

**LEGACY EFFECTS OF LONG-TERM MANURE APPLICATIONS ON  
SOIL-DERIVED NITROUS OXIDE EMISSIONS**

A Thesis Submitted to the College of Graduate Studies and Research  
In Partial Fulfillment of the Requirements  
For the Degree of Master of Science  
In the Department of Soil Science  
University of Saskatchewan  
Saskatoon

By  
Ryan John Pearce

## **Permission to Use**

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of the University of Saskatchewan may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professors who supervised my thesis work or, in their absence, by the Head of the Department of Soil Science or the Dean of the College of Graduate Studies. It is understood that any copying or publication or use of the thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due to recognition shall be given to me and to the University of Saskatchewan in any scholarly use that may be made of any material in my thesis. Requests for permission to copy or to make other uses of materials in this thesis, in whole or part, should be addressed to:

Head, Department of Soil Science  
University of Saskatchewan  
Saskatoon, Saskatchewan  
Canada, S7N 5A8

## **DISCLAIMER**

Reference in this thesis to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

## ABSTRACT

Termination of the manure application treatments at the Dixon long-term manure research site in Humboldt, Saskatchewan provided a unique opportunity to explore how a change in management regime to annual urea applications would affect nitrous oxide ( $\text{N}_2\text{O}$ ) emissions. My hypotheses were that long-term manure applications would produce a legacy (or priming) effect that would result in enhanced  $\text{N}_2\text{O}$  emissions following the changeover to a more readily available nitrogen source and that this effect would be relatively short-lived. The impacts of long-term manure application and change in fertility management in the sub-humid prairies of Saskatchewan has not been investigated in great depth, this work provided an opportunity for greater insight into changes in N transformation and gaseous N loss from a manured agroecosystem. Nitrous oxide fluxes associated with the long-term manure and fertilizer application from the Dixon site were measured during a 37-month period (i.e., from May 2011 to June 2014). In addition, denitrification enzyme activity (DEA) was measured in a subset of the plots starting in June 2011 and continuing three times per year (i.e., prior to and after the spring fertilizer application and again in early fall).

Treatment-induced  $\text{N}_2\text{O}$  emissions for the various historical amendment treatments indicate that past management can result in considerable N being lost from the system as  $\text{N}_2\text{O}$ . Indeed, summed over the three-year post-manure period (i.e., from fertilizer application in 2011 through the 2014 spring thaw),  $\text{N}_2\text{O}$ -N losses accounted for 2% to 6% of the total applied fertilizer-N. Moreover, under environmental conditions that optimize denitrification,  $\text{N}_2\text{O}$ -N losses can be even greater. For example, high DEAs coupled with warm moist soil conditions resulted in large  $\text{N}_2\text{O}$  emission events following the spring 2013 fertilizer application and during the 2014 spring thaw. As a result, cumulative annual  $\text{N}_2\text{O}$ -N losses in 2013/14 were much greater than those in previous years — with emissions from the liquid swine manure (LSM)-amended plots ranging from 3% to 15% of applied N. These data support my earlier hypothesis that long-term applications of manure-N can — especially at high application rates and following frequent application — produce a ‘priming’ effect that exacerbates  $\text{N}_2\text{O}$  emission when a more available form of N (e.g., urea fertilizer) is applied to the soil. Moreover, this priming effect appears to be relatively long-lived — persisting in the soil more than four and a half years after the last manure application. In any given year, however, the impact of the priming effect on

cumulative N<sub>2</sub>O emissions depends on environmental conditions — being greatest during years with above average precipitation and temperature during the spring thaw period and a seeding/fertilizer application. Overall, my data demonstrate that management history can have a significant impact on soil N turnover in agricultural soils, and that long-term annual application of manure-N at high rates can produce a N<sub>2</sub>O ‘priming’ effect that — under appropriate environmental conditions — can significantly intensify N<sub>2</sub>O emissions long after the manure applications have ceased.

## ACKNOWLEDGEMENTS

I would like to acknowledge my supervisors Drs. Rich Farrell and Reynald Lemke for their continuous help and support throughout my tenure at the University of Saskatchewan. Without their guidance I wouldn't have been able to excel in my studies and research. I would also like to thank my committee members Drs. Diane Knight and Bobbi Helgason. Dr. Helgason was paramount to my understanding of the complicated world of soil microbes and the different methods of analysis. This extends to Sarah Kuzmicz of the soil micro-lab and AAFC who provided technical help, as well as laboratory analysis used in this study, and to Hannah Korschuh who filled in that role while Sarah was away on leave. They all provided the kindness and reassurance to keep me on track and focused to finish.

I would also like to extend my thanks to the lab group in 5E19 (Darin, Frank, Mark, Sharon, Dwayne, and Amanda) as well as all the summer students (especially Nicolas and Zheng, Adam and Kelsey), who helped me complete my field and lab research. To Cody David for all the help with R and Nils Yannikos for making the weather figure work. To Dr. Jeff Schoenau for providing the supplementary information for my research site, and to all my fellow grad students who provided me the much needed breaks and adventures. It was all a positive contribution to my being able to complete this degree.

I also acknowledge my funding agencies and support graciously provided by the Agriculture and Development Fund through the government of Saskatchewan, Agriculture and Agri-Food Canada, and Fertilizer Canada. I would also like to say thanks to Wayne Stock at the Prairie Agriculture and Machine Institute for helping keep my field site marked and easily accessible.

Finally, I would like to acknowledge my mom and dad (Ellice and Joe), my brother, Devon, and extended family for their support and time in pushing me to excel. I would also like to thank all the friends who continually encouraged me throughout this postgraduate degree.

