil moisture (A, B, C) and air and soil temperature (D, E, F) during 2012, 2013 and 2014 measured either manually (●) or automatically (—). Air temperature is shown with grey lines. Bars indicate standard error of mean. Horizontal lines indicate periods used for data analysis: cold and snow season (CSS, mid-November to March 31, pre-growing season (PGS, April 1 to planting), early growing season (EGS, planting to flowering), late growing season (LGS, flowering to harvest).

3.3.2 Soil Ammonium and Soil Nitrate

Soil ammonium intensity was consistently affected by timing across years, with the highest values for fall-applied plots (3-yr average of 0.67 vs. 0.28 g NH_4^+-N d kg^{-1} soil for spring applied manure; Appendix: Table A1). Soil nitrate intensity was affected by year and method and the 3-way interaction between year and application time and application method (Appendix: Table A1), with the highest values for injected plots (average of 4.26 g NO_3^--N d kg^{-1} soil; Appendix: Table A1) and the lowest values for SB plots (1.76 g NO_3^--N d kg^{-1} soil; Appendix:

Table A1). When the data was analyzed on year-basis timing did affect SNI during 2013 and 2014, with the highest values for fall applied treatments compared to spring applied treatments.

Soil NH_4^+ reached higher concentration peaks in fall-applied (Fig. 3.2 A, B and C) than in spring-applied plots (Fig. 3.2 D-F) following manure application and this was consistent across years. In the case of soil NO_3^- concentration, the peak magnitude varied by period and by year (Fig. 3.2 G-L). Overall, NO_3^- concentration following fall manure application peaked twice (PGS and EGS) (Fig. 3.2 G-I) whereas there was only one peak in NO_3^- concentration after spring application (Fig. 3.2 J-L).

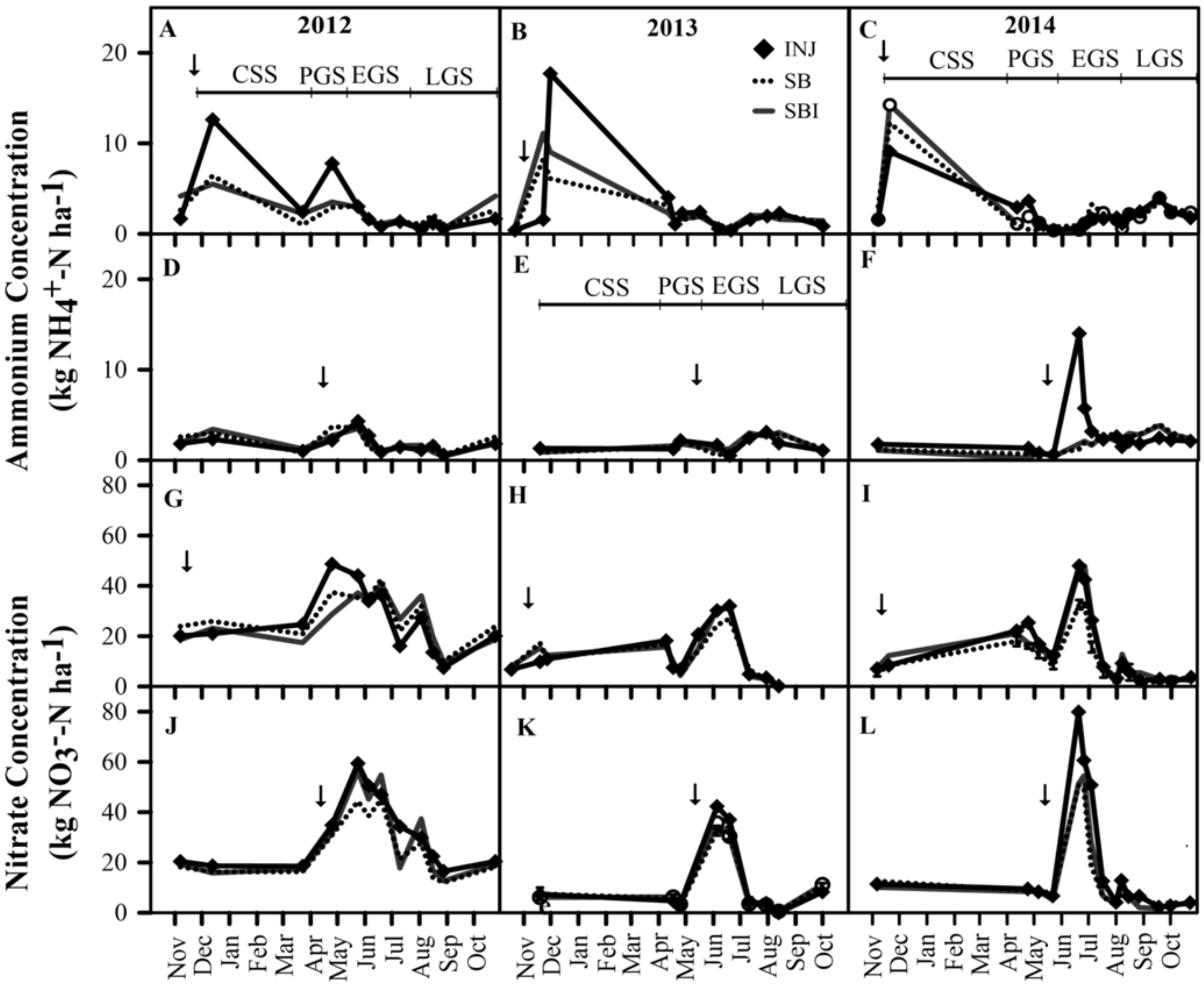

Figure 3.2. Mean soil ammonium and nitrate concentration in the 0-15 cm layer after fall (A, B, C, G,H and I) and spring applications of manure (D, E, F, J, K and L), during three years. Methods of manure application: INJ, injected; SB, surface broadcasted; SBI, incorporated.. Arrows indicate date of manure application. Periods as defined in Fig. 3.1

The method of application affected soil NH_4^+ and NO_3^- concentrations with some interaction with the time of year. Soil NH_4^+ concentration produced by fall-injected manure was significantly higher than that of control plots over all years during CSS (Appendix: Table A2) and for 2013 and 2014 during PGS. Fall-broadcasted and fall-incorporated plots had less consistent effects on NH_4^+ concentration with higher values than the control in 2013 and 2014 during CSS, and only in 2013 during PGS (Appendix: Table A2). During EGS and LGS, NH_4^+ concentration in plots receiving fall manure were not significantly ($p > 0.05$) different than the control. As for spring-applied plots, NH_4^+ concentration was not significantly increased above the control for any of the treatments or periods analyzed (data not shown). Soil NO_3^- concentration was consistently higher than the control for fall-injected plots during PGS in all years (Appendix: Table A2) and during CSS only in 2013. The two broadcast methods had less consistent effects on soil NO_3^- during both PGS and CSS (Appendix: Table A2). During the EGS, soil NO_3^- was higher than the control for all treatments in 2013 and 2014, with the highest values for INJ plots, while no effects were observed for the LGS (Appendix: Table A2). Nitrate concentration produced by spring-applied manure during EGS was higher than that of control plots consistently across years (data not shown). A similar effect was only observed for SB in 2013 and SBI in 2013 and 2014.

3.3.3 Nitrous Oxide Daily Emissions

Nitrous oxide emission peaks following fall application occurred during CSS (6 to 60 g N_2O-N ha^{-1} across years) and PGS (6 to 20 g N_2O-N ha^{-1} d^{-1} in 2012, 31 to 58 g N_2O-N ha^{-1} d^{-1} in 2013 and 72 to 270 g N_2O-N ha^{-1} d^{-1} in 2014) (Fig. 3.3 D-F). Compared to the control during CSS, fall application with SBI as well as SB had significantly higher average daily emissions during all years, and INJ only in 2012 (Table 3.4). Only fall-injected manure had higher average daily N_2O emissions than control plots during PGS 2012, whereas all fall-applied plots had higher average daily N_2O emissions than the control during PGS 2013, with the highest value for INJ, and no effect observed for PGS 2014 (Table 3.4). During EGS 2012 and 2014, all fall-applied methods produced low emissions similar to those of control plots; however, in EGS 2013, all treated plots produced greater emissions than those of control plots, with the highest value for INJ (Table 3.4). Daily N_2O emission was similar to the control during the LGS period for all fall-applied (Table 3.4) and spring applied plots (data not shown). In spring-applied plots, the greatest N_2O peaks were observed mainly during PGS (38 to 150 g N_2O-N ha^{-1} d^{-1} in 2012, 9 to 27 g N_2O-N ha^{-1} d^{-1} in 2013 and 30 to 90 g N_2O-N ha^{-1} d^{-1} in 2014) and EGS (65 to 278 g N_2O-N ha^{-1} d^{-1} across all years) (Fig. 3.3 G-I). Compared to the control, spring-applied treatments only had significant increases in N_2O emissions during the PGS period for SB plots in one year (2013) and during EGS for INJ in 2 years (2012 and 2014) (data not shown).

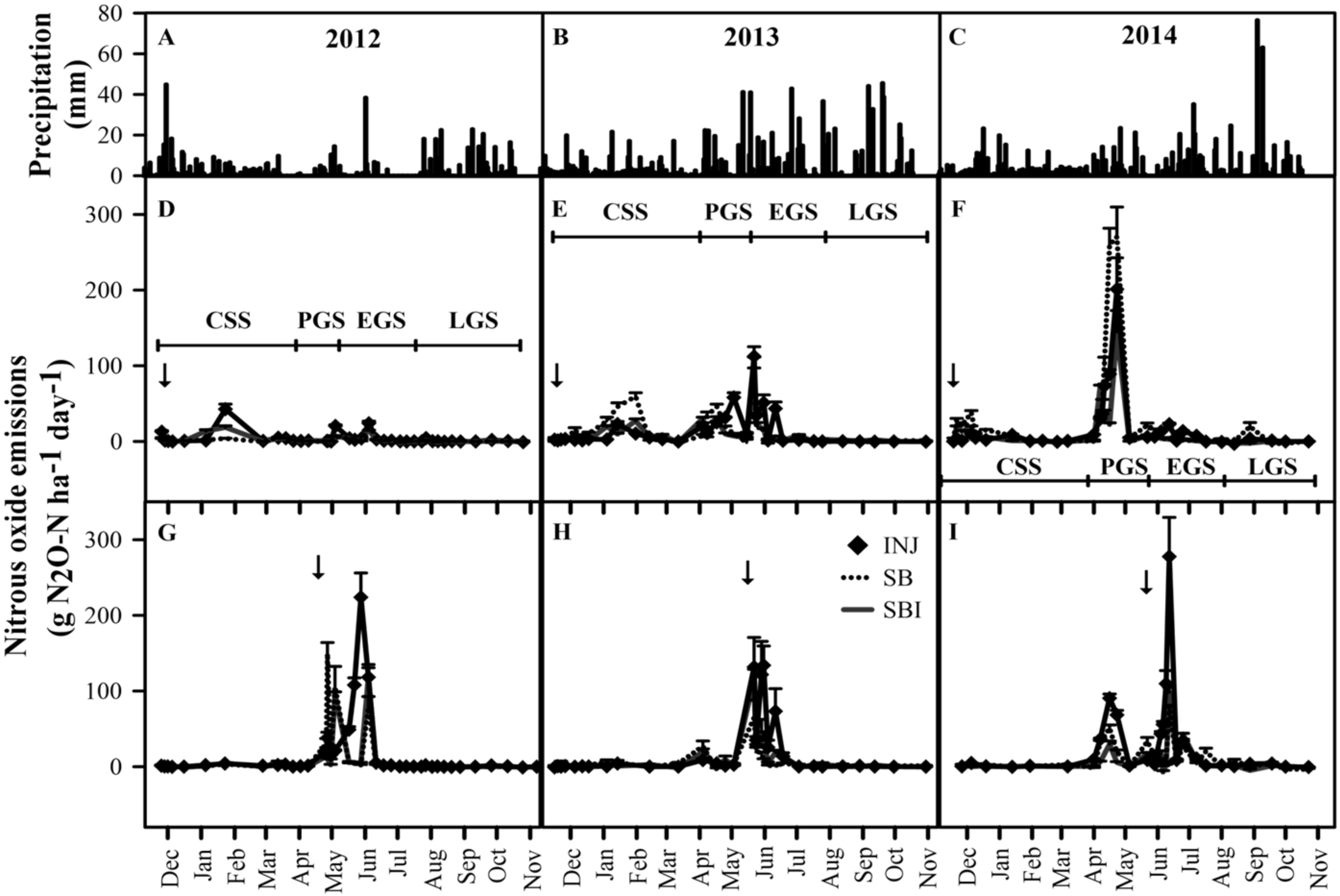

Figure 3.3. Precipitation (A, B, C) and dynamics of nitrous oxide emissions for fall application (D, E, F) and spring application of manure (G, H, I) for three methods of application (INJ, injected; SB, surface broadcasted; SBI, incorporated) over three consecutive years. Horizontal scales indicate periods considered for study: cold and snow season (CSS), and pre- (PGS), early (EGS) and late (LGS) growing season. Arrows indicate date of manure application.

Table 3.4. Average daily N_2O emissions after fall application for specific time periods (CSS, Cold and Snow season; PGS, Pre-Growing Season; EGS, Early Growing season; LGS, Late Growing Season) in each year depending on method of manure application (INJ, manure injection; SB, manure broadcasting; SBI, manure incorporation). Mean comparison among treatments by orthogonal contrasts for each period, according to Year. Data were ln-transformed for the statistical analysis when non normal distributed.

Year	Method	Average daily N_2O emissions			
		CSS (Nov to Mar)	PGS (Apr to mid-May)	EGS (mid-May to late Jul)	LGS (late Jul to late Oct)
		-------------------------g N_2O-N ha^{-1} day^{-1}	--------------------------		
2012	Control	1.34 b[†]	0.84 b	2.05	0.43
	INJ	6.37 a	3.93 a	3.93	0.61
	SB	2.22 a	1.10 b	3.64	0.21
	SBI	4.43 a	1.47 b	2.85	0.21
2013	Control	1.97 c	2.66 d	7.5 c	0.06
	INJ	5.02 c	22.3 a	30.7 a	0.04
	SB	14.4 a	16.4 b	19.2 b	0.08
	SBI	7.58 b	12.5 c	16.6 b	1.44
2014	Control	1.68 b	28.0	8.58	0.89
	INJ	2.90 b	56.2	9.19	0.18
	SB	11.6 a	91.6	5.34	2.84
	SBI	5.77 a	40.4	3.23	-0.63

[†]Different letters among methods within a year and period indicate difference at $p < 0.05$.

3.3.4 Cumulative N_2O Emission and Emission Factor

Cumulative N_2O emissions were affected by year (Table 3.5), attaining the highest value during 2014 (3.77 kg N_2O-N ha^{-1}) and the lowest value during 2012 (2.05 kg N_2O-N ha^{-1}) (Appendix: Table A3). A significant interaction between year and timing of manure application was observed (Table 3.5), with spring manure application resulting in higher cumulative emissions than fall application in 2012 (2.90 vs. 1.19 kg N_2O-N ha^{-1}) but not in the other years (Fig. 3.4 A). The effect of method on N_2O emissions was found to be significant in 2012 and 2014, and across years (Table 3.5), with INJ resulting in cumulative emissions that were significantly higher than SBI, but not different than SB (Fig. 3.4 B).

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Table 3.5. p-values for cumulative N_2O emissions, emission factor, grain yield and N_2O intensity as affected by timing and method of manure application during 2012, 2013, 2014 and pooled for the three years. Data were ln-transformed for the analysis when non-normal distributed.

Source of variation	Cumulative N_2O emissions				Emission Factor				Grain Yield				N_2O intensity			
	2012	2013	2014	Pooled years	2012	2013	2014	Pooled years	2012	2013	2014	Pooled years	2012	2013	2014	Pooled years
Year	-	-	-	<0.01	-	-	-	<0.01	-	-	-	0.04	-	-	-	<0.01
Timing	<0.01	0.10	0.31	0.16	<0.01	0.34	0.93	0.15	0.05	0.06	0.02	0.12	<0.01	0.52	0.11	0.28
Method	<0.01	0.80	0.02	0.02	<0.01	0.64	0.30	0.10	0.02	0.23	<0.01	<0.01	0.02	0.75	0.12	0.09
Timing X Method	0.25	0.42	0.11	0.25	0.46	0.52	0.15	0.14	0.43	0.59	0.71	0.70	0.66	0.39	0.38	0.27
Year X Timing	-	-	-	<0.01	-	-	-	<0.01	-	-	-	0.32	-	-	-	<0.01
Year X Method	-	-	-	0.05	-	-	-	0.34	-	-	-	0.02	-	-	-	<0.01
Year X Timing X Method	-	-	-	0.20	-	-	-	0.29	-	-	-	0.68	-	-	-	0.19

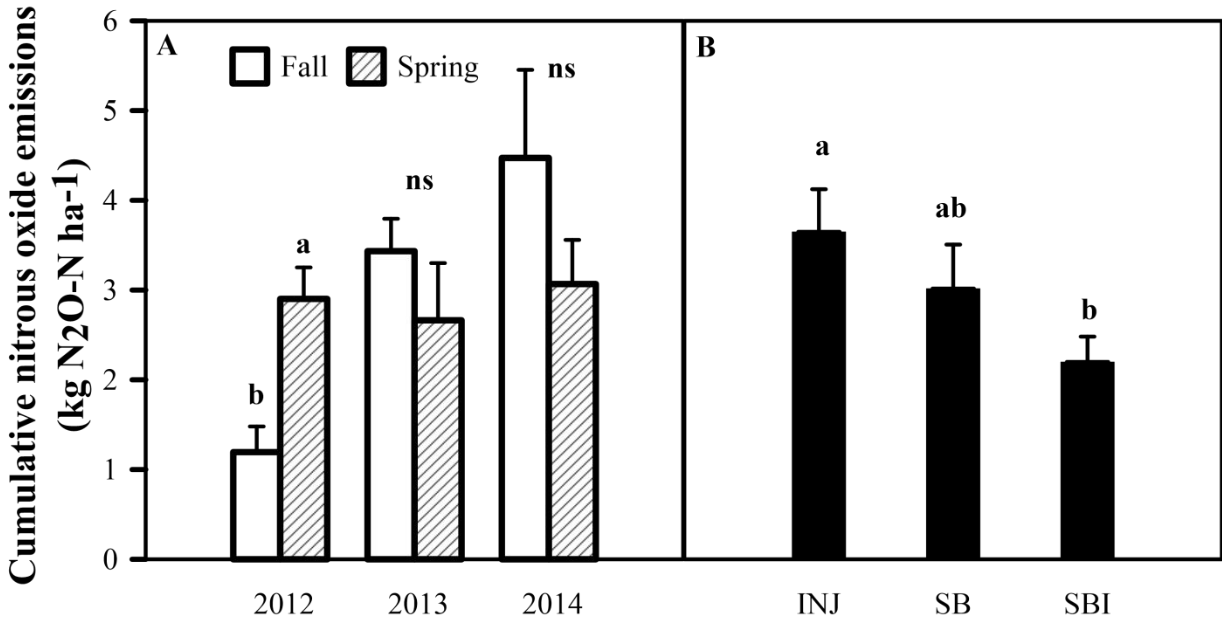

Figure 3.4. Cumulative mean annual N_2O emissions as influenced by: A. Year x timing interaction and B. Method of manure application. A: Bars with different lowercase letter indicate significant differences (LSD, p<0.05) between timings within year. ns indicates no significant effect of timing within year. B: bars with the same lower-case letter indicate no significant difference for method of manure application (LSD, p<0.05). Methods of manure application: INJ, injected; SB, surface broadcasted; SBI, incorporated. Error bars indicate standard error.

Emission factors were also affected by year with the highest value for 2013 at 1.92% (Fig. 3.5 B). The interaction between year and timing was significant (Table 3.5) with spring-applied plots having higher EF than fall-applied plots in 2012, but no differences were found in the other years (Fig. 3.5 A). Method of application only affected EF in 2012 (Table 3.5) when injected plots had an EF of 1.76%, which was significantly higher than SB (0.66%) or SBI (0.85%) (Appendix: Table A3).

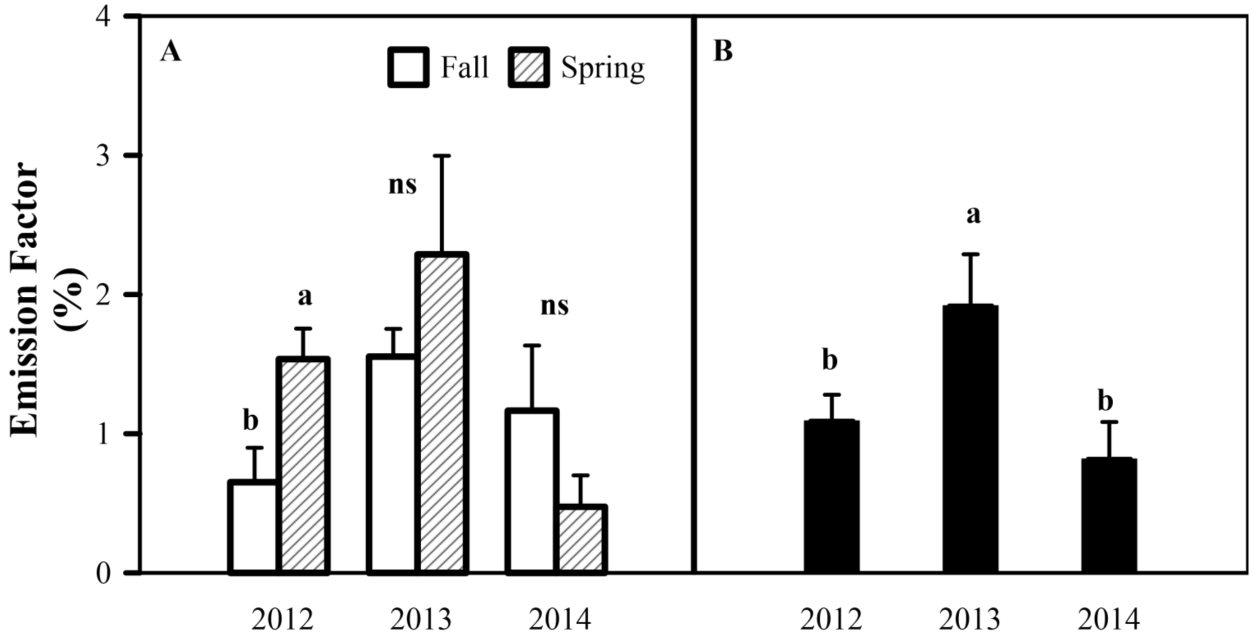

Figure 3.5. Emission factor as influenced by: A. Year x timing interaction and B. Year. A: Bars with different lowercase letter indicate significant differences (LSD, $p<0.05$) between timings within year. ns indicates no effect of timing within year. B: bars with the same lowercase letter indicate no significant difference between years (LSD, $p<0.05$). Error bars indicate standard error.

3.3.5 Grain Yield and Nitrous Oxide Intensity

Year had a significant effect on yield with 9.8 > 8.3 = 8.2 t ha^{-1} observed for 2012, 2013 and 2014, respectively. Corn grain yields were affected by method and year by method interaction when pooled years were analyzed (Table 3.5), with higher yields for INJ vs. SB or SBI plots during 2012 and lower yields for SB vs. INJ or SBI in 2014 (Fig. 3.6 A). Overall, the injection treatment had significantly greater yields than SB or SBI (Fig. 3.6 B). Timing affected yield only in 2014, with the highest values for spring-applied plots (8.7 t ha^{-1} for spring vs 7.7 t ha^{-1} for fall, data not shown).

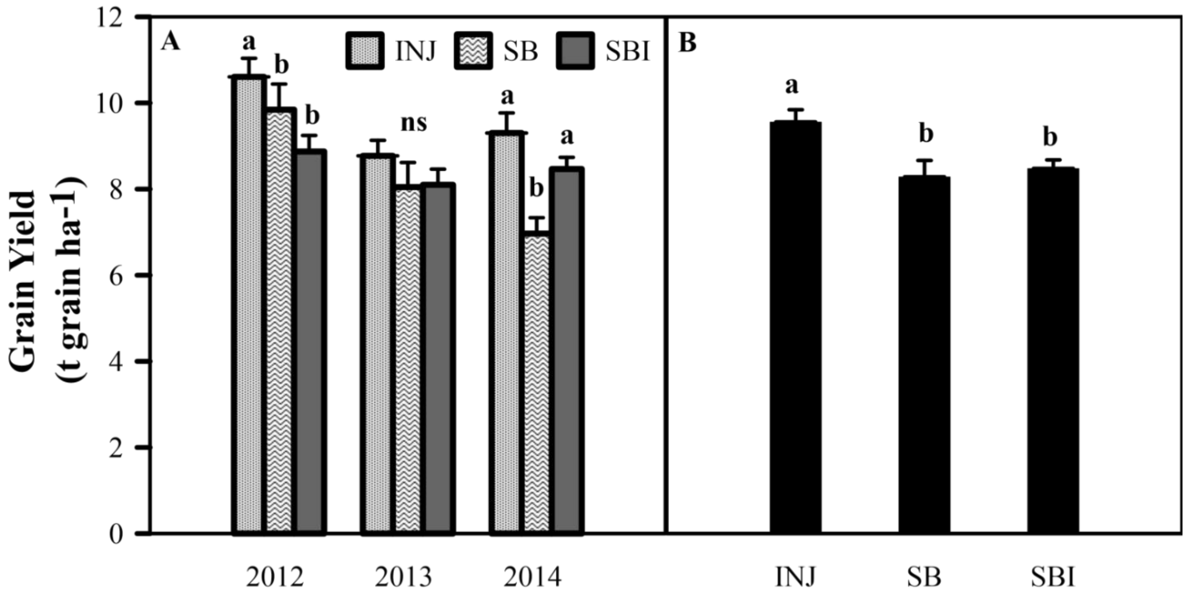

Figure 3.6. Grain yield as influenced by: A. Year x method interaction and B. Method. A: Bars with different lowercase letter indicate significant differences (LSD, $p<0.05$) between methods within year. ns indicates no significant effect of method within year. B: bars with the same lowercase letter indicate no significant difference between methods (LSD, $p<0.05$).Methods of manure application: INJ, injected; SB, surface broadcasted; SBI, incorporated. Error bars indicate standard error.

Nitrous oxide intensity was significantly affected by year and there were significant interactions of year by timing and year by method (Table 3.5). For the three years, NOI was 203<367 = 440 g N_2O-N t grain^{-1} for 2012, 2013 and 2014, respectively (data not shown). Both timing and method of manure application affected NOI only during 2012, with the greatest value for spring compared to fall (Fig. 3.7 A) and INJ compared to SB and SBI methods (Fig. 3.7 B).

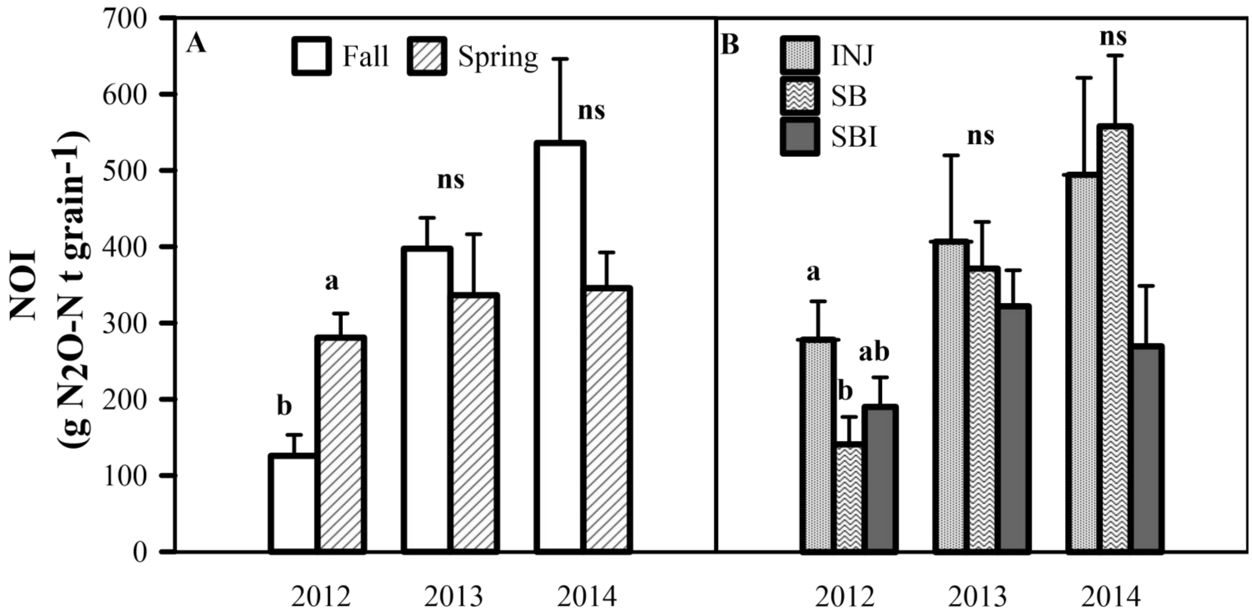

Figure 3.7. Nitrous oxide intensity as influenced by: A. Year x timing interaction and B. Year X method interaction. A: Bars with different lowercase letter indicate significant differences (LSD, $p<0.05$) between timings within year. ns indicates no significant effect of timing or method within year. B: bars with the same lowercase letter indicate no significant difference between methods within year (LSD, $p<0.05$). Methods of manure application: INJ, injected; SB, surface broadcasted; SBI, incorporated. Error bars indicate standard

3.4 Discussion

3.4.1 Timing of manure application

Nitrous oxide emissions did not respond to timing of manure application and no interaction between timing and application method was observed, but a year x timing interaction was found. Although differences in soil characteristics and previous crop between years (Table 3.1), could have contributed to contrasting N_2O emissions between years, it is improbable that these differences caused the observed year x timing interaction since both application timings would have been affected in a similar way each year. Inter-annual variability of fall vs. spring weather conditions likely caused the observed interaction. A significant effect of timing was observed in 2012, a year with particularly dry CSS and PGS, when fall-applied manure produced lower cumulative N_2O emissions, EF and NOI than spring-applied manure (Figs. 3.4, 3.5 and 3.7 respectively). This was probably due to the warm winter reducing freeze-thaw cycles and low soil water content conditions during PGS in 2012 (Fig 3.1 A) decreasing the emission event for fall application despite higher SAI values in fall compared to the spring application treatment (Appendix: Table A1). Contrary to fall application, when manure is applied in spring, manure NH_4^+ can be rapidly nitrified due to warmer soil conditions leading to lower SAI. The lack of conditions conducive to denitrification due to dry soil conditions during 2012 was also evident in the low cumulative N_2O emissions of control plots, compared to 2013 and 2014 (Appendix: Table A3). These results agree with Rochette *et al*. (2004) who found that cumulative N_2O emissions after a fall application of incorporated pig manure were low compared to spring application due to dry soil conditions in early spring reducing emissions for the fall-applied treatment. Similarly, Hernandez-Ramirez *et al*. (2009) found larger cumulative N_2O emissions in corn receiving manure in spring than in fall-manured plots (8.17 vs 3.29 kg N_2O-N ha^{-1}, respectively). Both studies did not measure N_2O fluxes during the winter months, so emission estimates for the fall-applied treatment could have been underestimated. Indeed, our emission estimates for 2012 would have been 58% lower if winter emissions after fall application had not been considered, despite the relatively unfavourable conditions for N_2O production for this year. In contrast, the spring treatment would have been underestimated by only 9%.

The dry conditions in 2012 were unusual with a probability of occurrence of ~10 % (1 year every 10 years, Environment Canada 2016). In contrast to 2012, soil water content was conducive for denitrification during 2013 and 2014 (Fig. 3.1 B, C), which was evident in the higher values of cumulative N_2O emissions for control plots compared to 2012 (Appendix: Table

A3). Under these wetter conditions, cumulative N_2O emissions induced by the treatments were expected to be higher for fall application than for spring application because applying manure in the fall leads to a longer gap between N input and N utilization by the crop, compared to spring application. Soil ammonium intensity and SNI behaved consistently with our hypothesis in 2013 and 2014, with fall application having higher values than spring application (Appendix:Table A1). Contrary to our hypothesis, cumulative N_2O emissions and NOI were similar between application timings (Figs. 3.4 and 3.7), suggesting that the relationship between cumulative N_2O emissions and SAI or SNI was weak. This partially contradicts the results of Asgendom *et al.* (2014), who found a strong correlation between SNI and cumulative N_2O emissions in rapeseed and spring wheat fertilized with both manure and urea, but is consistent with the results of Maharjan and Venterea (2011) who found that cumulative N_2O emissions were not related to SAI or SNI in corn. In our study, the N_2O vs. SNI or SAI relationship in fall-applied plots may have been weakened by processes competing for NO_3^- and NH_4^+ such as re-immobilization due to low temperatures (Jensen *et al*. 2000, Clark *et al*. 2009) or by complete denitrification and leaching (Granli and Bøckman 1994, Jayasundara *et al*. 2010, Butterbach-Bahl *et al*. 2013), which could have occurred during the winter. Indeed, in a similar climate to our study site, Schwager *et al*. (2016) observed higher non-growing season N leaching losses from fall compared to spring applications and no differences in N_2O emissions between timing of application. In agreement with Schwager *et al*. (2016), the proportion of annual N_2O emissions occurring during CSS was larger following fall application (20 - 58%) compared to spring application (9 - 19%). However, the pattern was inversed during the EGS resulting in no difference between timing treatments. Our study emphasizes the importance of considering non-growing season emissions, particularly for treatment that may result in enhanced emissions during CSS and PGS.

Although differences in grain yield were observed between years, likely due to a combination of weather, soil and previous crop effects, grain yield was not affected by timing of manure application. Significant N losses over winter have been suggested to result in larger grain yields with spring application of liquid dairy manure compared to fall application (Zebarth *et al*. 1997). Contribution of N from soil organic matter through mineralization during early summer could have counteracted the potential N losses over winter for plots receiving the fall application treatment (Jensen *et al.* 2000).

3.4.2 Method of manure application

Manure injection resulted in the highest corn grain yields (Fig. 6B) as also observed by Klausner and Guest (1981). A possible explanation for this is that injection method led to lower NH_3 volatilization compared to surface broadcasted manure, whose losses usually range from 24 to 33% of N applied (Beauchamp *et al.* 1982). Additional measurements taken in our plots in a separate study for 20 days after manure application (not shown) indicated that INJ registered the smallest NH_3 loss (~12 kg N ha^{-1} day^{-1}) compared to SB plots (~27 kg N ha^{-1}). Avoiding this N loss with manure injection should have produced higher quantities of inorganic N (SAI and SNI) for both N_2O production and plant growth. Indeed, we did observe higher SNI for manure injection compared to SB and SBI treatments, however, SAI was not significantly different among methods (Appendix; Table A1). The absence of method effect on SAI could be associated with an initial NH_4^+ immobilisation period after injection as suggested by Comfort *et al*. (1988, 1990) under field and lab conditions.

We expected that N_2O emissions from INJ would have been higher than SB plots given the decreased N substrate availability induced by higher NH_3 losses associated with SB (Dosch and Gutser 1996). However, cumulative N_2O emissions were not different between these two treatments (Fig. 3.4 B). Considering our study included fall manure application likely explains this result as cool and wet conditions would have minimized NH_3 emissions causing less difference between substrate availability in INJ and SB treatments. Even if N_2O production was higher for INJ plots, the relatively wet conditions in fall could have resulted in slow N_2O diffusion allowing part of N_2O to be converted to N_2 (Markfoged *et al*. 2011), in contrast to spring studies where the produced N_2O would have been emitted more easily from INJ plots (Dosch and Gutser 1996). We did observe a delay of 18 ± 12 days in the date of the first N_2O emission peak event for fall-injected plots compared to SB plots (Fig. 3.3). In addition, fall-SB plots had high cumulative N_2O emissions (Appendix: Table A3), particularly during PGS in 2014, when the soil thaw created conditions conducive to denitrification at the surface (Granli and Bøckman 1994, Butterbach-Bahl *et al*. 2013) (Fig 3.3 F); consequently, year-round cumulative N_2O emissions for SB were not different from INJ.

Surface broadcasted manure followed by incorporation resulted in the smallest cumulative N_2O emissions, likely due to a better level of soil aeration which produced conditions not suitable for denitrification and N_2O production (Butterbach-Bahl *et al*. 2013). This treatment also has the advantage of reduced NH_3 emissions (Meissinger and Jokela 2000). From an N_2O emission reduction point of view, SBI should be recommended but effects on yield should also be considered.

Overall, scaled N_2O emissions (EF and NOI) were not significantly affected by method (Table 5). However, the interaction between method and year did affect NOI (Table 5), with INJ having the highest NOI value in 2012 (Fig. 3.7 B) and no difference among methods during the wet years. Recalling that NOI is the ratio between cumulative N_2O emissions and grain yield, the observed behaviour in INJ plots during 2012 was due to different relative increases for N_2O emission and grain yield between methods. For example, cumulative N_2O emissions for INJ plots were 100% larger but grain yield was only 25% larger than for SB plots, resulting in a higher NOI for injected plots during 2012. Although no differences in EF were detected between methods over the 3-years, injection had the highest EF in 2012, the driest study-year (Appendix: Table A3). Potentially larger retention of N due to lower NH_3 loss could have led to higher N_2O loss per N applied in INJ compared to SB and SBI plots during the dry year.

3.5 Conclusions

Nitrous oxide emissions did not respond to timing of manure application as expected and no interaction between timing and application method was observed, but, instead, a year and timing interaction was found. Also, N rate-scaled (emission factor) as well as yield-scaled emissions (N_2O intensity) had the same response to timing as cumulative N_2O emissions. Although in 2012, the driest year, fall application lead to lower cumulative N_2O emissions compared to spring application, N_2O emissions did not respond to manure application timing in years with close to normal precipitation (2013 and 2014).

Daily N_2O emissions followed a different temporal pattern according to manure application timing: for fall application, N_2O emissions were concentrated in the non-growing season, while for spring-applied plots emissions were concentrated during the Early Growing Season. Including N_2O emissions for the non-growing season in the total annual estimates was quite important particularly for the fall application treatments where non-growing season emissions accounted for 20 to 60% of total N_2O emissions, while for spring treatment this proportion was only 9-19%. This suggests that studies of fall treatments in cold climates which did not measure winter N_2O emissions likely underestimated emissions and presented biased treatment comparisons.

From the point of view of N_2O emission mitigation, incorporating raw dairy manure is recommended, but injection is the better practice for grain yield increases. Given that manure injection appears to lead to more N retained in the soil compared to other methods, there is a potential opportunity to adjust N rates for economical corn grain yields and decreased N_2O intensity.

CHAPTER 4: ANAEROBICALLY DIGESTED DAIRY MANURE AS AN ALTERNATIVE NITROGEN SOURCE TO MITIGATE NITROUS OXIDE EMISSIONS IN FALL-FERTILIZED CORN

The following Chapter was submitted to the Canadian Journal of Soil Science on August 19, 2016 and is currently being revised. I am the primary author of the paper, with Dr. Claudia Wagner-Riddle, Dr. Craig Drury, Dr. John Lauzon and Dr. William Salas acting as supervisors, and appearing as coauthors on the journal article.

4.1 Introduction

Global milk production increased by 23% from 2003 to 2013 which resulted in a 5.3% increase in greenhouse gas (GHG) emissions from dairy farms (FAOSTAT 2015). Although the dairy industry in Ontario (Canada) decreased its GHG emissions by 24% in 2011 compared to 1991, the dairy manure-induced N_2O emissions have increased by 17% based upon unit of product (Jayasundara and Wagner-Riddle 2014). About 30% of the carbon footprint of milk (GHG emission per unit of milk produced) on Ontario dairy farms is due to crop production with 23% of this contribution in the form of soil N_2O emission associated with manure application as fertilizer (Jayasundara and Wagner-Riddle 2014). In Canada, 29% of the dairy manure is applied during fall due to practical reasons such as limited manure storage, time constraints and/or risk of soil compaction in the spring prior to growing season (Beaulieau 2004). Fall application of liquid raw dairy manure has been proven to increase N_2O emissions when compared to no nitrogen (N) application (Wagner-Riddle *et al.* 1997, Rochette *et al*. 2000, Hao *et al.* 2001, Kariyapperuma *et* *al.* 2012, Chapter 3). However, anaerobically digesting the manure may be a mitigation strategy which could be used to reduce N_2O emissions (Clemens *et al.* 2006, Lemke *et al.* 2012).

Anaerobic digestion is a controlled biological process that results in the production of renewable and sustainable energy (biogas) and a stabilized digestate, which may be used as fertilizer if certain quality requirements are met such as low activity of pathogens and low content of heavy metals (Holm-Nielsen *et al*. 2009, Møller *et al*. 2009, Comparetti *et al*. 2013). During the digestion, carbon (C) and N forms present in RM undergo numerous changes from complex organic compounds to simpler molecules (CO_2 and NH_4^+), resulting in anaerobic digestate (AD), a substrate with a lower C:N ratio than RM (Möeller and Müller 2012). In addition, AD often undergoes solid-liquid separation as the solid fraction is recycled as bedding (Baldé *et al*. 2016). Organic C contained in AD is less metabolizable than that of RM, which may limit soil microbial growth and oxygen demand, leading to an aerobic soil environment and limiting denitrification after land application (Petersen 1999). Thus, it has been suggested that AD is an useful substitute for synthetic fertilizers since it has been shown to reduce N_2O emissions (Amon *et al*. 2006, Clemens *et al*. 2006, Petersen 1999) and also produce corn grain yields equal to or greater than untreated manure (Loria *et al*. 2007, Lemke *et al.* 2012).

Even though AD has been proposed as an alternative source of N to reduce N_2O emissions relative to RM, there have been few field studies comparing the effect of these sources on N_2O emissions. Clemens *et al*. (2006) found that cattle-AD in Germany produced lower N_2O emissions after field application compared with cattle-RM and calcium ammonium nitrate over a 56 day period in pastures. Similarly, in a study performed in Saskatchewan (Canada), Lemke *et al.* (2012) reported a reduction in N_2O emissions after fall application of swine-AD compared to swine-RM. In contrast, Amon *et al.* (2006) found no significant differences in N_2O emissions between AD and untreated cattle slurry after applying approximately 100 kg N ha^{-1} in late summer. Clemens *et al*. (2006) and Amon *et al*. (2006) applied AD directly on the surface or banded on the surface while Lemke *et al*. (2012) used an experimental applicator that injected AD at 10 cm depth. Wulf *et al*. (2002) compared N_2O emissions resulting from co-fermented and untreated slurry applied in spring using several application methods in arable land and in grassland. We are not aware of any studies which have compared AD to RM across several methods for fall application.

Both N_2O emissions and crop yields can be affected by the method of manure application (Dosch and Gutser 1996, Ahmed *et al*. 2013). The interaction between source of N and method of manure application should be considered as part of the framework for a mitigation plan. There are 3 primary methods for applying manure including: (i) surface broadcasting, (ii) surface broadcasting followed by immediate incorporation into the soil, and (iii) injection (Meisinger and Jokela 2000). Even though injection is a recommended method to reduce ammonia volatilization losses (Dosch and Gutser 1996, Meisinger and Jokela 2000) and also increase crop yields (Ahmed *et al.*2013), its effect on N_2O emission from AD should also be investigated. Several studies reported significant increases in N_2O emissions from liquid RM injection compared with other methods (Comfort *et al*. 1988, Dosch and Gutser 1996, Flessa and Beese 2000, Velthof *et al*. 2003, Chapter 3). Some studies evaluated N_2O emissions and crop yields with AD application in cold climates, but did not investigate possible interaction with the application method. For example, Lemke *et al.* (2012) found a consistent decrease in N_2O emissions with swine-AD, but an inconsistent response in barley (*Hordeum vulgare*) yields across years following fall or spring injection. Studies focusing on the interaction between N source and manure application method are needed to better understand N_2O emission dynamics and crop growth after a fall application in cold climates where emission events due to spring thaw are significant (Wagner-Riddle and Thurtell 1998). Under this framework, we hypothesize that AD will produce lower cumulative N_2O emissions than RM across typical application methods due to its low concentration of easily metabolizable organic C.

The main goal of this study was to compare the effect of fall application of AD and RM using three application methods (broadcast, broadcast and incorporated, injected) on N_2O emissions. We aimed to evaluate: 1) whether the effect of manure application method on yearly cumulative N_2O emissions varied according to the source of manure used (AD or RM) ; 2) whether the proportion of N emitted as N_2O (emission factor, EF), crop yield, yield-scaled N_2O emissions (N_2O intensity), and N uptake changed according manure application method and source.

4.2 Material and methods

4.2.1 Experimental Site and Set up

The study was conducted from 2012 to 2014 at the Elora Research Station (University of Guelph), Elora, ON, Canada (43.85º N,-80.42º W). The historical average precipitation is 874 mm and the average temperature is 6.7 ºC (period 1981-2010) for this site. A new field was chosen each fall to establish the experimental plots and measurements were conducted from November to October. The soils of the experimental site belong mostly to the Guelph loam series, classified originally within the group of Grey-Brown Podzolics according to the Canadian System of Soil Taxonomy (Hoffman 1963, OMAFRA 1999) and as Haplic Glossudalf according to the USDA classification system (USDA-NCSS 2012). Soil attributes (0-15 cm) were 12.5 g organic C kg soil $^{-1}$, 7.8 pH, 162 g kg^{-1} clay , 491 g kg^{-1}silt and 2.9 mg NO_3^- kg^{-1}for the 2013 field site, and 21.4 g organic C kg soil $^{-1}$, 7.8 pH, 196.3 g kg^{-1} clay, 520 g kg^{-1} silt and 3.6 mg NO_3^- kg^{-1} for the 2014 site.

Corn (*Zea mays*) was planted on May 17, 2013 and May 27, 2014 (Dekalb DKC39-97RIB, 2700 CHU, glyphosate tolerant) and barley (*Hordeum vulgare*) was the preceding crop for each field site. The plots were tilled with a C-tined swept tooth cultivator prior to planting. The average seeding rate was 80,000 plants ha^{-1}, with rows spaced at 0.75 m. Phosphorus (triple super phosphate 0–46–0), potassium (potassium sulphate 0–0–50) and sulphur (dry ammonium sulphate, 20.5-0-0-24) were applied at 80 kg P_2O_5 ha^{-1}, 110 kg K_2SO_4 ha^{-1} and 25 kg SO_4 ha^{-1}, respectively, prior to planting. Glyphosate was used as a post-emergent herbicide at labelled rates to control weeds. Corn was harvested on October 27, 2013 and November 17, 2014.

A factorial randomized complete block design with 4 replications was used each year. Factors analysed were the source of nitrogen (RM vs. AD) and the three methods of application examined were broadcasted, broadcast and incorporated and injected. Control plots receiving no nitrogen fertilizers were included in each block. Fall applications of RM and AD were performed on November 15, 2012 and November 13, 2013, using a research applicator unit set up with 4 fan outlets, totalling a 3 m wide application. Both RM and AD were provided by a local farm (140 milking cows producing 28 L milk cow^{-1}day^{-1}) located near Drayton, Ontario (approx. 20 km from the research site) (Ngabwie *et al*. 2014). The process of manure management and AD production was as follows: (i) RM was pumped into a mixing tank along with off-farm organic materials; (ii) the mixture was pumped into a two-stage anaerobic digester and it was retained in the digestor for 60 days, (iii) the liquids were separated from the solids and then liquids were transferred to a concrete storage tank.

The application methods were as follows: (i) surface broadcasting (SB), which consisted of spreading directly onto the soil and leaving either RM or AD on the soil surface; (ii) surface broadcasting followed by incorporation (SBI) with a C-tined sweep-tooth cultivator to 12 cm depth within 2 hours after application; (iii) injection (INJ) was performed using a traveling shoe plus a disk opener with injection lines spaced at 75 cm width and 20 cm depth. The equipment available did not allow for placement of manure in SBI and INJ treatments at the exact same depth, but we considered that the difference in depths was small (20 cm vs. 12 cm) and did not have a major influence on N_2O emissions, as found by Maharajan and Venterea (2014).

An average volume of 50.2 m^3 of manure was applied every fall with a target application rate of 150 kg N ha^{-1} according to total Dumas N (TDN, %) in samples previously collected from the storage tanks. To check for the consistency of TDN analysis of the manure, 4 samples were collected during each application. The manure samples were analyzed by the Agri-Food Laboratories (SGS, Guelph, Canada). Variations from the targeted rated occurred due to differences in the manure-N concentration between sampling from the storage tank prior to application and in the field during application (Table 4.1). Therefore, N rate was included as a covariate in the statistical analyses as explained below.

Table 4.1. Chemical properties of anaerobically digested (AD) and raw manure in 2013 and 2014. Mean ± standard deviation.

Source	Year	DM[a] (%)	EC[b] (mmohs cm^{-1})	OC[c] (%)	C:N ratio	N (%)	TAN[d] (mg NH_4^+ kg^{-1})	N rate (kg N ha^{-1})
AD manure	2013	4.2±0.16	18.0±0.43	1.4±0.05	5.9±1.4	0.25±0.07	1520±96	126.7
	2014	5.6±0.02	16.4±0.27	2.0±0.01	4.6±0.44	0.42±0.04	1750±16	228.2
Raw manure	2013	7.5±0.16	16.9±0.21	2.5±0.05	7 ±0.68	0.36±0.03	1343±15	181.7
	2014	6.4±0.12	13.9±0.025	2.6±0.04	6.9±1.31	0.38±0.08	1360±52	209.5

[a]: DM, dry matter

[b]: EC, electrical conductivity

[c]: OC, organic carbon

[d]: TAN, total ammoniacal nitrogen

4.2.2 N_2O gas sampling and flux calculation

Nitrous oxide fluxes were sampled 34 times in each year, using non steady-state circular chambers. An experimental unit of one chamber per plot was used, with chambers centered between corn rows and over the injection band in the INJ treatment. The frequency of sampling was weekly from November to December, twice per month from January to February, weekly from March to mid-May, biweekly from mid-May to July and weekly until harvest. The non-steady state chambers consisted of a combination of collar plus lid and these were designed following protocols and guidelines suggested in previous studies (Rochette and Bertrand 2008, Parkin and Venterea 2010, Snider *et al.* 2015). Soon after every manure application, PVC collars (44.2 cm inner diameter X 19 cm height) were buried in the soil to a 9 to 13 cm depth; the headspace volume was measured regularly in each collar and used in the flux calculations. Collars were removed 1 day before manure application and re-inserted 1 day after manure application. Aluminum bubble foil-covered PVC lids (44.2 cm inner diameter x 8.3 cm height) were placed over the collars to enable the collection of gas samples from each plot. Syringes (20 mL polypropylene; Becton Dickinson, Rutherford, NJ) were used to extract the gas samples through a lid sampling port at four times (0, 12, 24 and 36 min) during chamber deployment and these samples were injected into 12 ml pre-evacuated (-10 mbar) glass vials (Labco, High Wycombe, UK). The gas samples were analyzed on a Varian 3800 gas chromatograph (GC, Varian, Mississauga, ON, Canada) fitted with a Combi-PAL auto-sampler as described by Drury *et al*. (2006) and Guo *et al*. (2011) at the Agriculture & Agri-Food Canada research station in Harrow, Ontario.

Nitrous oxide fluxes were calculated using the equation $F=(dC/dt)*(V/A)*\ p*M/\ (R*$

*T)*k*, where F is the N_2O flux in g N_2O-N ha^{-1}, *dC/dt* is the slope or change of concentration of N_2O over time (µg N_2O-N g gas^{-1} min^{-1}), V is the volume of chamber headspace considering the distance between the top of the lid and the first solid surface (either snow or bare soil, according season) (m^3), A is the area occupied by the chamber (m^2), p is the barometric pressure (Pa), M is the molar mass (N_2O-N = 28 g mol^{-1}), R is the Universal Gas Constant (8.314 J $mol^{-1}K^{-1}$), T is the absolute temperature (K) and k is a constant (14.4 g μg^{-1} m^2 ha^{-1} min d^{-1}) to obtain the flux in g N_2O-N ha^{-1} d^{-1}.

The slope *dC/dt* was calculated through a hybrid method which combined linear and quadratic approaches (Parkin *et al.*2012) and the Hutchinson-Mosier equation (1981). More details about the flux calculation method are given in Chapter 2.

4.2.3 Supporting environmental measurements

Soil samples were collected prior and during the corn growing season each year, from November 2012 to October 2013 (12 sampling dates) and from November 2013 to October 2014 (16 sampling dates). Samples were analyzed to determine soil ammonium (NH_4^+-N) and nitrate (NO_3^--N) concentration. Plots receiving SB and SBI treatments were sampled by taking 8 soil subsamples with a sampler (0-15 cm depth) within a 1-m radius of the chamber. Plots receiving injection treatment had 4 subsamples taken from the injection zone and 4 subsamples taken outside the injection zone. These 8 subsamples were thoroughly mixed in a bucket to obtain 1 sample per plot. The soils were stored frozen at -18 ºC until they were analyzed. Once thawed, samples were extracted under a protocol based on Maynard *et al.* (2008) by sieving the samples with a 4.75 mm mesh sieve and taking a 10 g soil subsample, adding 50 mL of a solution 2.0 M of KCl and shaking it for 1 hr at 300 rpm and then filtering the solution through filter papers (Whatman no. 42). An auto-analyzer (AACE 6.07 software, SEAL Analytical Inc. Wisconsin) was used to determine concentrations of NO_3^--N and NH_4^+-N in the extracts. Additional soil subsamples (10 g) were placed in an oven at 105 ºC for approximately 24 h to determine soil water contents on a weight-loss basis. Ammonium and nitrate contents are reported on a dry-weight basis. A "Field Scout" time domain reflectometer (TDR, model TDR 300, Spectrum Technologies, Inc., Plainfield, IL, USA) was used to manually measure volumetric water content (VWC) at 8 points per plot at every gas sampling date for the 0-12 cm depth layer, except when there was snow present and/or frozen soil conditions. Permanent TDR probes were used in 8 plots, inserted into the soil at a 22º angle to capture the water contents in the 0-12 cm depth. All permanent TDR probes were connected to a datalogger (CR23X Micrologger, Campbell Scientific Inc.), which measured average VWC over 30 minutes. Soil bulk density (BD) was determined at the beginning of the experiment (November) and around harvest each year. Stainless steel cylinders (4.72 cm i.d. X 5 cm height) were used to collect soil samples. The samples were dried at 105 ºC for 24 h and then weighed. Water filled pore space (WFPS) was calculated for each plot using WFPS = VWC /(1-(BD/PD))*100, where VWC is the volumetric water content (%), PD is the particle density (2.65 g cm^{-3}, Lynn and Doran 1984) and BD is soil bulk density. WFPS was calculated for manually (field scout) and automatically (datalogger) measured VWC.

Manual and automatic measurements were also made for soil temperature. For manual measurements, one copper-constantan thermocouple was installed per plot to measure soil temperature at 5 cm depth during every gas sampling event. Data were read using a digital thermometer (Type J-K-T thermocouple, Model HH23, OMEGA) and manually recorded.

Permanent thermocouples were buried at 5 cm depths in 8 plots and connected to the datalogger (CR23X Micrologger, Campbell Scientific Inc.). Daily mean air temperature, precipitation and barometric pressure were collected from the Elora Research Station weather station which is located ~500 m from the experimental plots.

4.2.4 Plant sampling for grain yield

Corn grain yield (GY) and corn stover biomass (CSB) were determined at maturity by harvesting a 7.5 m^2 area (2 rows 5 m long and 0.75 m between rows), between the 4th and 5th row of each plot. Cobs were hand-harvested and stover was hand-clipped, and weighed in the field, thus obtaining fresh weight. Sub-samples of 10 cobs per plot were weighed and dried at 60 ºC until constant weight. Dried ears were sheared and the kernels weighed. Grain yield was calculated as $GY=m*FW*(DWs/FWs)*(c/A)$, where *GY* is grain yield (kg grain ha^{-1}), *m* is the correction for moisture content (i.e. yields are reported at 15.5% moisture content), *FW* is the fresh weight of sample (g), *FWs* is the fresh weight of subsample (g), *DWs* is the sub-sample dry weight (g), *c* is a conversion factor (10 kg g^{-1} m^2 ha^{-1}) and *A* is the harvested area (m^2). Stover sub-samples from 7 plants in each plot were weighed in the field, ground to coarse pieces, and then dried at 60 ºC to determine *CSB*, which was calculated similarly to GY but expressed on a dry weight basis instead of 15.5% moisture.

4.2.5 Nitrogen Uptake and N in grain

A subsample of ~1 kg was taken from both kernels and stover, after the total weights were determined. The subsamples were ground using a Brinkman-Retsch hammer mill to pass through a 1 mm mesh sieve, collecting sub-samples of 1 oz (28.3 g) per sample. From these sub-samples, an aliquot of 100-150 mg was taken to be analyzed in an autoanalyzer (TruSpec® CN Analyzer, LECO®). Variables obtained were: nitrogen concentration in grain (Ng, %) and N concentration in stover (Ns, %); then total Nitrogen uptake (TNU) (kg N ha^{-1}) was calculated as the sum of nitrogen in the stover (*CSB*Ns*/100) and the nitrogen in the grain (*GY*Ng*/100) as follows: *TNU= (Ns/100)*CSB + (Ng/100)* GY*

4.2.6 Data and statistical analyses

Nitrous oxide flux calculations were performed using a spreadsheet program (Excel®,Microsoft 2010) and R-Studio 0.99.465 (2015). It was assumed that N_2O flux measured between 0900 and 1200 h on a sampling date was representative of the average daily N_2O flux. Fluxes for the injected plots were corrected to account for potential overestimation of area-scaled fluxes due to placement of the chamber over the injection area. The equation used was as follows:

$$F_{inj} = a*F_{chamber} + b*F_{outside}$$

Where F_{inj} is the N_2O flux for the injected plots (g N-N_2O ha^{-1}), $F_{chamber}$ is the N_2O flux inside the chamber area, $F_{outside}$ is the N_2O flux in the surrounding area (average of control plots), a = 0.46 and b = 0.54 are coefficients indicating the fraction of 1 m^2 covered and not covered by the chamber, respectively.

Annual cumulative N_2O emissions were calculated as the summation of daily estimates of N_2O fluxes obtained by linear interpolation between sampling events. Emission factor (EF, %) was calculated by subtracting the average cumulative emission produced by control plots from emissions for manure-applied plots and dividing the output by the rate of N applied (Asgendom *et al*. 2014). Soil NH_4^+ and NO_3^- concentrations were time-scaled to obtain intensities as measures of the hypothetical accumulations of these variables in the soil over time (Burton *et al*. 2008). Both soil ammonium and soil nitrate intensity (SAI and SNI, respectively) were calculated by the daily summation of concentrations, obtained through linear interpolation, between the beginning and the end of the experimental periods (Zebarth *et al.* 2008, Burton *et al*. 2008).

We analyzed the data with a combined ANOVA including all years, since variances were homogeneous among years, according to Bartlett's test, and year interactions were not significant. Cumulative emissions, EF, TNU, Ng, CGY, SAI, SNI and N_2O intensity (NOI) (cumulative N_2O emissions yield-scaled, in g N_2O-N t grain^{-1}) were analyzed with an ANOVA, using a sub-sub-plot model (main plot: year, sub-plot: source, sub-sub-plot: method). Nitrogen rate was considered as a covariate in the model to better estimate the mean square error, provided its direct and positive relationship with N_2O emissions (Halvorson *et al*. 2014).

The analyses were run using the function *aov* in R-Studio (2015), and the assumptions of the ANOVA were tested with Shapiro-Wilks test (normality of residuals) and Bartlett test (homogeneity of variance). If model residuals were not normally distributed, the data were transformed using natural logarithm. Least significant difference ($p<0.05$) test was performed to compare means when significant effects were found.

4.3 Results

4.3.1 Weather conditions

In 2014, the average air temperature was -7.3 ºC during the cold season (November 1-March 31) while in 2013 the air temperature was - 2.4 ºC for the same period (Fig. 4.1 A, B). Average soil temperature during the cold season in 2014 was -0.9 ºC, while it was 0.7 ºC in 2013. During 2014 the snow layer was thicker than during 2013 (32 cm vs 13 cm, respectively), reaching peaks of approximately 50 cm (Fig. 4.1 A, B).

The soil was wetter in 2013 than in 2014. The accumulated precipitation between November 1 and March 31 was 329 and 228 mm for 2012/2013 and 2013/2014, respectively. The 2013 growing season (mid-May to late October) was wetter than 2014 (683 mm vs 508 mm, respectively) (Fig. 4.1 C, D). Soil water content was highest at the start of the growing season (March to mid-May) with WFPS of 100% and 90% for 2013 and 2014, respectively. Soil moisture fluctuations were greater for the 2013 growing season than for 2014, with WPFS ranging from 38 to 70 % in 2013 and from 43 to 60 % in 2014 (Fig. 4.1 C, D).

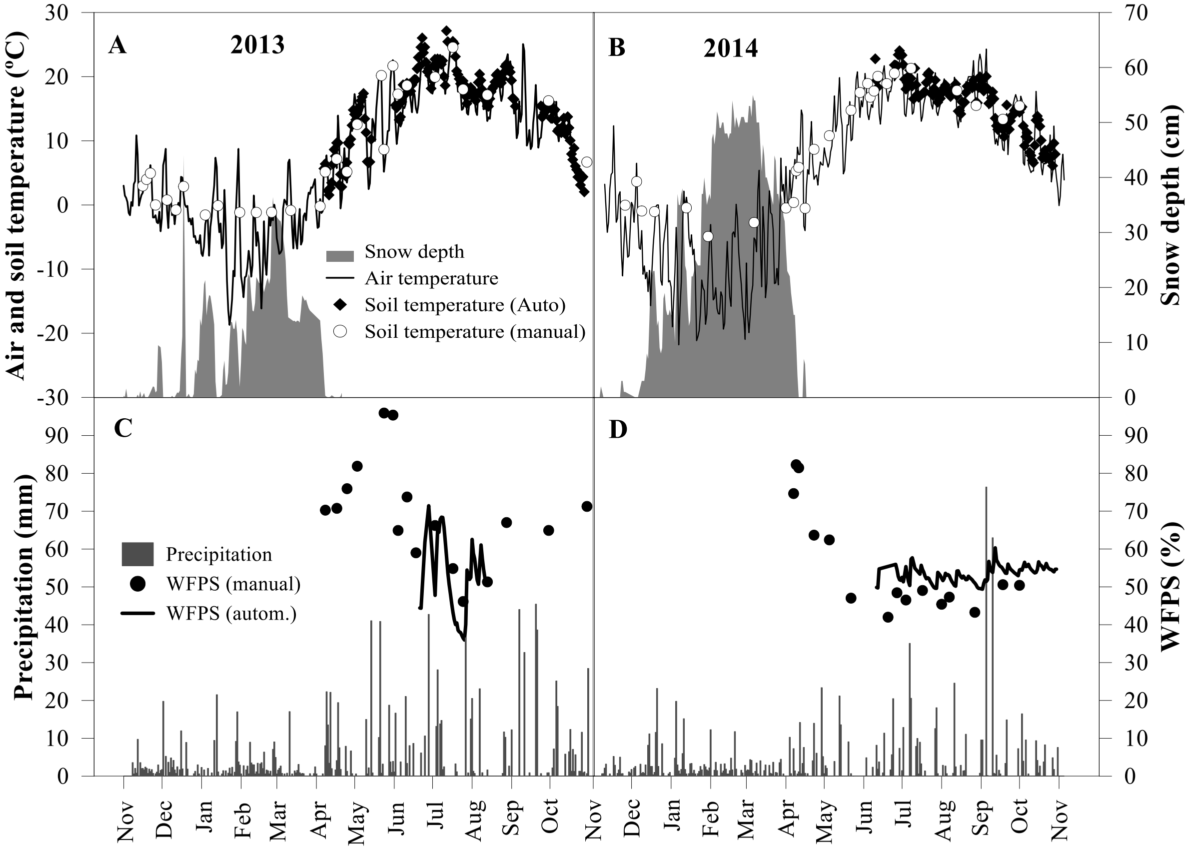

Figure 4.1. Environmental variables: Air and soil temperature and snow depth for 2013 (A) and 2014 (B). Precipitation and water filled pore space (WFPS) for 2013 (C) and 2014 (D). Manual measurements were made during the gas sampling day. Automatic measurements were recorded using sensors fitted to an automated datalogger.

4.3.2 Soil ammonium and nitrate

During the week following application, AD and RM had soil NH_4^+ concentration with increased peak values compared to that of control plots consistently across years (24.0 ± 2.8 and 14.7 ± 2.9 vs. 1.4 ± 0.1 kg NH_4^+-N ha^{-1}, respectively) (Fig. 4.2 A-D). After reaching the peak concentrations, the values decreased and were less than 10 kg NH_4^+-N ha^{-1}, except for April 2014 when injected AD still had values at ~12 kg NH_4^+-N ha^{-1} (Fig. 4.2 C).

The temporal patterns followed by soil NO_3^- concentration were not consistent across years (Fig. 2 E-H). In 2012/2013, during the week following application, AD and RM-treated plots had higher NO_3^- concentration peaks compared to that of control plots (16.0 ± 1.2 and 14.1 ± 2.2 vs. 3.7 ± 0.9 kg NO_3^--N ha^{-1}, respectively) (Fig. 4.2 E, F), while in 2013/2014 treated and control plots had similar NO_3^- concentration peaks within the week after application (9.0 ± 1.4 and 9.7 ± 1.3 vs. 7.7 ± 0.9 kg NO_3^--N ha^{-1}) (Fig. 4.2 G, H). In 2013, the dynamics followed by soil NO_3^- was similar among treatments, with concentration peaks attained in April (16.4 ± 1 kg NO_3^--N ha^{-1}) and June (30.6 ± 1 kg NO_3^--N ha^{-1}) (Fig. 4.2 E, F). In 2014, injected AD produced NO_3^- concentration peaks that overpassed those of the other treatments, including RM plots, with the first peak in early May (39.5 ± 6.2 kg NO_3^--N ha^-1) and the second peak at end of June (62 ± 5.3 kg NO_3^--N ha^{-1}(Fig. 4.2 G, H).

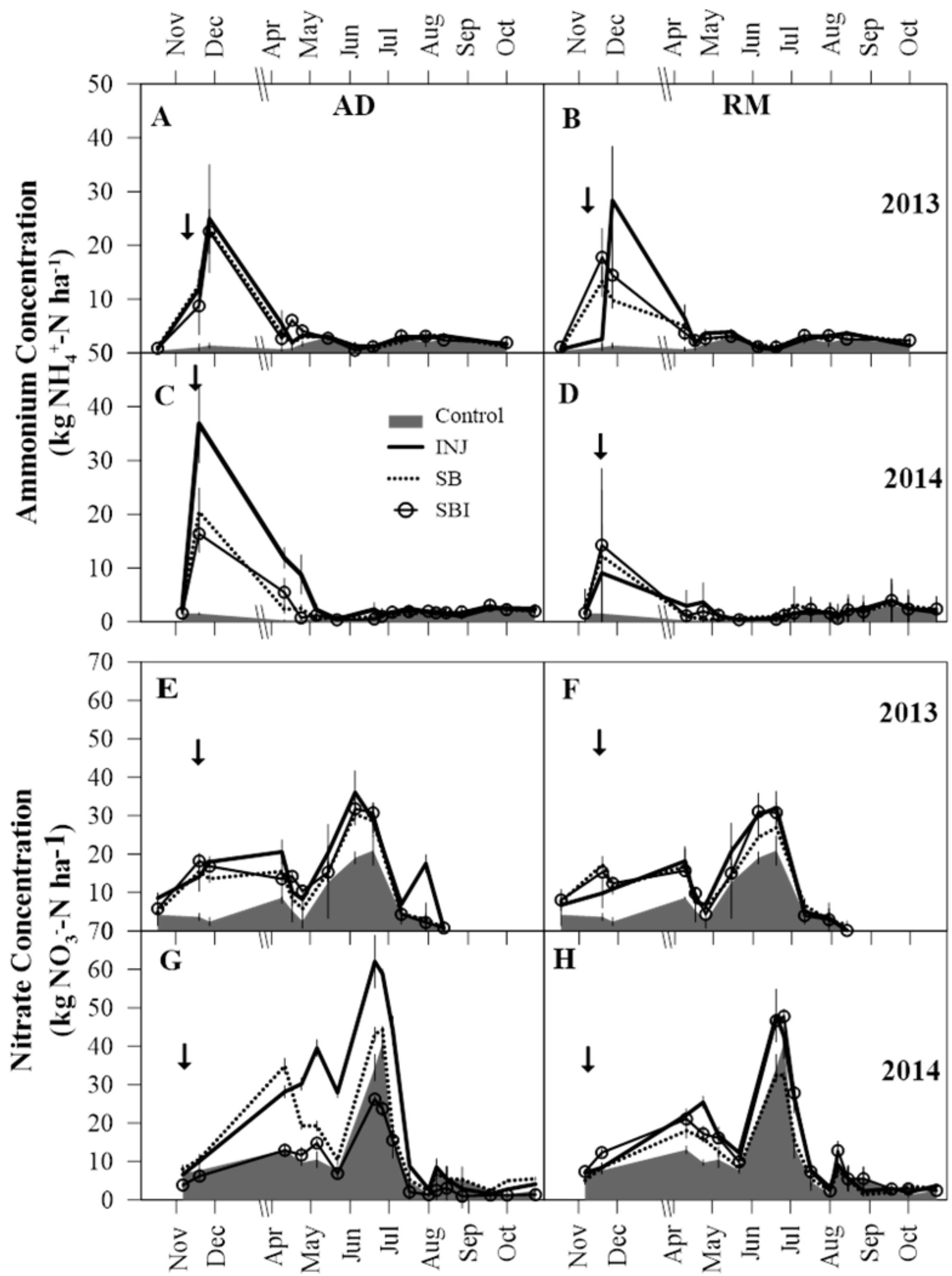

Figure 4.2**.** Soil ammonium (A-D) and soil nitrate concentration (E-H) in the 0-15 cm layer after AD manure (A, C, E, G) and raw manure (B, D, F, H) applications during 2013 and 2014, using three application methods (INJ, injection; SB; surface broadcasting and SBI, incorporation) and controls (no N added plots). Bars indicate standard error of mean. Arrows indicate the day manure was applied.

The ANOVA results revealed source affected SAI, with AD having the highest values (1.21 ± 0.12 g NH_4^+-N day kg $soil^{-1}$) (Table 4.2). There was a trend of interaction among year, source and method (*F* = 3.3, *df* = 24, *p*=0.05) on SAI (Table 4.2), resulting in a significant interaction between source and method on SAI only during 2014 (*F* = 7.4, *df*= 15, *p*<0.01, not shown), with the highest value for AD-INJ (2.15 ± 0.20g NH_4^+-N day kg $soil^{-1}$, not shown). Soil NO_3^- intensity tended to be affected by the interaction between year and source (*F* = 5.8, *df* = 24, *p*=0.05), with significant effects in 2014, when AD showed highest values (2.67 ± 0.16 g NO_3^--N day kg $soil^{-1}$) (Table 4.2). Year also affected SNI, with the largest values for 2014 (2.84 ± 0.12 g NO_3^--N day kg soil $^{-1}$) (Table 4.2).

Table 4.2. Mean comparison according to year, source and method of manure application for soil ammonium and nitrate intensities.

Source of variation/ treatments	NH_4^+ intensity	NO_3^- intensity
	g NH_4^+-N day kg soil $^{-1}$	g NO_3^--N day kg soil $^{-1}$
Year		
2013	0.86 ± 0.06	2.00 ± 0.09 B
2014	1.06 ± 0.12	2.84 ± 0.13 A
LSD (0.05)	ns	0.38
Source		
AD	1.21± 0.12a	2.67 ±0.16A
RM	0.72± 0.03b	2.17 ±0.09B
LSD (0.05)	0.18	0.26
Method		
INJ	1.12 ± 0.26	2.61±0.26
SB	0.88 ± 0.08	2.26±0.25
SBI	0.89 ± 0.11	2.39±0.20
LSD (0.05)	ns	ns
Year	0.32	<0.01
Source	<0.01	<0.01
Method	0.46	0.11
Source X Method	0.06	0.27
Year X Source	0.21	0.05
Year X Method	0.17	0.48
Year X Source X Method	0.05	0.58

Different lowercase letters between rows indicate significant differences between manure sources for SAI ($p<0.05$, LSD). Different uppercase letters between rows indicate difference between sources for SNI ($p<0.05$, LSD). The ANOVA summary included below shows the effects of treatments for each year and for the three years pooled together (data log-transformed when needed). Note: (*), (**) and (***) indicate p values lower than 0.05, 0.01 and 0.001 respectively. The Sum of Squares due to N rate was calculated in the model to better estimate Residual Sum of Squares but N rate effect was removed from the analysis.

4.3.3 Temporal pattern of daily N_2O emissions

Daily N_2O emissions had a different temporal pattern according to source and method of manure application (Fig. 4.3). In 2013, the main N_2O emissions peak for AD was produced during January with the SB method (115 ± 38 g N_2O-N ha^{-1} d^{-1}) while the main peak for RM was produced with either INJ (112 ± 51 g N_2O-N ha^{-1} d^{-1}) or SB (107 ± 17 g N_2O-N ha^{-1} d^{-1}) at the end of May (Fig. 3 a, b). All the treatments overpassed the peak attained by the control plots (26 ± 16 g N_2O-N ha^{-1} d^{-1}, Fig. 4.3 A, B). In 2014, the highest N_2O emissions peak for AD (448 ± 211 g N_2O-N ha^{-1} d^{-1}) was attained by SBI and the highest peak for RM (270 ± 158 g N_2O-N ha^{-1} d^{-1}) was reached with SB treatment at the end of April, while control plots had a maximum of 62 ± 16 g N_2O-N ha^{-1} d^{-1} (Fig. 4.3 C, D).

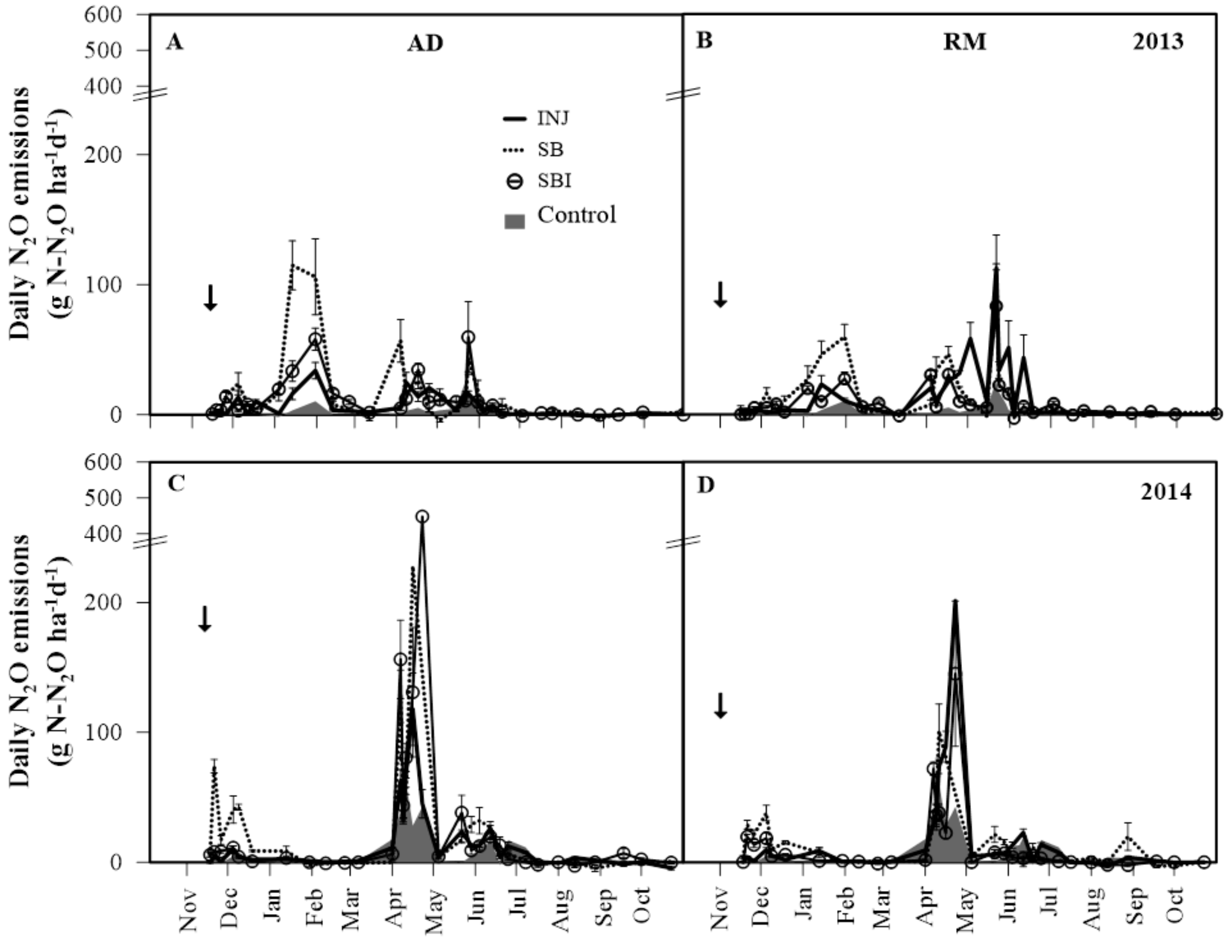

Figure 4.3. Nitrous oxide emissions according to manure source and application method for 2013 and 2014. N_2O emissions from AD manure for different methods of application (A and C), using three application methods (INJ, injection; SB; surface broadcasting and SBI, incorporation). N_2O emissions from raw manure for different methods of application (B and D). Bars indicate standard error of mean X 0.5. Arrows indicate date of manure application.

4.3.4 Cumulative N_2O emissions

Cumulative N_2O emissions were affected by the interaction between source and method (*F=4.0*, *df = 24*, *p=0.03*, Table 4.3), with the highest value for AD-SB (6.4 ± 1.3 kg N2O-N ha-1) and the lowest value for AD-INJ (2.6 ± 0.6 kg N_2O-N ha^{-1}) (Figure 4.4 A). For AD, injection reduced cumulative N_2O emissions compared to SB and SBI, while for RM, SBI reduced emissions compared to SB but produced similar emissions to injection (2.8 ± 0.9 vs. 5.3 ± 1.4 and 3.8 ± 1.3 kg N_2O-N ha^{-1}, respectively) (Fig. 4.4 A). Significant differences between sources were found only for SBI plots with AD having larger N_2O emissions than RM (5.4 ± 1.4 vs. 2.8 ± 0.9 kg N_2O-N ha^{-1}, Fig. 4.4 A). Manure application method affected cumulative N2O emissions (F=6.6, df = 24, p < 0.01, Table 4.3), with the largest value for SB plots (5.8 kg N_2O-N ha^{-1}, not shown). The interaction between year and source tended to affect emissions (*F=5.29, df=6, p=0.06*, Table 3), with a trend of largest value for AD in 2014 (5.9 ± 0.8 kg N_2O-N ha^{-1}, Appendix: Table A4). There was also an overall trend of manure source effect on N_2O emissions (*F = 4.7, df = 6, p = 0.07*, Table 4.3), with a tendency of highest emissions for AD (4.8 ± 0.57 kg N2O-N ha-1, Table S1). Source also tended to affect EF (*F = 3.2, df = 24, p = 0.06*, Table 4.3), with the highest value for AD (2.1 %, data not shown) and the lowest value for RM (1.3 %, data not shown). Method of application affected EF (*F=5.5, df=24, p = 0.01*, Table 4.3), with 1.5 = 1.2 >0.9 % observed for INJ, SBI and SB plots, respectively (Figure 4.4 B).

Table 4.3. The *p*-values for cumulative N_2O emissions, emission factor, grain yield and N_2O intensity as affected by manure source and method of application during 2013, 2014 and pooled over the years.

Source of variation	Cumulative N_2O emissions	Emission factor	Grain yield	N_2O intensity
Year	0.13	0.20	0.49	0.02
Source	0.07	0.06	0.21	0.34
Method	*<0.01*	0.01	0.49	*<0.01*
Source X Method	0.03	0.09	0.03	0.04
Year X Source	0.06	0.62	0.29	0.50
Year X Method	0.89	0.95	0.09	0.39
Year X Source X Method	0.35	0.27	0.25	0.45

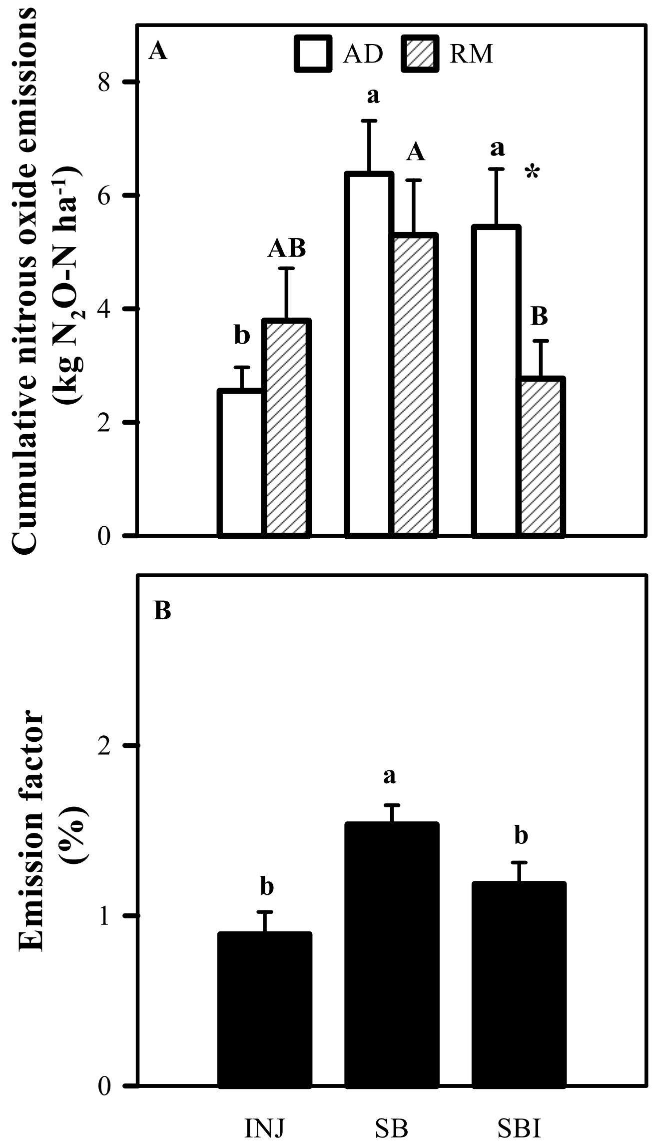

Figure 4.4. A: Cumulative mean annual N_2O emissions as influenced by the interaction between manure source and application method. B: Emission factor as affected by method of manure application. Error bars indicate standard error of the mean. A: Bars with different lowercase letter indicate significant differences (LSD, $p<0.05$) among methods for AD manure. Bars with the different upper-case letters indicate differences among methods for raw manure (LSD, $p<0.05$). * indicate significant difference between sources within the same method. B: columns with different lowercase letter indicate significant differences (LSD, $p<0.05$) among application methods; INJ: injected, SB: broadcasted and SBI: broadcast-incorporated manure.

4.3.4 Grain yield, N_2O intensity and N uptake

The interaction between source and method also affected GY (*F=4.1, df = 24, p=0.03*, Table 4.3), with the highest values for injected AD (9.2 ± 0.3 t grain ha^{-1}) while the lowest values were reached using RM and SB treatment (7.8 ± 0.7 t grain ha^{-1}) (Fig. 4.5 A). This interaction was detected only in 2013 (*F=7.8, df=12, p<0.01*, Table 4.3), with the highest yields for RM-SBI and AD-SB (9.8 ± 0.5 and 9.7 ± 0.7 t grain ha^{-1}, respectively; Appendix: Table A4).

Nitrous oxide intensity was also affected by the interaction between source and method (*F=3.5, df = 24, p=0.04*), with the highest intensities for AD-SB, RM-SB and AD-SBI (746 ± 386, 682 ± 251 and 670 ± 335 g N_2O-N t $grain^{-1}$, respectively) and the lowest values for RM-SBI and AD-INJ (Fig. 4.5 B, Appendix: Table A4). Nitrogen uptake and N in grain were affected only by the method of manure application, with the greatest value for injected plots with no significant differences between SB and SBI (Fig. 4.6).

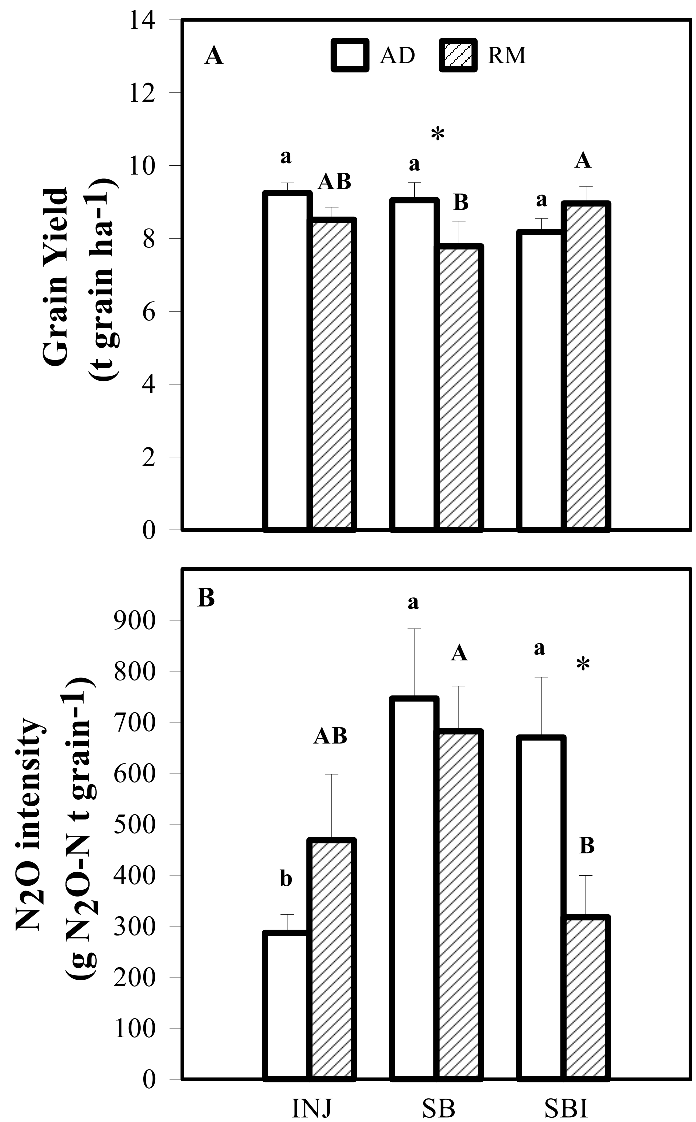

Figure 4.5. Grain yield (A) and Nitrous oxide intensity (B) as influenced by the interaction between manure source and method. Bars with the same lowercase letter indicate no significant differences (LSD, p<0.05) among methods for AD manure. Bars with the same upper-case letter indicate no differences among methods for raw manure (LSD, p<0.05). * indicate significant difference between sources within the same method. Error bars indicate standard error of the mean. INJ: injected, SB: broadcasted and SBI: incorporated manure.

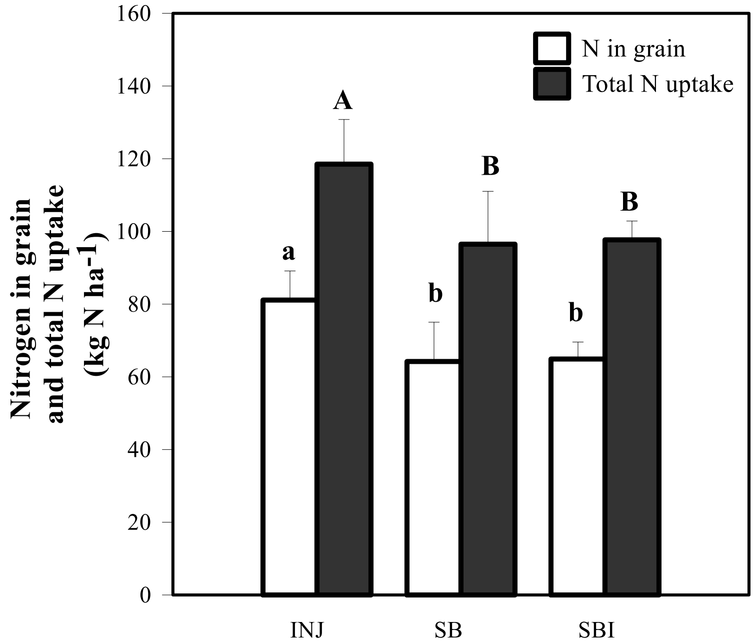

Figure 4.6**.** Nitrogen concentration in grain and N uptake as affected by method of application. Columns with the same lowercase letter indicate no significant differences (LSD, $p<0.05$) among methods for N in grain. Columns with the same upper-case letter indicate no differences among methods for total N uptake (LSD, $p<0.05$). Error bars indicate standard error of the mean. INJ: injected, SB: broadcasted and SBI: incorporated manure.

4.4 Discussion

4.4.1 Nitrous oxide emissions

It is well known that manure injection promotes soil N_2O emissions by creating an anaerobic environment in the injection line (Comfort *et al*. 1990, Dosch and Gutser 1996, Flessa and Beese 2000, Velthof *et al*. 2003, Markfoged *et al*. 2011); however, in our study, injecting AD during fall reduced cumulative N_2O emissions compared with that of broadcasted or broadcasted and incorporated AD (Fig. 4.4 A). Generally, AD has been characterized by its low organic C concentration, low dry matter content, low viscosity, high infiltration rates and high NH_4^+ concentration (Petersen 1999, Möeller and Müller 2012). These properties of AD, and particularly the low organic C concentration, have been shown to reduce N_2O production compared to RM in several lab and field studies (Petersen *et al*. 1992, Petersen and Andersen 1996, Petersen 1999); however, no study has shown whether or not this N_2O reduction by AD remains across application methods, so that our results shed some light in that regard. Also, it has been suggested that the low viscosity of AD facilitates the infiltration and the transport of NH_4^+ outside the injection zone (Petersen 1999, Frey *et al*. 2012), which would decrease the concentration of substrate available to produce N_2O. This contributes to O_2 diffusion to the injection lines and reduces denitrification rates (Petersen *et al*. 1996, Markfoged *et al*. 2011). High N_2O emissions for RM with INJ complement our previous findings at the Elora site (Chapter 3), where we found RM injection promoted N_2O emissions consistently across years, albeit this study included spring treatments and an additional year of measurements. Our results suggest that injecting AD during the fall is a N_2O mitigation practice that should be considered, since the low organic C in AD could contribute to reduce N_2O emissions compared to RM.

Even though the overall trend for cumulative N_2O emissions was to increase with AD addition ($p = 0.07$), there was no effect of source in either year. This was due to the fact that there was a confounding effect of source plus N rate each year, especially during 2013, when N rate for RM was larger than that of AD (182 vs. 127 kg N ha^{-1}, respectively, Table 1). When the N rate effect was isolated in the pooled-year analysis ($p = 0.14$, not shown), we were able to estimate the interaction of source by method effect on cumulative N_2O emissions ($p = 0.03$, Table 4.3). The response of N_2O emissions to N rate was likely limited by ammonia losses as discussed below.

Broadcasting caused similar N_2O emissions for AD and RM (Fig. 4.4 A). This can be explained by the relationship between NH_4^+ levels and the observed values for NH_3 volatilization for both AD and RM. Even though AD had higher levels of NH_4^+ (Table 4.1), the observed levels of NH_3 volatilization were also higher than for RM. Surface broadcasting exposed NH_4^+ contained in both AD (mean = 86 kg NH_4^+-N ha^{-1}, calculated from Table 4.1) and RM (mean = 66 NH_4^+-N kg ha^{-1}, calculated from Table 4.1) to be volatilized as NH_3, when maximum temperatures reached 7-15˚C, within the week after application (Misselbrook *et al.* 2005). Additional measurements taken in our plots confirmed a larger average cumulative NH_3 loss for AD than for RM in broadcasted plots (48 vs. 28 kg NH_3-N ha^{-1}; data not shown). This left ~38 kg NH_4^+-N kg ha^{-1} on the soil surface or to be infiltrated in the soil for both manures providing the same amount of substrate to the soil microorganisms. Our results suggest that if AD is broadcasted its low C concentration does not reduce N_2O emissions compared to RM presumably because C for denitrification is not limiting in the surface layer or because nitrification is the main N_2O producing processes (Baggs and Philippot 2010, Butterbach-Bahl *et al*. 2013). Hence, fall broadcasting of AD is not recommended for mitigating N_2O emissions because its net effect is that of NH_4^+ promoting both ammonia losses and N_2O emissions. Future studies will need to consider the measurement of additional explanatory variables such as NH_3 volatilization or NO_3^- leaching to give a more detailed overview of N fate.

Incorporating AD after broadcasting during fall increased cumulative N_2O emissions compared to fall-incorporation of RM (Fig. 4.4 A). This was likely due to AD providing soil microorganisms with additional amounts of NH_4^+ compared to RM (Table 4.1) and perhaps also to soil organic carbon becoming available after soil tillage and mixing (Linn and Doran 1984, Baggs and Philippot 2010). Thus, the combination of high NH_4^+ addition and carbon respiration resulted in larger N_2O emission peaks (Fig. 4.3 C, D) and consequently in larger cumulative N_2O emissions (Fig. 4.4 A) compared to RM. It has been suggested that soil microorganisms immobilise manure NH_4^+ throughout the late fall to winter period and then release and oxidize the N in the following spring (Jensen *et al.* 2000); however, the occurrence of net immobilisation during winter months after manure application was not detected in other studies (Clark *et al.* 2009, Jayasundara *et al.* 2010). Our results are in contrast with Petersen (1999), who working in spring barley with incorporated digested and undigested slurry found that the greatest N_2O emissions were produced by untreated manure; however, in their study, manure was applied in spring and N_2O fluxes measurements were performed only during the growing season. Therefore, our results suggest that fall-incorporated AD stimulates N_2O emissions compared to RM because AD provides higher amounts of substrate during the spring thaw to soil microorganisms.

4.4.2 Grain yield, nitrous oxide intensity and N uptake

Grain yield was also affected by the interaction between manure source and application method (Table 4.3), but in a different fashion than N_2O emissions. For AD application, grain yield was consistent across methods, while for RM, grain yield produced by SB was lower than that of SBI and similar to that of INJ (Fig. 4.5 A). This difference between SB and SBI for RM-applied plots was likely due to SB plots attaining a very low grain yield during 2014 (7.49 ± 1.28 t grain ha^{-1}), caused by an exceedingly high NH_3 loss (47 kg NH_3-N ha^{-1}). Grain yields attained with injection of either source were not consistent with crop N uptake observed for the same method (Fig. 4.6). This inconsistency suggests that grain yield was not associated with crop N uptake and that it may have been constrained by other factors such as planting date (May 17 in

2013 vs. may 27 in 2014), growing season length and cumulative solar radiation interception (Hay and Porter 2006). These results are in contradiction with our previous findings (Chapter 3), where injection promoted higher yields mostly due to results associated with a dry year, when grain yield in injected plots surpassed those of the other treatments. Additionally, grain yield for injected AD was similar to that of injected RM, and the same trend occurred for yield in SBI plots. These results are in agreement with other studies evaluating AD vs. RM under different manure application methods. For example, Lemke *et al*. (2012) compared the effects of fall injected AD and RM and did not find significant differences in corn yields, suggesting that fall-injected AD had an agronomic performance similar to untreated manure. Saunders *et al*. (2012), working on reed canary grass (*Phalaris arundinacea*), found that neither aboveground biomass nor N uptake were increased with a subsurface incorporation of manure, regardless if the manure was raw or anaerobically digested; however, the dry matter was <4% for slurries in their study and the authors attributed the absence of differences between methods to plant-growth disturbances caused by injectors.

Nitrous oxide intensity behaved consistently with all the results discussed above, being affected by the interaction between source and method (Table 4.3) and injected AD was the practice with the least N_2O intensity compared to the other treatments (Fig. 4.5 B). This was due not because AD injection promoted higher grain yields, but because it promoted quite lower N_2O emissions than the other treatments as discussed above (Fig. 4.5 B). Therefore, our results suggest that grain yields obtained with fall applied AD are not improved with injection; however, the use of fall-injected AD can lead to lower N_2O intensity.

4.5 Conclusions

Our hypothesis was refuted, since AD produced similar or higher N_2O emissions than RM with SB and SBI treatments, respectively. However, AD was proven to reduce cumulative N_2O emissions when it was fall injected into the soil. Manure properties such as NH_4^+ concentration, infiltration rate and viscosity should be taken into account when deciding the source of N to be land applied. The injection of AD not only helps to mitigate N_2O emissions when properly applied but also results in corn yields with a low N_2O intensity. Since our conclusions are applied to wet years in cold climates, the response of N_2O emissions and yield during dry years should be considered in future studies.

CHAPTER 5: SHORT AND LONG TERM ANALYSES OF MANURE MANAGEMENT PRACTICES FOR N_2O MITIGATION IN CORN

5.1 Introduction

Nitrous oxide (N_2O) is a greenhouse gas of increasing importance due to its high warming potential (Ravishankara *et al.* 2009). Nitrous oxide is primarily emitted by agricultural soils (Hartmann *et al*. 2013) as a by-product of either nitrification or incomplete denitrification (Baggs and Philippot 2010, Butterbach-Bahl *et al*. 2013). In cropland, the addition of nitrogen (N) through liquid manure enhances soil N_2O emissions compared to those of unfertilised soils (Kariyapperuma *et al*. 2011, Halvorson *et al*. 2014, Agendom *et al*. 2014). Even though manure injection is a practice recommended to reduce NH_3 volatilization, it may promote N_2O emissions if warm and wet soil conditions are met (Comfort *et al.* 1988, Chapter 3). Laboratory studies have been developed to explain the processes occurring at the injection zone (Petersen *et al*. 1993, Petersen *et al.* 1996, Markfoged *et al*. 2011). Among the first approaches, Petersen *et al.* (1996) modelled NO_3^- transport in the manure-soil interface and its effects on denitrification, and they concluded that denitrification is limited by low NO_3^- availability during the first week after manure application. In another study, Markfoged *et al*. (2011) simulated manure injection under lab conditions, modeled their results and found that peak N_2O emissions occurred within 7 to 27 h after manure application. However, we are not aware of any models that have been calibrated and validated to predict N_2O emissions after manure injection under field conditions. The consistency of the prediction across different field situations should also be evaluated to better understand the processes controlling N_2O emissions.

Manure management practices such as application timing, method, and source affect N_2O emissions in different ways. Previous studies at Elora, Ontario (Canada) showed that manure application timing does not affect N_2O emissions during 'normal' weather years, rather N_2O emissions are influenced by the application method, the source of manure and the inter-annual variability of soil moisture (Abalos *et al*. 2015, Chapters 3 and 4). A few studies have compared fall and spring application (Rochette *et al.* 2004, Hernandez-Ramirez et al. 2009, Abalos *et al*. 2015, Chapter 3), but considered the inter-annual variability of weather for only a few years (short-term) so that a long-term evaluation with varying weather patterns is also required. Also, the inter-annual variability of weather in the long term under various climatic conditions may modify the effects of potential N_2O emissions mitigation techniques. In particular, the rapid incorporation of broadcast raw manure (RM) into the soil and the injection of anaerobically digested manure (AD) were found to have lower N_2O emissions compared to the injection of raw manure (Chapters 3 and 4) but the effectiveness of these practices may vary with annual variations in weather. In addition, the application of AD has been encouraged as it has decreased denitrification rates due to its low organic carbon (OC) content compared to RM (Pedersen 1999). Therefore, both short- and long-term assessments are needed to better evaluate the effects of manure application timing, method and source on grain yields as well as on N_2O emissions. Long-term field studies are costly and use of properly calibrated and validated process-based models are an alternative to obtain estimates of N_2O emissions over >10 years.

Process-based models were developed to simulate the interaction among climate, soil and agronomic variables. They have been used to predict grain yield as well as to model the dynamics of soil N and greenhouse gases emissions from agricultural soils (Li *et al*. 1992, Giltrap *et al*. 2009). The DeNitrification-DeComposition model (DNDC) belongs to this category of models and it was originally developed to simulate and predict N_2O emissions from cropped soils in the US (Li *et al*., 1992). It has since been used by many research groups, covering a wide range of countries and production systems, and it is in a continuous evaluation-improvement process. Previous Ontario studies have evaluated DNDC using different approaches. For example, Smith *et al.* (2002) evaluated DNDC v. 7.1 for the Ontario environment using data from static chambers, finding that the model predicted manure-induced N_2O emissions in corn reasonably well, albeit they only modelled N_2O emissions for the snow-free season. In another study, Kariyapperuma *et al*. (2011) considered the snow season and used N_2O data estimated with the flux gradient-method. They re-parameterized DNDC v. 9.1 to model freeze-thaw induced emissions over 5 years, although this study modelled N_2O emissions from corn plots receiving inorganic fertilizer. Currently, DNDC has evolved towards a 'family of models' (Global Research Alliance Modelling Platform, http://gramp.org.uk/models/), including a version for Canada environments (DNDC-CAN). This version was calibrated and validated in Elora (Southwestern Ontario), where the response of N_2O emissions and corn yields to fertilization was evaluated using several fertilization times and application practices such as injection of anhydrous ammonia or urea-ammonium nitrate, with a good performance (Abalos *et al*. 2016). To date, no study has evaluated DNDC performance for simulating N_2O emissions after manure injection in corn in comparison to other techniques such as RM incorporation or AD use.

The objectives of this study were: (i) to evaluate the ability of DNDC-CAN to simulate cumulative N_2O emissions and corn grain yields by comparing the model results to measured results over a 3-year period as influenced by manure application timing, method of application and source of dairy manure (RM vs. AD) and (ii) to determine whether the relationships observed over the 3-year period persist over longer simulations using 26 years of historic weather data.

5.2 Material and methods

5.2.1 Model description

The original version of DNDC model was developed to simulate nitrogen and carbon dynamics, with a particular focus on N_2O emissions (Li *et al*. 1992). The core of the latest version of DNDC model (v. 9.5) consists of interactions among sub-routines such as soil thermal-hydraulic flows, organic matter decomposition, nitrification/denitrification and plant growth (Li *et al.* 1992, Giltrap *et al.* 2010). Three groups of agro-ecological drivers are required as inputs by DNDC: (i) climate variables (maximum and minimum temperature, precipitation, wind speed, solar radiation and relative humidity); (ii) soil properties (texture, field capacity, wilting point, bulk density, pH, hydraulic conductivity, mineral N and organic carbon); and (iii) land use and agricultural practices (crop rotation, tillage, N fertilizer application details, planting and harvest dates) (Li *et al.* 1992, Kariyapperuma *et al.* 2011).

Currently, there is a Canadian version of DNDC (DNDC-CAN), which was derived from the standard DNDC (Li *et al*., 1992) and adapted for Canadian agro-ecosystems. DNDC-CAN was originally denominated as DNDC-CSW and included modified equations to predict wheat biomass growth, N partitioning in the plant and the effects of water stress on wheat yield (Kröbel *et al.* 2011). Following this initial version, other developments were included in DNDC-CAN to simulate corn and soybean growth under heat stress or elevated CO_2 conditions (Smith *et al*. 2013, Uzoma *et al*. 2015). The latest algorithms incorporated to DNDC-CAN include routines to improve the water balance (Grant, pers. comm.). As the recent versions of DNDC as well as DNDC-CANs include an option for simulating manure application within the component of crop management, requiring inputs such as manure application date, application depth, dry matter concentration, N rate, C:N ratio, manure pH and NH_4^+ concentration. Even though, both models can simulate as manure injection as well as manure incorporation, the procedure is different in both cases: DNDC simulate injection and incorporation as direct options, while in DNDC-CAN, injection is a direct option and incorporation is generated after broadcasting manure and tilling the soil on the day of manure application (Grant pers. comm.); all these options are available within the 'farm practices' dialog box. For our study we simulated manure injection at 20 cm depth and incorporation at 12 cm depth. Further details about differences between DNDC and DNDC-CAN can be found in the Global Research Alliance Modelling Platform (http://www.gramp.org.uk), in Kröbel *et al.* (2011), and in Congreves *et al*. (2016).

5.2.2 Field experimental measurements

In this study, we used measured daily N_2O emissions, soil water content (SWC) and corn yield from an experiment conducted at Elora, ON, Canada (43.85º N, -80.42º W) during the period November 2011 – November 2014, where two application times (fall, spring), three methods (injection, surface broadcasting, incorporation) and two manure sources (RM and AD and) were evaluated (Chapters 3 and 4). The measurements also included plots with no N applied (control plots), which were used to calibrate the model according to the protocol described in Li (2013). Model calibration was performed for every year of the study, since corn was planted in a new site every year, resulting in control plots with different characteristics every year. The site was changed every year to emulate farmer's decisions with soybeans grown as the previous crop to corn in 2011 and barley in 2012 and 2013. Site characteristics for every year including soil organic carbon and bulk density, and management details such as previous crop, planting dates, corn hybrid, and manure application rates are given in Table 5.1.

Table 5.1. Soil, crop management and manure data used in DNDC v. CAN simulations.BD: bulk density. SOC: soil organic carbon. RM: raw manure. AD: Anaerobically digested manure. DM: dry matter content. TAN: total ammoniacal nitrogen

Soil characteristics and crop management data								
Year	BD	SOC	Clay	Soil pH	Previous crop	Corn hybrid	Planting date	Harvest date
	g cm^{-3}	%	%					
2012	1.35	1.85	18.5	7.7	Soybean	39 D85	10-May-12	25-Oct-12
2013	1.53	1.25	16.2	7.8	Barley	DKC39-97RIB	17-may-13	27-Oct-13
2014	1.30	2.14	19.6	7.8	Barley	DKC39-97RIB	27-may-14	17-Nov-14
Manure data								
Year	Timing	Date	Type	DM	pH	TAN	N rate	
				%		%	kg N ha^{-1}	
2011	Fall	24-Nov-11	RM	6.7	7.2	0.13	116	
2012	Spring	24-Apr-12	RM	6.4		0.26	160	
2013	Fall	15-Nov-12	RM	7.6	6.9	0.13	182	
			AD	4.2	6.6	0.15	127	
	Spring	16-May-13	RM	4.0	7.4	0.08	90	
			AD	3.6	8.2	0.09	123	
2014	Fall	13-Nov-13	RM	6.4	7.2	0.13	209	
			AD	5.6	7.5	0.17	228	
	Spring	26-May-14	RM	9.2	6.9	0.18	218	
			AD	5.6	6.9	0.50	317	

Nitrous oxide fluxes were measured using non-steady state circular chambers consisting of a partially buried PVC collar (44.2 cm inner diameter X 6-10 cm height aboveground) and a lid (8.3 cm thick), which was also made of the same material but covered with insulating material. Gas sampling was performed on 39, 34 and 34 events for 2012, 2013 and 2014, respectively. Nitrous oxide samples were collected in sealed vials and analyzed using a gas chromatograph (3800 GC, Varian, Mississauga, ON, Canada) fitted with a Combi-PAL auto-sampler as described by Drury *et al*. (2006). Fluxes were calculated as $F = \frac{dC}{dt} * \frac{V}{A} * \frac{pM}{RT} * k$, where F is the

N_2O flux in g N_2O-N ha^{-1}, $\frac{dC}{dt}$ is the change of N_2O concentration over time in µg N_2O-N g gas^{-1} min^{-1}, V is the volume of chamber headspace in L , A is the chamber area (m^2), p is the barometric pressure (Pa = J m^{-3}), M is the molar mass (N_2O-N = 28 g mol^{-1}), R is the Universal Gas Constant (8.314 J $mol^{-1}K^{-1}$), T: absolute temperature (K) and k= constant (14.4 m^2 min $ha^{-1}d^{-1}$) to obtain the flux in g N_2O-N ha^{-1} d^{-1}. The slope $\frac{dC}{dt}$ was calculated through a hybrid method which combined linear and quadratic approaches and Hutchinson-Mosier equation (1981). Further details about the chambers, experimental design and the flux calculation method can be found in Chapter 2.

Soil volumetric water content (SWC) was measured on 8 subsamples per plot during every gas sampling date with a portable time domain reflectometer (TDR; model TDR 300, Spectrum Technologies, Inc., Plainfield, IL, USA) for the 0-12 cm depth layer, except during the cold months, due to snow presence and frozen soil conditions.

Daily maximum and minimum air temperature, precipitation, solar radiation, wind speed and air relative humidity were collected from the Elora Research Station weather station, located less than 500 m from the experimental plots.

5.2.3 Model calibration and validation

5.2.3.1 Calibration procedure

The control plots were used to calibrate the model and the values predicted by DNDC-CAN were compared to the measured values of soil water content (SWC, cm^3 cm^{-3}, %), and daily N_2O emissions (g N_2O ha^{-1}) during 2011, 2012, 2013 and 2014. The calibration was performed in an iterative fashion as suggested by Li (2013), inputting datasets derived from measured and reference values (Table 5.2) and reading the outputs with R-Studio (2015). Given that the site changed every year and there were different previous crops per site as mentioned above, we simulated a soybean-corn sequence for calibrating the model in 2011/12 and a barley-corn sequence for 2012/13 and 2013/14. Each simulation was initialized considering model default values for soil ammonium (NH_4^+) and soil nitrate (NO_3^-) concentration for each soil. The management of the previous crop to corn was assumed to be under a best agricultural practices scheme (OMAFRA 2009), and inputs such as planting date, fertilization and maximum grain yield were based on variety trial results conducted at the Elora Research Station (OOPSCC 2012, OCCC 2013, OCCC 2014). Parameters such as fraction of residues left by the previous crop, soil field capacity (FC), wilting point (WP) and corn water demand were calibrated (Table 5.2). Measured and reference values were used for corn parameters such as maximum biomass, biomass partitioning, C:N ratio and thermal requirements (Table 5.2).

Table 5.2.Soil and crop parameters used as input for DNDC v. CAN calibration.

Sub-model	Parameter	Units	Used values	Sources / References
Soil	Field capacity (WFPS)	%	93[a], 77[b], 55[c]	Fallow et al. 2003, McCoy *et al*. 2006, Von Bertoldi (pers. com.)
	Wilting point (WFPS)	%	22	
	Hydraulic conductivity	$m\ h^{-1}$	0.025	Model default
Crop (Corn)	C concentration	$kg\ C\ kg\ DM^{-1}$	0.42	Jarchow and Liebman. 2012
	Maximum biomass production			
	Grain	$kg\ DM\ C\ ha^{-1}\ yr^{-1}$	4700-5800	Calculated from measured yield, own data
	Leaves + shoot		1200-1400	Jarchow and Liebman 2012
	Root		180	
	Biomass fraction			
	Grain		0.49-0.59	Calculated from measured biomass, own data
	Leaves + shoot		0.36-0.43	
	Root		0.05	Dietzel 2014
	C:N ratio			
	Grain		60	Jarchow and Liebman 2012
	Leaves + shoot	%	77	
	Root		25	Dietzel 2014
	Annual N demand	kg N ha-1 yr-1	140[a], 150[b], 180[c]	De Bruin *et al*. 2013
	Thermal degree days to maturity	ºC day	2070	www.pioneer.com , www.deekalb.ca
	Optimum temperature	ºC	30	Sanchez *et al*. 2014.
	Annual water demand	$g\ water\ g\ DM^{-1}$	200[a], 150[b,c]	Sadras *et al* 2010

[a]: value for 2012, [b]: value for 2013, [c]: value for 2014

After each DNDC run, a R-Studio script read DNDC outputs and calculated root mean square error (RMSE = $\sqrt[2]{\frac{1}{n}\sum_{i=1}^{n}(S_i - M_i)^2}$, where S_i is the ith simulated value and M_i is the ith measured value) and normalized RMSE (NRMSE = RMSE / r * 100, where r is the difference between maximum and minimum measured values) for the response variables (SWC and daily N_2O emissions). The process was considered to be finished when the combination of inputs tested lead to the smallest NRMSE.

5.2.3.2 Validation strategy

The validation was focused on the ability of DNDC-CAN to simulate daily N_2O emissions following manure injection. The injection routine of DNDC v. CAN was evaluated for each application time (fall or spring), source (RM or AD) and site / year combination. Prediction capability of DNDC-CAN for SWC and daily N_2O emissions after manure injection was evaluated using RMSE, NRMSE and the modified index of agreement ($d' = 1 - \frac{\sum_{i=1}^{n}|M_i - S_i|}{\sum_{i=1}^{n}(|S_i - \bar{M}| + |M_i - \bar{M}|)}$ where $\bar{M}$ is the mean of the measured values) (Legates and McCabe 1999). The index of agreement *d*' varies from 0 to 1, with values closer to 1 indicating a better agreement between the predicted and measured values. The reliability of *d*' has been improved by changing the squaring of the sums by the absolute value (Legates and McCabe 1999). Even though *d'* was initially used as a measure of the accuracy of hydro-climatic models, it has also been used to validate yield responses in corn with other models (Hsiao *et al.* 2009, Liu *et al*. 2014). Additionally, we evaluated the performance of the model for predicting daily soil NH_4^+, NO_3^- concentration.

5.2.4 Short-term scenario analysis for cumulative N_2O emissions and grain yield

Six scenarios were set up to verify the trends found in our field studies for cumulative N_2O emissions and grain yield (Chapters 3 and 4), including simulations for manure incorporation method, for the site/year combinations mentioned above. The first 3 scenarios considered the following comparisons: i) fall injected vs. spring- injected RM, ii) fall incorporated vs. spring incorporated RM and iii) injected vs. incorporated RM. The other 3 scenarios were based upon the following comparisons: iv) fall-injected vs. fall-incorporated AD, v) fall injected vs. fall-incorporated RM and vi) AD vs RM. Simulated daily N_2O emissions were time-scaled in R-Studio (2015) to obtain cumulative N_2O emissions for each year / site combination. The averages of three years (2012, 2013 and 2014) were used to perform the comparisons using descriptive stats in scenarios i, ii and iii, while in scenarios iv, v and vi only data from 2013 and 2014 were used. Student's t-test was performed in R-Studio (2015) to compare the means of interest.

5.2.5 Long-term assessment

A long-term simulation period (26 years) was run with DNDC-CAN for the scenarios mentioned above, to assess the consistency of cumulative N_2O emissions over a longer period of time. For this assessment we used fixed application dates and constant manure rates and quality. Manure injection dates evaluated for each timing were November 15 (Fall) and May 9 (Spring), which were based upon the dates used in our field experiments. Manure application rate was fixed at 150 kg N ha^{-1}. Manures differed in dry matter content, 6 % for raw manure and 4 % for AD manure, based on our previous manure quality analysis. To add robustness to the scenarios, we included crop rotations considered for DNDC validation analysis (soybean/corn and barley/corn), performing simulation runs for a period of 26 years, from 1982 to 2007.

Cumulative N_2O emissions and grain yields were simulated for the whole rotation, but analyzed only for the corn phase of the rotation (i.e. half of the years for each rotation system), from November to October. The simulation runs were performed for first (2012) and the third site (2014) since they were the most contrasting environments during field experiments. Weather data for Elora were obtained from Environment Canada website (http://climate.weather.gc.ca/historical_data/search_historic_data_e.html). The outputs of each rotation system were pooled for each year, averaged and exported to R-Studio (2015), totaling a sample size of 13 corn years per scenario. Cumulative N_2O emissions and grain yields were evaluated according to the time series used for the simulation. Averages and standard errors were calculated to compare different scenarios and paired t-tests were performed in R-Studio (2015) to compare means.

5.3 Results

5.3.1 Predicted vs. measured N_2O emissions

Overall, the temporal variability of daily N_2O emissions after manure injection was captured by DNDC-CAN model for RM-injected plots (Fig. 5.1) as well as for AD-injected plots (Fig. 5.2), although with discrepancies in time and the magnitude of the main N_2O emission peaks. In 2012, the predicted emission peak for fall-injection occurred 56 days later than the measured peak and it was overestimated by the model (294 vs. 43 g N_2O-N ha^{-1} day^{-1} for predicted and measured data, respectively); however, spring injection had the reverse scenario with the predicted emission peak occurring 22 days earlier than the measured peak, and being underestimated by the model (62 vs. 224 g N_2O-N ha^{-1} day^{-1}). In 2013, the model tended to underestimate maximum emission peaks for fall-injected RM plots (86 vs. 112 g N_2O-N ha^{-1} day $^{-1}$) as well as spring-injected RM plots (80 vs. 132 g N_2O-N ha^{-1} day $^{-1}$), with few days of temporal discrepancy (Fig. 5.1 C, D). Predicted N_2O emission peak for fall-injection of AD was also overestimated by the model for fall-injection (80 vs. 24 g N_2O-N ha^{-1} day $^{-1}$) and it occurred 44 days later than the measured peak (Fig. 5.2 A). For spring injection of AD in 2013, the model produced an emission peak which was in good agreement with the measured value (262 vs. 279 g N_2O-N ha^{-1} day $^{-1}$) although there was a temporal discrepancy of 21 days between predicted and measured emission peaks (May 21 vs Jun 11, respectively) (Fig. 5.2 B). In 2014, the temporal discrepancies between predicted and measured emission peaks were reduced compared to the previous years; however, the differences between predicted and measured emissions were larger for fall-injected RM (597 vs. 201 g N_2O-N ha^{-1} day $^{-1}$, respectively) (Fig. 5.1 E) and AD (1591 vs. 118 g N_2O-N ha^{-1} day $^{-1}$, respectively) (Fig. 5.2 C). The model produced reasonable estimates of daily N_2O emission peaks for spring-injected RM (252 vs. 271 g N_2O-N ha^{-1} day $^{-1}$) (Fig. 5.1 F), while spring injection of AD produced predicted values that were greater than measured values (732 vs. 252 g N_2O-N ha^{-1} day $^{-1}$) (Fig. 5.2 D).

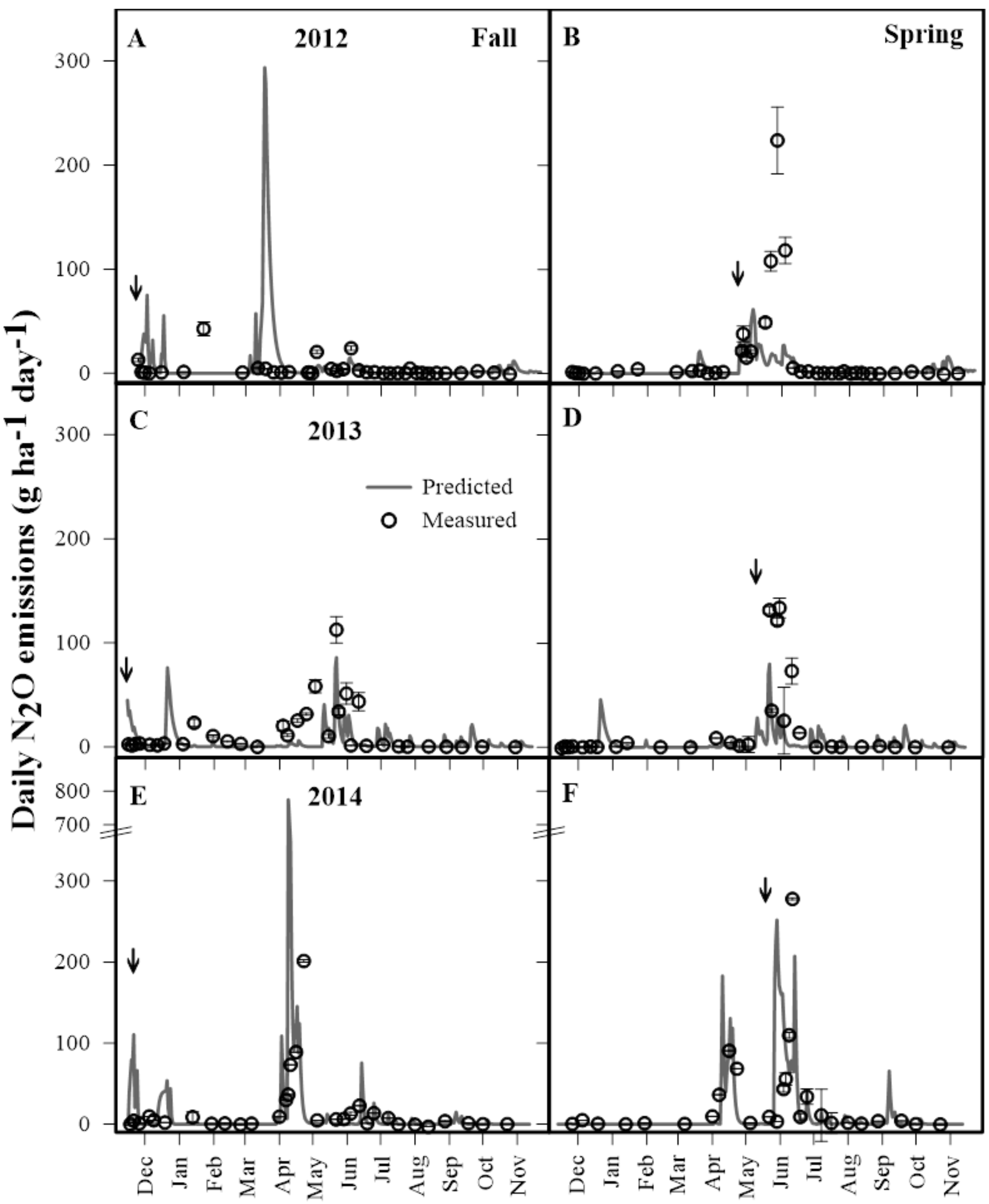

Figure 5.1. Predicted vs measured N_2O emissions after RM injection according to year and timing of application (A, C, E: fall application; B, D, F: spring application). Bars indicate standard error.

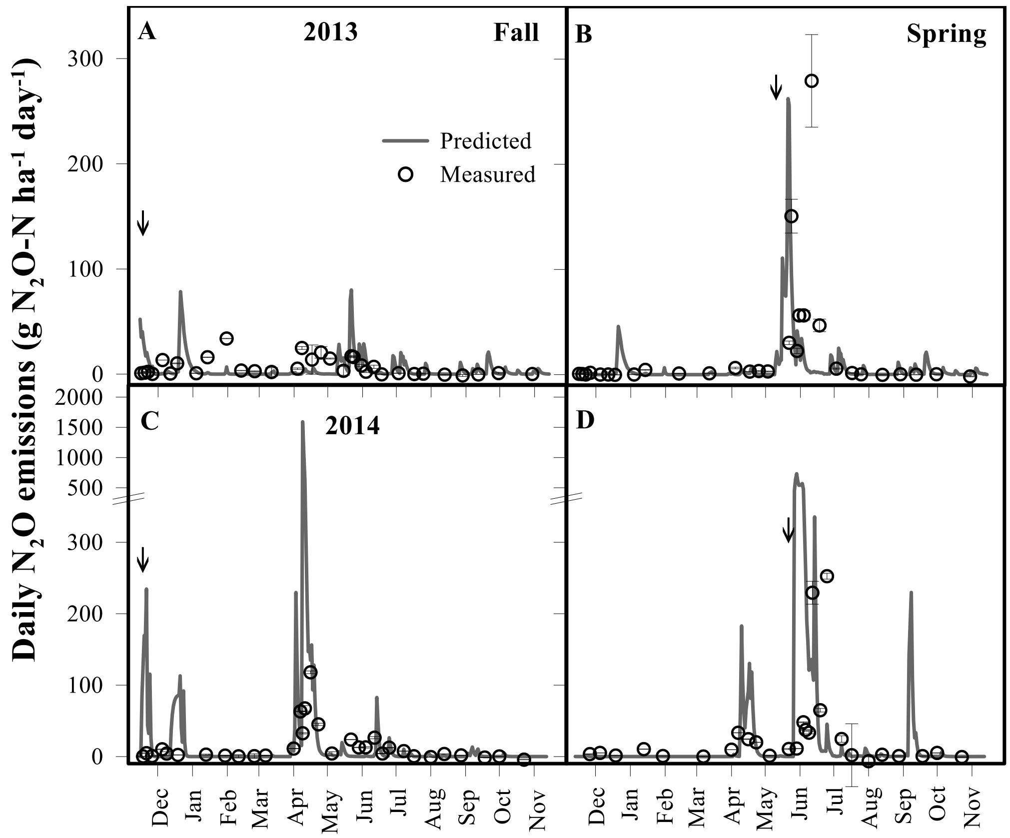

Figure 5.2. Predicted vs measured N_2O emissions after AD injection according year and timing of application. Bars indicate standard error.

5.3.2 Model performance

5.3.2.1 Soil ammonium and soil nitrate simulation

The performance of DNDC-CAN to predict soil ammonium concentration after manure injection varied broadly across sources and years, which was reflected in the range observed for *d'* (0.10 to 0.60) (Table 5.3). The fall injected-RM plots NRMSE varied from 21 % in 2012 to 186 % in 2014 while *d'* varied from 0.17 in 2014 to 0.60 in 2012. Plots fall-injected with AD had NRMSE values of 28 and 77%, while *d'* values were 0.56 and 0.40 for 2013 and 2014, respectively. Spring-injected RM plots had values of NRMSE ranging from 58 % in 2013 to 152 % in 2014 and values of *d'* going from 0.10 in 2014 to 0.33 in 2012. Plots spring-injected with

AD had NRSME values of 71 and 27 %, while *d'* values were 0.37 and 0.44 for 2013 and 2014, respectively (Table 5.3).

The model predicted soil nitrate concentration more accurately than soil NH_4^+, since NRMSE and *d'* showed less variability across sources and years (Table 5.3). Fall-injection of RM had values of NRMSE ranging from 41 % in 2014 to 66 % in 2012, while *d'* went from 0.28 in 2012 to 0.43 in 2014 (Table 5.3). Plots injected with AD in the fall had values of NRSME of 49 and 43 %, with values of *d'* of 0.34 and 0.49 for 2013 and 2014, respectively. For spring-injected RM plots, NRMSE went from 36 % in 2014 to 65 % in 2012, while *d'* varied between 0.32 in 2012 to 0.51 in 2013 and 2014. Plots spring-injected with AD had NRMSE values of 47 and 46 %, respectively, while *d'* was 0.51 in both 2013 and 2014 (Table 5.3).

Table 5.3. Performance of DNDC v. CAN for predicting daily soil ammonium (NH_4^+) and soil nitrate (NO_3^-) after liquid dairy manure injection. Root mean square error (RMSE) is expressed in kg N ha^{-1}. In brackets: RMSE normalized by range (NRMSE). The index of agreement (*d'*) is unit-less.

Year	Timing	Source	Soil NH_4^+ concentration		Soil NO_3^- concentration	
			RMSE	*d'*	RMSE	*d'*
			g NH_4^+-N ha^{-1} day^{-1}	-----	g NO_3^--N ha^{-1} day^{-1}	
2012	Fall	RM	2.53 (20.9)	0.60	27.2 (65.9)	0.28
	Spring		4.87 (131.7)	0.33	27.7 (64.6)	0.32
2013	Fall	RM	7.6 (27.3)	0.49	14.9 (46.7)	0.41
		AD	6.79 (28.0)	0.56	17.2 (48.6)	0.34
	Spring	RM	1.49 (57.8)	0.30	18.3 (43.3)	0.51
		AD	1.61 (71.4)	0.37	32.4 (46.6)	0.51
2014	Fall	RM	16.1 (186.4)	0.17	18.7 (40.6)	0.43
		AD	28.0 (76.9)	0.40	28.5 (42.6)	0.49
	Spring	RM	3.19 (152)	0.10	27.9 (35.9)	0.51
		AD	7.16 (26.8)	0.44	90.0 (45.9)	0.51

5.3.2.2 Soil water content (SWC) and daily N_2O emissions

The model predicted SWC and N_2O emissions with different levels of accuracy, depending on year and site combinations, as well as the manure source and application timing. For SWC, the values of NRMSE ranged from 22.5 % in 2013 (calibration) to 53.2 % in 2012 (validation) and the values of *d'* ranged from 0.33 in 2014 (calibration) to 0.48 in 2013 (calibration) (Table 5.4). Regarding model calibration for daily N_2O emissions, NRMSE ranged from 27.4 % in 2014 to 37.5 % in 2013 and *d'* ranged from 0.45 in 2012 to 0.63 in 2014. Validating DNDC-CAN for daily N_2O emissions after manure injection produced different performances according to the scenario set. The highest value of NRMSE for fall-injection of RM was attained in 2012 (107 %) and the lowest in 2013 (17 %), while the highest and the lowest *d'* occurred in 2013 and 2012 (0.58 and 0.2, respectively). For the validation of spring-injection of RM, NRMSE varied from 19 % in 2012 to 25 % in 2013 and *d'* ranged from 0.58 in 2012 to 0.68 in 2013 (Table 5.4). For fall AD-injected plots, the highest value of NRMSE was attained in 2014 and the lowest in 2013 (243 % and 49 %, respectively) while the highest and lowest *d'* were 0.3 in 2013 and 0.23 in 2014. For spring-injected AD plots, the highest value of NRMSE was attained in 2014 (28 %) and the lowest value was attained in 2013 (20 %) with 2013 having the highest *d'* (0.64) and 2014 the lowest *d'* (0.37) (Table 5.4).

Table 5.4. Performance of DNDC v. CAN for predicting daily volumetric Soil Water Content (SWC) and daily N_2O emissions after liquid dairy manure injection. Root mean square error (RMSE) is expressed in m^3 m^{-3} (SWC) or g N_2O-N ha-1 (N_2O). In brackets: RMSE normalized by range (NRMSE). The index of agreement (*d'*) is unit-less.

Year	Timing	Source	SWC				Daily N_2O emissions			
			Calibration		Validation		Calibration		Validation	
			RMSE	*d'*	RMSE	*d'*	RMSE	*d'*	RMSE	*d'*
			------------ m^3 m^{-3}	------------	------------	------------	g N_2O-N ha^{-1} day^{-1}	------------	------------	------------
2012	Fall	RM	0.16 (53.2)	0.4	0.14 (48.5)	0.47	2.8 (32.0)	0.45	46.4 (106.6)	0.2
	Spring				0.16 (53.2)	0.44			41.9 (18.6)	0.58
2013	Fall	RM	0.05 (22.5)	0.48	0.05 (22.5)	0.47	10.4 (37.5)	0.45	19.1 (16.9)	0.58
		AD							17.2 (48.9)	0.3
	Spring	RM							34.0 (25.5)	0.68
		AD							56.7 (20.2)	0.64
2014	Fall	RM	0.06 (30.7)	0.33	0.06 (30.7)	0.37	17.7 (27.4)	0.63	145.1 (71.0)	0.35
		AD							296.6 (242.5)	0.23
	Spring	RM							66.5 (23.9)	0.62
		AD							69.6 (28.5)	0.37

5.3.3 Short-term scenarios for cumulative N_2O emissions and grain yield

Overall, predicted cumulative N_2O emissions for short term scenarios were in good agreement with measured data (Fig. 5.3); however, DNDC-CAN tended to predict emissions with greater variability for AD applied plots (Figs 5.3 D, F). Predicted cumulative N_2O emissions were similar (t = 0.49, p = 0.65) between spring and fall injection of RM (2.4 ± 1 vs. 3.0 ± 0.8 kg N_2O-N ha^{-1}, respectively) (Fig. 5.3 A) as well as for fall and spring broadcast-incorporated RM (*t* = 0.07, *p* = 0.94) (2.3 ± 0.2 vs 2.1 ± 0.6 kg N_2O-N ha^{-1}, respectively) (Fig. 5.3 B), agreeing with the trend found in the measured values where there was no difference between treatments(Chapter 3; Table 4.5). However, DNDC-CAN tended to underestimate the values of cumulative N_2O emissions for spring-injection (Fig. 5.3 A). Even though simulated injection produced values of cumulative N_2O emissions not significantly different (*t* = 0.88, *p* = 0.4) than simulated incorporation (2.7 ± 0.3 vs 2.2 ± 0.3 kg N_2O-N ha^{-1}, respectively), simulated values tended to replicate the trend of the observed data (Fig. 5.3 C). Predicted values of cumulative N_2O emissions were not different (*t* = 0.53, *p*=0.67) between application methods for AD (4.8 ± 3 vs. 3.0 ± 1.3 kg N_2O-N ha^{-1} for manure injection and manure incorporation, respectively) (Fig. 5.3 D), which differed from the trend found for measured data where injection had lower cumulative N_2O emissions values compared to incorporation (2.5 ± 0.4 vs. 5.4 ± 1.0 kg N_2O-N ha^{-1}, respectively). Even though the difference among treatments was not significant (*t* = 0.66, *p* = 0.61), the trend found for predicted cumulative N_2O emissions comparing between injected and incorporated RM (3.2 ± 1.4 vs. 2.3 ± 0.4 kg N_2O-N ha^{-1}, respectively) was in agreement with the measured data (3.8 ± 0.9 vs. 2.8 ± 0.7 kg N_2O-N ha^{-1}, respectively) (Fig. 5.3 E). There was also no significant difference between sources for predicted values of cumulative N_2O emissions (*t* = 0.76, *p* = 0.48), though AD tended to produce more N_2O emissions than RM (3.9 ± 1.4 vs. 2.7 ± 0.6 kg N_2O-N ha^{-1}, respectively), in agreement with the trend found for the measured values (Fig. 5.3 F).

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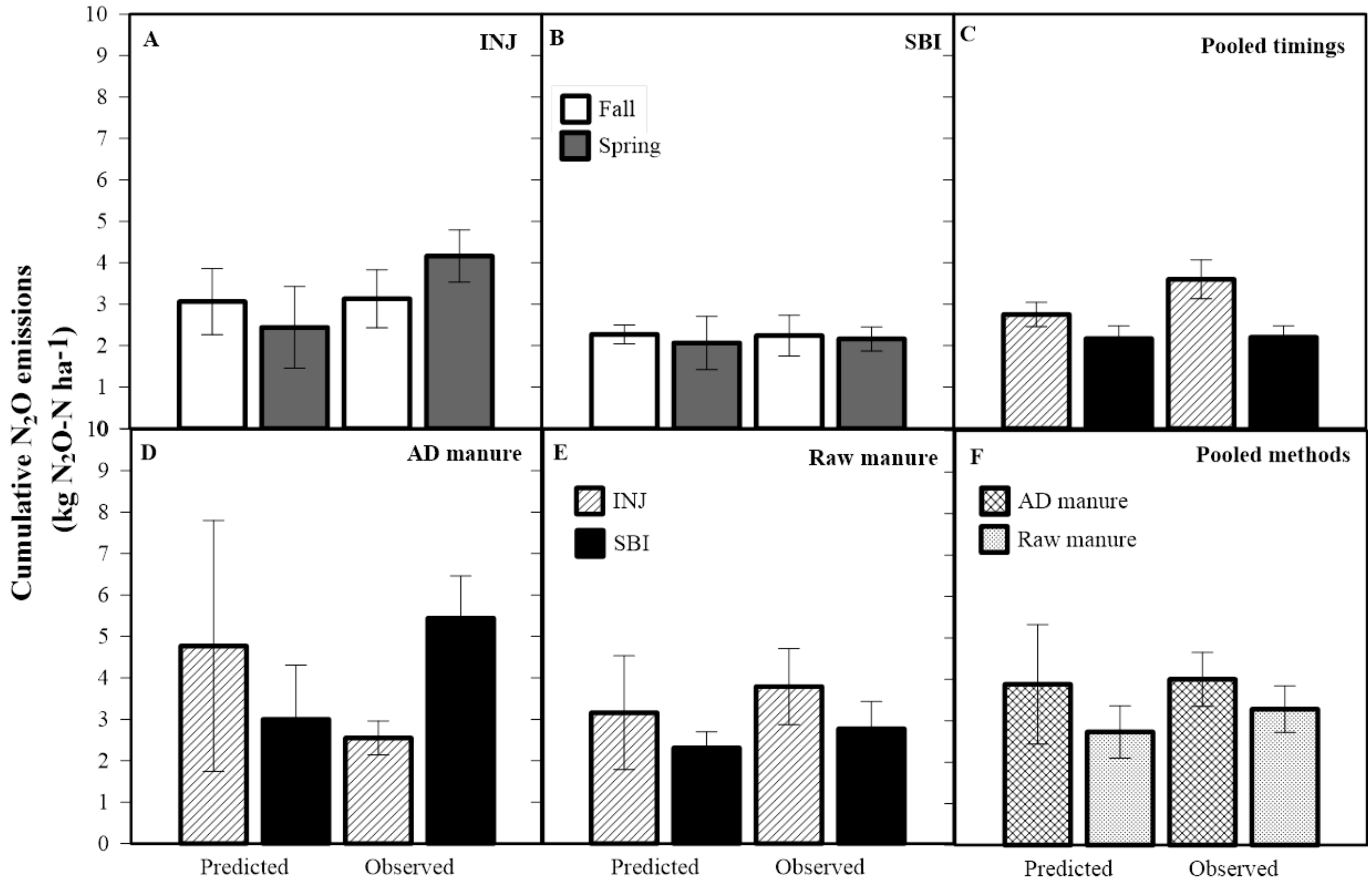

Figure 5.3. Predicted vs. observed year-round cumulative N_2O emissions. A: Fall vs. spring application with manure injection (INJ). B: Fall vs. spring application with manure incorporation (SBI). C: INJ vs. SBI. D: INJ vs. SBI for anaerobically digested (AD) manure. E: INJ vs. SBI for raw manure. F: AD manure vs. raw manure. Bars indicate standard error.

Predicted values of grain yield were smaller (t = -2.84, p = 0.06) for fall- than for spring-injected RM (6.2 ± 0.6 vs. 9.4 ± 1.0 t grain ha^{-1}, respectively) (Fig. 5.4 A) as well as they were smaller (t = -2.7, p = 0.05) for fall- than for spring-incorporated RM (6.5 ± 0.8 vs. 9.8 ± 0.9 t grain ha^{-1}, respectively) (Fig. 5.4 B). These differences were not observed during field experiments as measured yields were similar between fall and spring application for both injection and incorporation treatments. Even though DNDC-CAN underestimated grain yields for fall application, predicted values for spring application were in good agreement with measured values (Fig. 5.4 A and B). Simulated grain yields did not differ (t = 0.59, p = 0.57) between RM injection and RM incorporation methods (7.8 ± 0.9 vs. 8.2 ± 0.9 t grain ha^{-1}, respectively) (Fig. 5.4 C), which was in contrast to measured yields which were higher for injection compared to incorporation (Chapter 3). Simulated grain yields did not differ (t = -0.63, p = 0.6) between injection and incorporation of AD (6.5 ± 1.5 vs. 8.3 ± 2.5 t grain ha^{-1}, respectively) (Fig. 5.4 D) as well as they did not differ (t = -1.15, p = 0.42) for injection and incorporation of RM (5.8 ± 0.4 vs. 7.1 ± 1.1 t grain ha^{-1} for, respectively) (Fig. 5.4 E). This is in contrast with the measured values for AD, where grain yields for injection were higher than those for incorporation (Fig. 5.4 D), but in good agreement with measured grain yields for RM (Fig. 5.4 E). Finally, the model prediction for grain yield did not vary (t = 0.67, p = 0.54) between RM and AD (7.4 ± 1.3 vs. 6.4 ± 0.6 t grain ha^{-1}, respectively) which was in agreement with the trend found for the measured data, albeit the simulated values were lower than the measured values (Fig. 5.4 F).

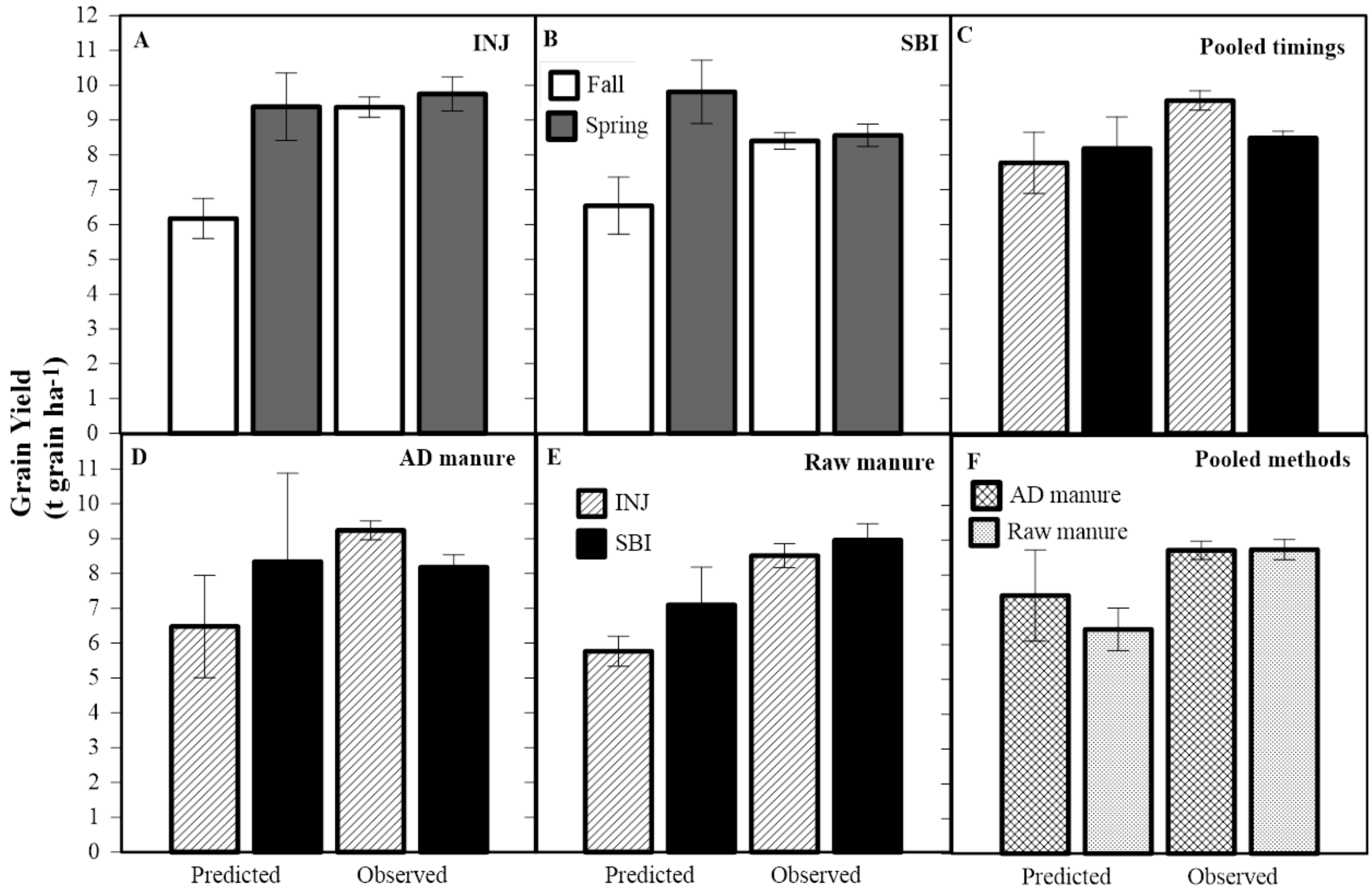

Figure 5.4. Predicted vs. observed grain yield. A: Fall vs. spring application with manure injection (INJ). B: Fall vs. spring application with manure incorporation (SBI). C: INJ vs. SBI. D: INJ vs. SBI for anaerobically digested (AD) manure. E: INJ vs. SBI for raw manure. F: AD manure vs. raw manure. Bars indicate standard error.

5.3.4 Long term assessment

The values of predicted cumulative N_2O emissions resulted in a different trend for the long term assessment than what was observed in the short-term. For RM injection, the model simulated higher values of emissions for fall than for spring (t = 5.83, p<0.01) (5.7 ± 0.4 vs. 2.9 ± 0.3 kg N_2O-N ha^{-1} yr^{-1}), and the same difference occurred for simulated N_2O emissions values of RM incorporation (t = 3.51, p<0.01) (5.5 ± 0.3 vs. 3.3 ± 0.3 kg N_2O-N ha^{-1} yr^{-1} for fall and spring application, respectively) (Fig. 5.5 A). Also, there was no difference (t= -0.23, p = 0.82) between RM injection and RM incorporation (4.3 ± 0.3 vs. 4.4 ± 0.3 kg N_2O-N ha^{-1} yr^{-1}, respectively). When simulated N_2O emissions were observed across simulation years, the gap between application times was negligible in 1987 and also decreased in 2003, 2005 and 2007 (Fig. 5.5 C). Even though cumulative N_2O emissions produced by AD and RM did not differ over the long term, AD tended to have a higher value of cumulative emissions than RM (6.6 ± 0.9 vs. 5.6 ± 0.5 kg N_2O-N ha^{-1} yr^{-1}, respectively) (Fig. 5.5 B). Within the AD application scenarios, incorporated AD produced higher N_2O emissions than injected AD (t = -2.2, p = 0.03) (7.9 ± 0.9 vs. 5.6 ± 0.5 kg N_2O-N ha^{-1} yr^{-1}, respectively), while application methods did not differ (t = 0.3, p = 0.77) between RM application scenarios (Fig. 5.5 B). Cumulative N_2O emissions produced by incorporated AD surpassed those of injected AD especially in 1985, 2001 and 2003 (Fig. 5.5 D).

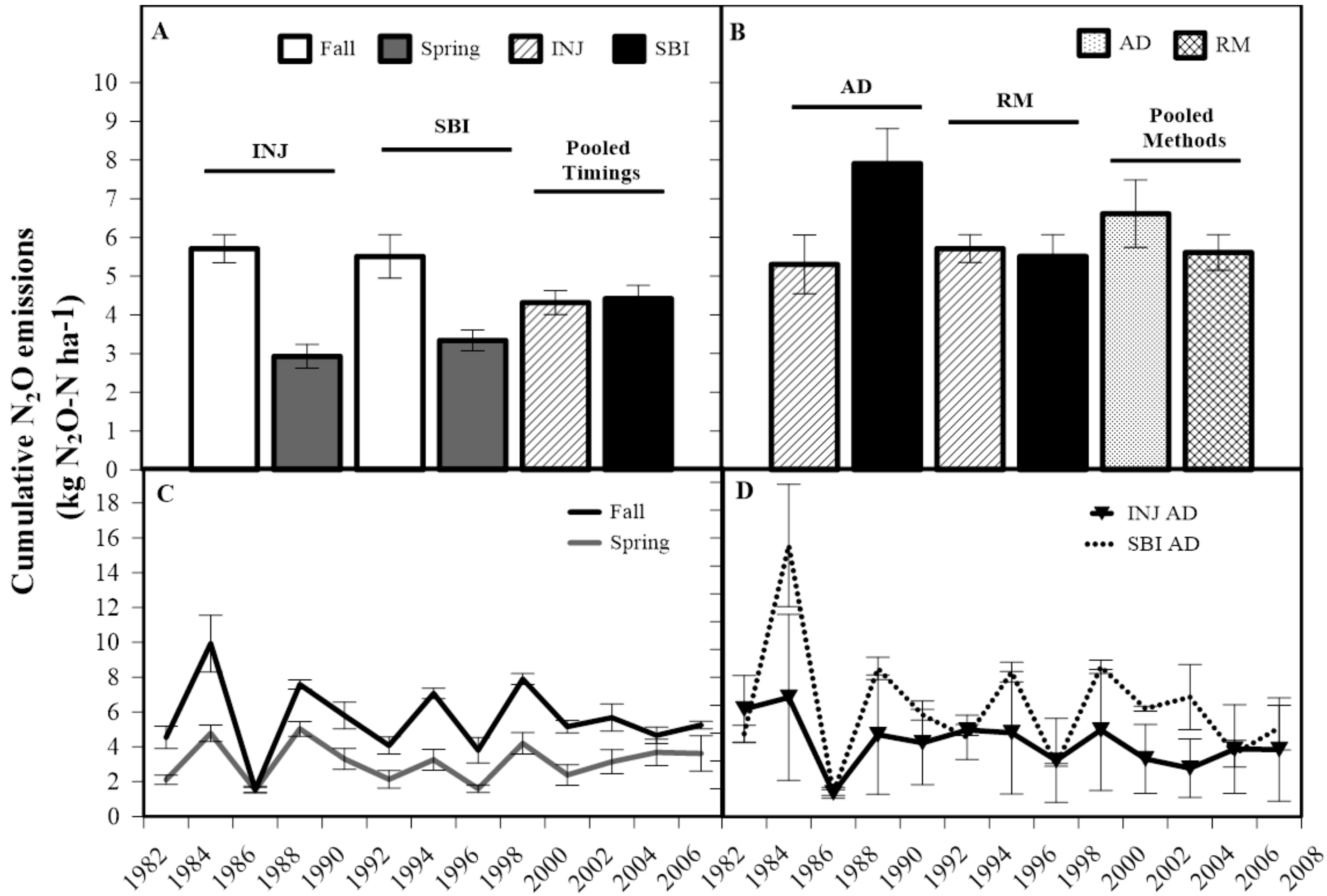

Figure 5.5. Cumulative N_2O emissions for long term scenarios according to (A) timing and method, (B) manure source and (C, D) year. RM: raw manure. AD: anaerobically digested manure. Error bars indicate standard error.

The model simulated higher values of grain yield for spring application than for fall application of manure as for injected RM (t = -8.4, $p < 0.01$) (7.3 ± 0.2 vs. 5.3 ± 0.2 t grain ha^{-1}) as well as for incorporated RM (t = -7.97, $p < 0.01$) (7.5 ± 0.2 vs. 5.4 ± 0.1 t grain ha^{-1}) (Fig. 5.6 A); however, when data were pooled by method similar grain yields were obtained for injected and incorporated manure (6.3 ± 0.2 vs. 6.5 ± 0.2 t grain ha^{-1}, respectively) (Fig. 5.6 A). Predicted grain yields for spring application were consistently greater than those of fall application across the years (Fig. 5.6 C). Grain yields did not differ between methods within the AD long-term scenario (t = -0.97, p = 0.34) nor did it for the RM long term scenario (t = -0.38, p = 0.7); however when data were pooled by source, higher grain yields were obtained for AD compared to RM (t= -5.6, $p < 0.01$) (5.9 ± 0.2 vs. 5.4 ± 0.1 t grain ha^{-1}, respectively). Anaerobically digested manure produced the highest grain yield for most of the years of the simulation period (Fig. 5.6 D).

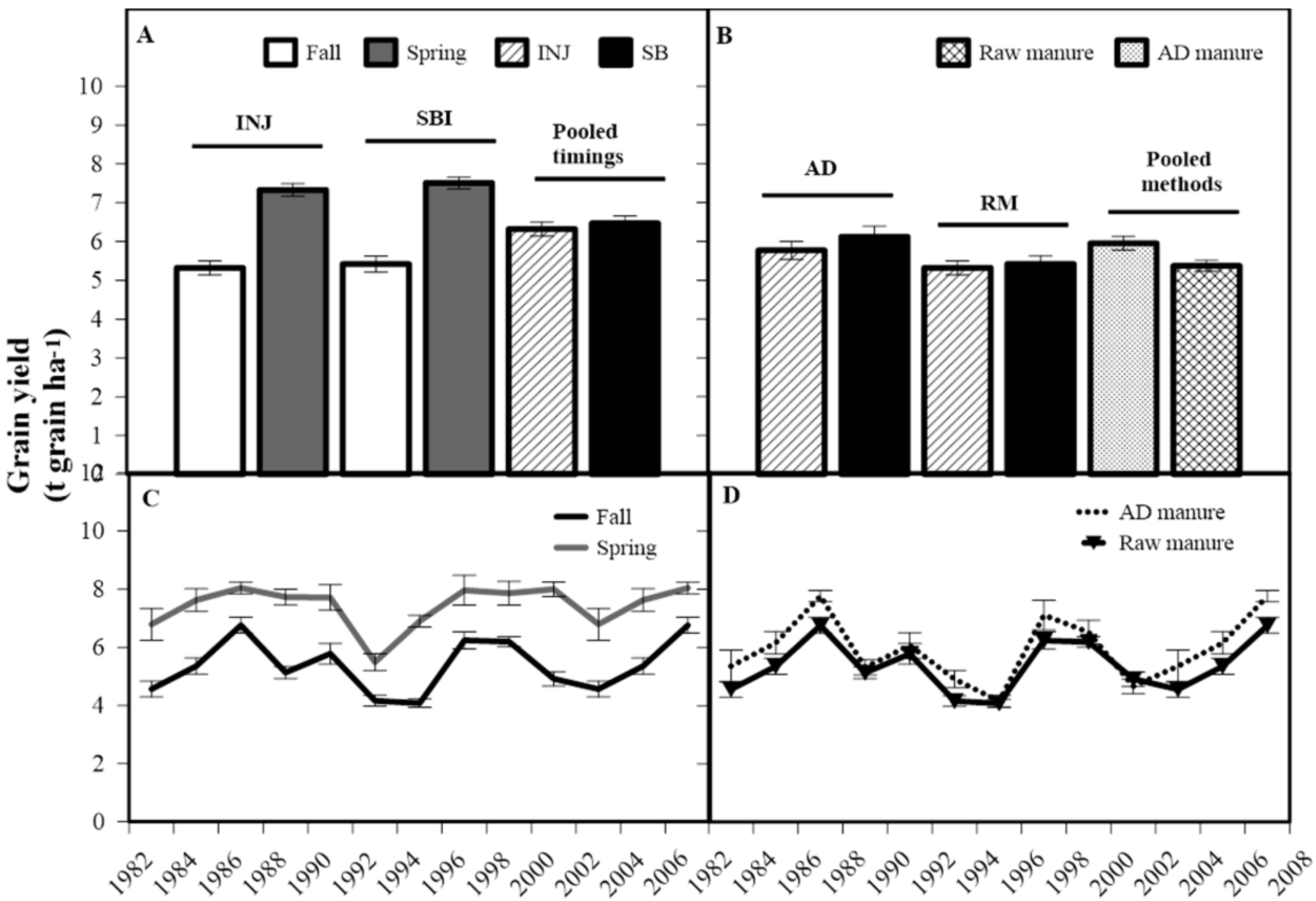

Figure 5.6. Grain yield for the scenarios according to (A) timing and method, (B) manure source and (C, D) year. RM: raw manure. AD: anaerobically digested manure. Error bars indicate standard error.

5.4 Discussion

5.4.1 Model performance

Model calibration resulted in values of RMSE comparable to other studies for daily N_2O emissions as well as for SWC. Our values of RMSE for daily N_2O emissions obtained with the calibration set (3.8 - 17.7 g N_2O-N ha^{-1} day^{-1}, Table 5.4) were lower than those obtained by Abalos *et al.* (2016) (39.6-68.3 g N_2O-N ha^{-1} day^{-1}) with DNDC-CAN at the same study site. This difference may be due to the use of unfertilized plots as the calibration set for every site-year combination, while Abalos *et al*. (2016) used data from fertilized plots from a particular year to calibrate the model. Our results indicate that the calibration protocol used in our analysis (Li 2013) improved the accuracy of predictions. For SWC, we obtained values of RMSE (0.05 – 0.14 m^3 m^{-3}, Table 5.4) that were similar to those obtained with DNDC-CAN by Abalos *et al.* (2016) (0.05-0.09 m^3 m^{-3}) and Congreves *et al.* (2016) (0.05 m^3 m^{-3}) for the same soil type but lower than that of Uzoma *et al.* (2015) (0.27 m^3 m^{-3}). Therefore, our results suggest that using data from control plots in the calibration procedure is a good strategy to provide a reasonable adjustment in DNDC-CAN. .

Overall, the calibrated model was able to capture the data variability and to predict daily N_2O emissions after manure injection; however, simulated N_2O emissions for fall-injected manure were greater than the measured values, particularly during dry years (Fig. 5.1, Fig. 5.2, Table 5.4). The values of RMSE (17.2 - 296.6 g N_2O-N $ha^{-1}day^{-1}$) (Table 5.4) in our validation set, were higher than those obtained by Abalos *et al.* (2016) (25.2 - 53.5 g N_2O-N ha^{-1} day^{-1}) and lower than that found by Beheydt *et al*. (2007) for corn (340 g N_2O-N ha^{-1} day^{-1}). However, if the highest RMSE in our study was not considered, then the range of values obtained (17 - 70 g N_2O-N ha^{-1} day^{-1}) was similar to Abalos *et al*. (2016). The high RMSEs attained in our study were primarily associated with fall-injection, as the N_2O emissions peaks during the subsequent spring-thaw period following fall injection were overestimated by the model in both 2012 when we compared simulated and measured fluxes (294 vs. 43 g N_2O-N ha^{-1} day^{-1}, respectively) and 2014 (600 - 1600 g N_2O-N ha^{-1} day^{-1} for simulated data vs. 120 - 200 g N_2O-N ha^{-1} day^{-1} for measured data) (Fig. 5.1 E and Fig. 5.2 B). We also found that the model did not simulate an increase in N_2O emissions following sudden changes in soil temperature, suggesting not only a revision of heat transfer equations of DNDC-CAN, but also an evaluation of how a sudden soil temperature increase affects other drivers such as soil moisture and mineral N content. Furthermore, the use of RMSE and *d'* for measuring model performance is limited in situations where the model largely overestimates measured N_2O emissions. These results may be due to inaccurate estimation of the microbial biomass (C_{bio}) and denitrifier growth rate (*dB/dt*) at spring thaw. Both C_{bio} and the denitrifier growth rate are used to simulate denitrifier activity and N_2O production, and they are controlled by the C assimilation through OC decomposition or through the consumption of soluble carbon left by dead microorganisms (Li *et al*. 1992). In our study, predicted N_2O emissions for fall application in the site with OC = 1.25% (2013) had a smaller NRMSE than sites with OC=1.85% (2012) and 2.14% (2014) (Table 5.4), regardless of the different N rates used for RM and AD in 2013 (Table 2). Furthermore, the largest C_{bio} overestimation occurred during the coldest year, when large concentrations of dissolved OC (DOC) and NH_4^+ may have simulated N_2O emissions exceedingly larger than measured N_2O emissions, as found by Kariyapperuma *et al.* (2011). Therefore, our results suggest that model parameters representing the response of denitrifiers activity should be adjusted depending on OC thresholds.

The model predicted soil NO_3^- concentrations (*d'*= 0.28 - 0.51) more accurately than soil NH_4^+ concentration (*d'* = 0.10 - 0.60) (Table 5.4). In addition, the lower RMSEs obtained for NH_4^+ and NO_3^- on the site with OC=1.25% compared to the other sites (Table 5.4) suggested again the need of consider OC thresholds for the algorithms. This supports the concepts discussed above that an overestimation of DOC by the model in sites with high OC also produces an overestimation of NH_4^+ and NO_3^-, given that DOC is an input used to estimate NH_4^+, and NH_4^+ is an input used to estimate NO_3^- (Li *et al*. 1992, Kariyapperuma *et al*. 2011).

5.4.2 Predicted cumulative N_2O emissions and grain yield in short term scenarios

Overall, the model could predict cumulative N_2O emissions trends in the short term, confirming the absence of manure application timing effect (Fig. 5.3 A, B) as well as the larger cumulative N_2O emissions for injection method compared to incorporation method as shown in our previous studies (Fig. 5.3 C) (Chapters 3 and 4). However, there was a discrepancy between predicted and measured cumulative N_2O emissions for spring-injection. Predicted cumulative N_2O emissions was slightly lower for spring-injection than for fall injection, while measured cumulative N_2O emissions was higher for spring injection than for fall injection (Fig. 5.3 A). This difference between predicted and measured cumulative N_2O emissions for spring injection is likely linked to the underestimation of the main N_2O emission peak, and it is also probably related to how the sudden changes of soil temperature interacted with other drivers, as previously discussed. The DNDC-CAN predicted that cumulative N2O emissions were greater with fall applied AD injection compared to fall incorporated AD, however the measured values were opposite with larger emissions for the fall incorporated AD. (Fig. 5.3 D). Likely the model did not accurately take into account the viscosity of AD during the fall and it likely underestimated NH_4^+ transport rate outwards the injection zone (Petersen 1999, Frey *et al*. 2012) which resulted in an overestimation of nitrification rate in the injection zone. Thus, the additional concentration of N and C added to the additional water coming with the manure and the C fraction mineralized from the organic matter increased denitrification rates in the injection zone (Markfoged *et al.*2011). Furthermore, the model underestimated cumulative N_2O emissions for AD incorporation, which may have been linked to an overestimation of substrate loss through NO_3^- leaching, based upon the low performance of the model to predict soil NH_4^+ as well as NO_3^- concentrations (Table 5.3) (Beheydt *et al*. 2007).

The model was able to simulate the cumulative N_2O emissions trend for injected and incorporated RM in the fall-applied RM scenarios (Fig. 5.3 E), and it was also able to predict the difference between AD and RM sources (Fig. 5.3 F). This is in agreement with our previous study, where cumulative N_2O emissions for injected RM were slightly greater than incorporated RM and emissions for AD were slightly greater than RM, though the differences were not statistically significant (Chapter 4). Our results suggest that DNDC-CAN has a reasonable performance to simulate cumulative N_2O emissions in the short term, although an improvement is needed to simulate emissions with fall-injected and incorporated AD, likely by improving simulation of soil N mineralization and nitrification, and microbial biomass changes under rapid changes in temperature.

Overall, DNDC-CAN captured the yield variability for Elora site, since the range of yields predicted for the short term scenarios (5.2-.9.8 t grain ha^{-1}) was similar to that obtained by Roy *et al*. (2014) (5.5-10.9 ha^{-1}) at the same study site. The model systematically underestimated grain yield for fall application of both RM (Fig. 5.4 A, B) and AD (Fig. 5.4 D, E), simulating grain yields 2 – 3 t lower than measured yields (Fig. 5.4 A). Overestimation of the NO_3^--N leaching losses from fall applied manure before corn planting could potentially explain these results. For example, the model simulated nitrate concentrations of 1.5, 0 and 3.8 kg NO_3^--N ha^{-1} for the sampling dates following spring thaw in 2012, 2013 and 2014, respectively, while the measured values were much higher at 49, 41 and 48 kg NO_3^--N ha^{-1}, respectively (data not shown); so that DNDC predicted that most of the nitrate was leached after a fall application of manure. Even though DNDC was calibrated for nitrate leaching in corn in several studies (Li *et al*. 2006, Tonitto *et al*. 2007, Deng *et al*. 2011, Li *et al*. 2014), they all measured and simulated NO_3^- leaching considering only a spring application of inorganic fertilizer, so that model performance for simulating leaching after a fall-injection to corn remains unexplored. Our results suggest that DNDC-CAN's performance for simulating grain yield after a fall application of manure is limited and that an improvement should be performed for the algorithms calculating NO_3^- leaching during the spring period prior to the cropping season.

5.4.3 Long term assessment

The magnitude of cumulative N_2O emissions predicted for fall-injection of manure in our study were lower (5.5-5.7 vs. 8.0 kg N_2O-N ha^{-1} yr^{-1}) than those predicted for inorganic fertilizer injection by Abalos *et al*. (2016), while predicted cumulative N_2O emissions for spring injection were higher (2.9-3.3 vs. 2.5 kg N_2O-N ha^{-1} yr^{-1}) than those from Abalos *et al*. (2016). This difference is likely due to the form of N used and the application depth, since Abalos *et al*. (2016) used an inorganic source of N injected at 10 cm depth, while in our study we used dairy manure as N source, applied at 20 cm depth. The greater depth of injection in our study increased the chances that N_2O was retained for longer into the soil and converted into N_2, thereby decreasing the amount of N_2O emitted (Markfoged *et al*. 2011). Trends in predicted cumulative N_2O emissions for the long term were different than those for the short term, with higher cumulative N_2O emissions predicted for fall than for spring application, and no difference between manure application methods. This difference between fall and spring application agrees with the premise that cumulative N_2O emissions induced by fall manure application are expected to be higher than for spring application, given the longer gap between N input and N utilisation by the crop with fall application (Venterea *et al*. 2012). The disagreement between short term and long term trends also reveals the role played by the inter-annual variability of weather, which was reflected in the predicted N_2O emissions for the period from 1982 to 2007 (Fig 5 C), confirming the importance of the interaction between manure application time and year which was found in our previous study (Chapter 3). As for the sources behaviour during the long-term, injection of AD did mitigate N_2O emissions compared to AD incorporation (Fig. 5.5 B), agreeing with our previous study at Elora (Chapter 4). Dry-wet cycles in the soil promote N_2O emissions (Granli and Bockman 1994, Butterbach-Bahl *et al*. 2013) which was also found in the model predictions especially for years with dry-wet cycles such as 1984-1985. The differences between injection and incorporation of AD for cumulative N_2O emissions were not consistent among years (Fig. 5.5 D), suggesting an interaction between year and method, which was not detected in Chapter 4. Therefore, our results suggest that presumably spring application of manure is a useful technique for N_2O mitigation but this effect is subject to the inter-annual variability and can only be detected in the long-term simulations. Inter-annual variability in the predicted cumulative N_2O emissions values override the impact of application method on the long term. Finally, injection of AD was found to be a good technique to mitigate N_2O emissions in the long term.

The yield trends predicted by DNDC-CAN for the short term were consistent with the long term simulations, and the predicted corn yields for spring application were found to be higher than those in the fall application regardless of the application method (Fig. 6 A), which was consistent across years (Fig. 6 C). This is because the model likely assumes that N is captured more efficiently after spring-manure injection compared to fall-injection. The model assumed a higher corn N use efficiency for spring injection (NUE= of ~29-31 kg grain kg N applied^{-1} ha^{-1}, not shown) than for fall injection (NUE = ~16-19 kg grain kg N applied^{-1} ha^{-1}, not shown). The decrease in NUE for fall-injection is based upon N losses during the period from manure application date to planting date (64-67 kg N ha^{-1}, not shown) and ~58-61 % of this loss was attributed to NO_3^- leaching and ~8-9 % to N_2O emissions (data not shown). These predicted N losses are consistent with what was found for the validation scenarios and they were related to the uncertainty of NO_3^- and N_2O prediction after a fall-injection of manure. The model systematically underestimated crop yields for fall manure application, which was likely due to the overestimation on N losses between fall application and crop planting.

5.5 Conclusions

DNDC-CAN is a useful tool to simulate the effects of manure injection on daily and cumulative N_2O emissions in corn, given that its performance, based on calibration in control plots, was comparable to other studies. However, denitrification rates during spring thaw were overestimated by DNDC-CAN when soils having a large concentration of organic C received manure during fall. Predicted short and long term trends for cumulative N_2O emissions were contradictory for some scenarios, confirming the influence of the inter-annual variability of weather on the evaluated treatments. For example, spring application of manure is a useful technique for N_2O mitigation but it benefit expresses more or less from year to year as a function of weather conditions; nevertheless, its global benefit becomes more obvious in the long-term . The inter-annual variability also negated the effects of method of application in the long term compared to the short-term. Finally, injection of AD was projected to be a good technique to mitigate N_2O emissions in the long term. The model simulations for grain yields after fall application of manure manure application appeared to be underestimated, as compared to measured values in the short term, likely by an overestimation of NO_3^- leaching and also by the previously mentioned overestimation of N2O losses during spring thaw. Further model improvements in the algorithms used to estimate N losses are required during the period between fall application and the subsequent cropping season.

CHAPTER 6: SUMMARY AND GENERAL CONCLUSIONS

In this research, we hypothesized that dairy manure management practices such as application timing and method, and manure source influenced N_2O emissions and corn grain yield and that the effects were predictable and consistent across years. To evaluate this hypothesis, we conducted a series of field studies in Southern Ontario. Our objectives were: (1) to evaluate a hybrid method for N_2O flux calculation, (2) to determine whether the effect of timing of manure application (fall or spring) on N_2O emissions and grain yield in corn varies with manure application method (injection, broadcasting or incorporation to soil); (3) to determine whether the effect of manure application method on N_2O emissions changed according to manure source (raw or anaerobically digested manure) after a fall application and (4) to evaluate the ability of model DNDC v. CAN to predict N_2O emissions according to changes in manure application timing, method and source for short and long term scenarios. The set of manure management practices included two application times (fall and spring), three application methods (surface broadcasting, incorporation and injection) and two manure sources (raw and anaerobically digested), evaluated during three (timing and method) and two experimental years (source and method) at Elora, Ontario.

Nitrous oxide emissions for each study were based on N_2O concentration measurements performed at four time-points (0, 12, 24 and 36 min) using non-steady state chambers. To calculate the change of N_2O concentration over time (dC/dt) and achieve objective 1, a novel methodology was developed (Chapter 2), consisting of a decision tree based model (DTBM) that combined linear and non-linear approaches and data type discrimination. We used Monte Carlo simulations to test DTBM against fluxes calculated with linear and quadratic regressions, and the equation of Hutchinson and Mosier (1981). Mean values of dC/dt estimated with DTBM were the least sensitive to the decrease of precision in measurements (increase of coefficient of variation) and, therefore, were more stable compared to the other methods.

Nitrous oxide emissions did not respond to the interaction between timing and application method but, instead, a year × timing interaction was found (Chapter 3, objective 2). Also, N rate-scaled (emission factor) as well as yield-scaled emissions (N_2O intensity) had the same response to timing as cumulative N_2O emissions. Hence, our results suggested that the inter-annual variation of soil moisture and soil temperature influenced the response of emissions to timing, regardless of substrate availability (NH_4^+ or NO_3^- intensity). In the driest year, fall application led to lower cumulative N_2O emissions compared to spring application, whereas N_2O emissions did not respond to manure application timing in years with precipitation close to normal.

In terms of sampling methodology, including N_2O emissions occurring during the non-growing season improved the estimation of timing and method effect on cumulative N_2O emissions, since they accounted for 20 to 60 % of yearly N_2O emissions. These proportions were linked to a different temporal pattern of N_2O emissions, which were concentrated in the non-growing season (Cold and Snow Season – Pre - Growing Season) for fall-applied manure, while emissions were concentrated during the Early Growing Season for spring-applied manure.

Injection of raw dairy liquid manure stimulated N_2O emissions, suggesting the importance of considering a trade-off between reduced N losses via NH_3 and increased N_2O emissions; however the reduction in NH_3-N loss appeared to be more important than increased N_2O losses from the agronomic standpoint as injection also resulted in greater corn yields than the other methods. Future studies should focus on a time horizon larger than 3 years, to verify the absence of response of N_2O emissions to manure application timing. Also, future studies should consider the influence of the dry periods on N_2O emissions response to manure application during early fall (before November).

Unexpectedly, fall-applied anaerobically digested (AD) manure did not decrease N_2O emissions compared to raw manure (Chapter 4, objective 3); however, we found an interaction effect between manure source and method of application on N_2O emissions that had not been reported in other studies, so that our results bring new information to properly mitigate N_2O emissions with AD manure. Anaerobically digested (AD) manure reduced cumulative N_2O emissions compared to raw dairy manure only when it was fall-injected into the soil; albeit, surprisingly, both surface broadcasting and incorporation of AD manure resulted in cumulative N_2O emissions larger than those produced by surface broadcasted or incorporated raw manure, which contradicts previous laboratory studies. Therefore, manure properties such as NH_4^+ concentration, infiltration rate and viscosity should be taken into account when deciding the method of field application. Further, the injection of AD manure not only helped to mitigate N_2O emissions when properly applied but also resulted in corn yields with a low N_2O intensity. For future studies, the focus should be on the ability of AD manure to mitigate N_2O emissions after a spring application of manure.

An aspect that should be addressed in future research is how crop rotation with different species (legumes, cereals, grasses, or cover crop) influence N_2O emissions in the long term and what is the influence of previous crop on emissions in the next year. Corn evaluated in our study had soybeans and barley as previous crops, so previous crop effects were confounded with the year effect, so it may be necessary to separate such effects in future studies. Also, in our experiments, we controlled the N rate effect statistically, but experiments with a constant N rate both within and between years would allow for a better approach to evaluate the environmental factors affecting N_2O emissions.

Regarding objective (4), the model DNDC v. CAN was used for simulating N_2O emissions in short and long term scenarios. The model DNDC v. CAN was a useful tool to simulate the effects of manure injection on daily and cumulative N_2O emissions in corn, given that its performance, based on calibration with control plots data, was comparable to other studies. However, the response of denitrifier activity above certain available C thresholds in the soil for spring thaw emissions need to be adjusted to avoid the overestimation of daily N_2O emission peaks after fall manure application. Short and long term projections for cumulative N_2O emissions were contradictory for some scenarios, confirming the influence of the inter-annual variability of weather on the short term effects of treatments. For example, spring application of raw dairy manure showed reductions in N_2O emissions over the long term, while this effect was not detected for the short term simulation. Predicted N_2O emissions produced by broadcast-incorporation of raw manure were smaller than those of injection for the short term, while there was no difference between methods for the long term. Finally, injection of AD manure was also projected as a mitigation technique for N_2O emissions in the long term, since it reduced N_2O emissions compared to incorporated AD manure. The model was limited to simulating grain yield after a fall application of manure due to an overestimation of NO_3^- leaching in comparison with measured soil NO_3 concentrations. Therefore, an improvement should be performed for the algorithms calculating NO_3^- leaching during the period previous to the crop season. Modelling studies with DNDC-CAN should be based upon improved algorithms for simulating N_2O emissions and corn grain yield. Improved algorithms should address issues such as the simulation of spring thaw N_2O emissions after fall application and NO_3^- leaching overestimation during the non-growing season.

In summary, our hypothesis was partially rejected since we obtained some unexpected results as was the case for the absence of timing and AD manure effect on N_2O emissions; however the interactions found such as year (environment) by timing and source by method and the analyses for short and long term helped to better understand the dynamics of N_2O after a field application of manure and the influence of the inter-annual variability of weather on N_2O emissions. The data and information generated by these studies will be of great interest for the dairy sector, which is fully committed to mitigate N_2O emissions associated with milk production.

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APPENDIX

Table A1. Mean comparison according to year, timing and method of manure application for Soil Ammonium (NH_4^+) and Nitrate (NO_3^-) Intensity. Different lowercase letters indicate between rows significant difference among treatments (p<0.05, LSD). The ANOVA summary included below shows the effects of treatments for each year and for the three years pooled together (data ln-transformed when needed). Sum of Squares due to N rate was calculated in the model to better estimate Residual Sum of Squares but N rate effect was removed from the analysis.

Source of variation / Treatments	NH_4^+ intensity				NO_3^- intensity			
	2012	2013	2014	3-yr mean	2012	2013	2014	3-yr mean
Timing	----------g NH_4^+-N day kg soil $^{-1}$----------				----------g NO_3^--N day kg soil $^{-1}$----------			
Fall	0.56 a	0.72 a	0.72 a	0.67 a	4.28	1.89 a	2.46 a	2.87
Spring	0.33 b	0.22 b	0.28 b	0.28 b	4.27	1.63 b	2.21 b	2.69
LSD (0.05)	0.11	0.10	0.26	0.11	ns	0.22	0.21	ns
Method								
Injection (INJ)	0.51	0.46	0.48	0.49	4.50	1.80	2.60 a	4.26 a
Surface broadcasted (SB)	0.40	0.46	0.51	0.45	4.13	1.74	2.06 c	1.76 c
Incorporated (SBI)	0.42	0.49	0.52	0.48	4.16	1.74	2.34 b	2.33 b
LSD (0.05)	ns	ns	ns	ns	ns	ns	ns	0.43
Timing X Method								
Fall X INJ	0.73	0.70	0.67	0.70	4.34	1.89	2.62 a	2.95
Fall X SB	0.45	0.71	0.71	0.62	4.42	1.89	2.04 b	2.78
Fall X SBI	0.49	0.75	0.79	0.68	4.07	1.88	2.70 a	2.89
Spring X INJ	0.30	0.21	0.30	0.27	4.65	1.70	2.58 a	2.98
Spring X SB	0.34	0.21	0.31	0.29	3.85	1.59	2.07 b	2.50
Spring X SBI	0.34	0.23	0.25	0.27	4.24	1.59	1.97 b	2.60
LSD (0.05)	ns	ns	ns	ns	ns	ns	0.37	ns
--------------------p-values--------------------								
Year	-	-	-	0.52	-	-	-	<0.01
Timing	<0.01	<0.01	<0.01	<0.01	0.83	0.03	0.03	0.05
Method	0.18	0.80	0.75	0.81	0.13	0.87	<0.01	<0.01
Timing X Method	0.05	0.96	0.24	0.43	0.07	0.90	0.01	0.12
Year X Timing	-	-	-	0.09	-	-	-	0.40
Year X Method	-	-	-	0.50	-	-	-	0.17
Year X Timing X Method	-	-	-	0.07	-	-	-	0.01

LSD, Least Significant Difference; ns, no significant difference

Table A2. Soil ammonium and nitrate concentration at 15 cm top soil. Mean comparison among treatments by orthogonal contrasts according Period by Method interaction after fall application.

		Soil NH_4^+ concentration at 15 cm top soil				Soil NO_3^- concentration at 15 cm top soil			
Year	Method	CSS (Nov to Mar)	PGS (April to mid-May)	EGS (mid-May to late Jul)	LGS (late Jul to late Oct)	CSS (Nov to Mar)	PGS (April to mid-May)	EGS (mid-May to late Jul)	LGS (late Jul to late Oct)
		----------------------kg NH_4^+-N ha^{-1}--------------------				----------------------kg NO_3^--N ha^{-1}--------------------			
2012	Control	2.2 b	3.8	1.4	0.9	17.8	31.4 c	29.2	19.9
	INJ	5.6 a	7.8	1.7	0.7	21.9	48.7 a	26.3	16.1
	SB	3.3 b	2.9	1.7	1.4	23.5	37.4 b	32.4	18.6
	SBI	4.0 b	3.5	1.7	1.0	19.7	28.7 b	33.8	21.8
2013	Control	1.0 b	1.6 b	1.9	2.1	3.4 b	5.5 b	12.6 b	5.3
	INJ	10.6 a	3.9 a	1.5	2.7	8.3 a	10.8 a	18.5 a	6.1
	SB	7.9 a	3.3 a	1.9	2.7	12.1 a	10.2 a	16.8 a	7.8
	SBI	9.9 a	3.3 a	1.6	2.7	11.9 a	9.9 a	16.9 a	6.4
2014	Control	1.6 c	0.4 b	1.6	2.2	7.6	10.2 d	19.1 c	4.0
	INJ	5.3 b	2.0 a	1.4	2.5	7.7	19.1 a	25.6 a	3.9
	SB	7.6 a	0.7 b	1.9	2.5	6.7	13.7 c	17.7 b	3.9
	SBI	7.9 a	1.2 b	1.4	2.2	9.8	16.1 b	26.4 a	5.3

CSS, Cold and Snow season; PGS, Pre-Growing Season; EGS, Early Growing season; LGS, Late Growing Season. INJ, manure injection; SB, manure broadcasting; SBI, manure incorporation. Different letters among methods indicate difference at $p < 0.05$. Data were ln-transformed for the statistical analysis when non normal distributed.

Table A3. Means for cumulative N_2O emissions, emission factor, grain yield and N_2O intensity as affected by timing and method of manure application during 2012, 2013, 2014 and three-year average.

Timing	Method	Cumulative N_2O emissions				Emission factor				Grain yield				N_2O intensity			
		2012	2013	2014	3-yr mean	2012	2013	2014	3-yr mean	2012	2013	2014	3-yr mean	2012	2013	2014	3-yr mean
		----------kg N_2O-N ha^{-1}---------				---------------%---------------				---------t grain ha^{-1}-----------				------g N_2O-N t $grain^{-1}$------			
Fall	INJ	1.82 (0.75)†	3.49 (0.80)	4.1 (1.81)	3.13 (1.21)	1.19 (0.65)	1.58 (0.44)	0.98 (0.86)	1.25 (0.62)	10.0 (0.50)	9.39 (0.20)	8.70 (0.57)	9.37 (0.50)	181 (71)	370 (85)	509 (258)	353 (163)
	SB	0.59 (0.13)	3.96 (0.35)	6.64 (1.75)	3.72 (1.59)	0.13 (0.11)	1.85 (0.19)	2.2 (0.83)	1.39 (0.65)	9.14 (0.91)	8.40 (1.12)	6.34 (0.24)	7.96 (0.98)	62.0 (7)	489 (58)	764 (98)	438 (162)
	SBI	1.17 (0.25)	2.85 (0.68)	2.68 (1.26)	2.24 (0.86)	0.63 (0.21)	1.24 (0.38)	0.31 (0.60)	0.73 (0.44)	8.79 (0.33)	8.25 (0.64)	8.16 (0.24)	8.40 (0.42)	134 (29)	333 (54)	335 (162)	267 (103)
Spring	INJ	4.17 (0.12)	3.55 (1.79)	4.77 (0.99)	4.16 (1.10)	2.33 (0.07)	3.27 (1.98)	1.26 (0.45)	2.29 (1.15)	11.2 (0.62)	8.15 (0.56)	9.90 (0.67)	9.75 (0.86)	376 (22)	444 (227)	480 (94)	433 (131)
	SB	2.37 (0.53)	1.91 (0.47)	2.64 (0.31)	2.31 (0.44)	1.2 (0.33)	1.45 (0.52)	0.28 (0.14)	0.98 (0.42)	10.5 (0.70)	7.70 (0.41)	7.58 (0.57)	8.61 (0.88)	220 (43)	254 (70)	353 (49)	276 (58)
	SBI	2.17 (0.49)	2.53 (0.77)	1.79 (0.15)	2.16 (0.51)	1.08 (0.31)	2.14 (0.86)	0.11 (0.07)	1.04 (0.68)	8.96 (0.73)	7.94 (0.43)	8.77 (0.47)	8.56 (0.56)	246 (63)	311 (86)	204 (12)	254 (61)
Control		0.44 (0.06)	0.60 (0.19)	2.03 (0.46)	1.02 (0.91)	-	-	-	-	7.50 (1.52)	5.00 (0.53)	7.10 (0.78)	6.50 (1.09)	77 (33)	114 (36)	293 (73)	161 (67)

† The number within brackets indicates the standard error

Table A4. Means ± standard error for cumulative N_2O emissions, emission factor (EF), grain yield and N_2O intensity as affected by source and method of manure application during 2013, 2014 and two-year average.

Source	Method	Cumulative N_2O emissions			EF			Grain yield			N_2O intensity		
		2013	2014	2-yr mean	2013	2014	2-yr mean	2013	2014	2-yr mean	2013	2014	2-yr mean
		------kg N_2O-N ha^{-1}-----			-----------%------------			-------t grain ha^{-1}-------			----g N_2O-N t $grain^{-1}$----		
AD[a]	INJ	2.06 ± 0.48[c]	3.05 ± 0.64	2.55 ± 0.59	1.15 ± 0.38	0.44 ± 0.28	0.80 ± 0.36	8.67 ± 0.20	9.82 ± 0.32	9.24 ± 0.79	267 ± 49	307 ± 58	287 ± 102
	SB	5.51 ± 1.46	7.24 ± 1.21	6.38 ± 1.32	3.88 ± 1.15	2.28 ± 0.53	3.08 ± 0.93	9.68 ± 0.47	8.42 ± 0.77	9.04 ± 1.36	586 ± 183	905 ± 192	746 ± 386
	SBI	3.43 ± 0.55	7.45 ± 1.36	5.44 ± 1.44	2.23 ± 0.44	2.37 ± 0.60	2.30 ± 0.49	8.00 ± 0.70	8.36 ± 0.32	8.18 ± 1.03	450 ± 101	890 ± 151	670 ± 335
	Mean	3.66 ±0.65	5.91 ±0.84	4.79 ±0.57	2.42 ±0.51	1.70 ±0.20	2.06 ±0.32	8.78 ±0.33	8.87 ±0.34	8.82 ±0.23	434 ±76	701 ±113	568 ±72
RM[b]	INJ	3.49 ± 0.80	4.09 ± 1.81	3.79 ± 1.30	1.58 ± 0.44	0.98 ± 0.83	1.28 ± 0.65	8.32 ± 0.43	8.70 ± 0.57	8.51 ± 0.98	428 ± 102	509 ± 258	469 ± 366
	SB	3.96 ± 0.34	6.64 ± 1.74	5.30 ± 1.37	1.85 ± 0.19	2.20 ± 0.86	2.02 ± 0.57	8.07 ± 0.74	7.49 ± 1.28	7.78 ± 1.96	507 ± 67	857 ± 109	682 ± 251
	SBI	2.85 ± 0.68	2.68 ± 1.26	2.77 ± 0.94	1.24 ± 0.37	0.31 ± 0.60	0.77 ± 0.53	9.76 ± 0.74	8.16 ± (0.24)	8.96 ± 1.33	300 ± 69	335 ± 162	317 ± 232
	Mean	3.43 ±0.36	4.47 ±0.98	3.95 ±0.52	1.56 ±0.37	1.16 ±0.47	1.36 ±0.25	8.72 ±0.41	8.12 ±0.45	8.42 ±0.31	412 ±50	567 ± 117	489 ±64

[a]: Anaerobically digested manure, [b]: Raw manure, [c]: Standard error