# TABLE OF CONTENTS

PERMISSION TO USE .....	i
DISCLAIMER .....	ii
ABSTRACT.....	iii-iv
ACKNOWLEDGEMENTS.....	v
TABLE OF CONTENTS.....	vi-vii
LIST OF TABLES .....	viii
LIST OF FIGURES .....	ix-x
1 Introduction .....	1
1.1 Background.....	1
1.2 Research Objectives .....	2
1.3 Organization of the Thesis.....	2
2 Literature Review .....	4
2.1 Nitrogen Cycle.....	4
2.1.1 Overview.....	4
2.1.2 Mineralization .....	5
2.1.3 Nitrification.....	6
2.1.4 Denitrification .....	7
2.2 Nitrous Oxide and the Environment.....	9
2.2.1 N <sub>2</sub> O and the atmosphere .....	9
2.2.2 N <sub>2</sub> O emissions and the soil environment.....	10
2.2.3 N <sub>2</sub> O emissions and N-fertilizer application .....	11
2.2.4 N <sub>2</sub> O emissions and organic carbon .....	13
2.3 Soil Environment and Microbes .....	14
2.3.1 Fertilizer amendments and microbial abundances.....	14
2.3.2 Fertilizer amendments and microbial activity.....	16
2.3.3 Priming effects .....	17
3 Long-Term Manure Fertilizer Amendments Produce a “Legacy” Effect of Increased Nitrous Oxide Emissions.....	19
3.1 Preface .....	19
3.2 Abstract.....	20

3.3	Introduction .....	20
3.4	Methods and Materials .....	25
3.4.1	Field site description .....	25
3.4.2	Experimental design.....	26
3.4.3	Greenhouse gas monitoring .....	30
3.4.4	Soil sampling and processing.....	32
3.4.5	Denitrification enzyme activity.....	33
3.4.6	Weather data .....	35
3.4.7	Statistical analysis.....	35
3.5	Results .....	36
3.5.1	Local weather.....	36
3.5.2	Denitrification enzyme activity.....	38
3.5.3	Daily N <sub>2</sub> O emission .....	42
3.5.4	Cumulative nitrous oxide loss and historical fertilizer amendments .....	45
3.6	Discussion.....	54
3.7	Conclusion.....	60
4	Conclusions and Future Research Needs .....	61
4.1	Summary and Conclusions .....	61
4.2	Future Research .....	63
5	References .....	65
	Appendix A.....	74
	Precipitation and temperature spatial representations .....	74
	Appendix B .....	76
	Daily N <sub>2</sub> O emissions .....	76
	Appendix C .....	80
	Kendall-tau tables for N <sub>2</sub> O losses and abiotic factors.....	80
	Appendix D.....	82

## LIST OF TABLES

Table 3.1 Manure fertilizer properties from Canadian studies including the Dixon site from selected literature .....	23
Table 3.2 Historical N treatments, timings, and application rates for liquid swine manure (LSM) and solid cattle manure (SCM) trials. All amendments applied from fall 1996 until fall 2009. Only the treatments included in the GHG sampling are listed. ....	27
Table 3.3 Total precipitation and mean annual temperature (based on Environment Canada data for Saskatoon, SK RCS Station). The fertilizer applications rates are for that growing season, as well as the crop seeded that year.....	37
Table 3.4 Mean DEA activities for the historical liquid swine manure (top table) and solid cattle manure (bottom table) N-fertilizer treatments for each year of analysis. The p values are probabilities from One-Way ANOVA performed for each year and trial separately.....	39
Table 3.5. Annual and total cumulative N <sub>2</sub> O-N loss measured between the spring fertilizer application in 2011 (May 27 <sup>th</sup> ) and the end of snowmelt period in 2014 (May 16 <sup>th</sup> ). Only data from the plots with a history of annual manure/urea applications were considered; data were analysed using a 2-way ANOVA with N-source and N rate as the main effects. ....	46
Table 3.6. Annual and total cumulative N <sub>2</sub> O-N loss measured between the spring fertilizer application in 2011 (May 27 <sup>th</sup> ) and the end of snowmelt period in 2014 (May 16 <sup>th</sup> ). Only data from the plots with a history of triennial manure/urea applications were considered; data were analysed using a 2-way ANOVA with N-source and N rate as the main effects. ....	46
Table 3.7. Cumulative annual N <sub>2</sub> O-N loss and the proportion of the cumulative annual N <sub>2</sub> O loss associated with the spring application of N fertilizer (N <sub>f</sub> ) and the snowmelt (SM) period preceding the next year's spring fertilizer application. Emissions associated with the spring application of N fertilizer were calculated based on a 20-day period immediately following snowmelt or fertilizer application. ....	51
Table C1. 2012 Kendall-tau correlation table of N <sub>2</sub> O values and abiotic Factors. The values listed are correlation coefficients, and the asterisks represent the probability values.....	80
Table C2. 2013 Kendall-tau correlation table of N <sub>2</sub> O values and abiotic Factors. The values listed are correlation coefficients, and the asterisks represent the probability values.....	81
Table D1. Yield data for the 2012 and 2013 cropping seasons at the Dixon long-term research site. Available N data were obtained in spring and fall 2013, and spring 2014. All values are the means for the respective historical N-treatment. ....	82

## LIST OF FIGURES

Fig. 2.1. Conceptual model of N cycling showing the important transformations of reactive N. Adapted from Prosser (2007)..... 5

Fig. 3.1. Plot map (map is not to scale) for the liquid swine manure (LSM) trial at the Dixon long-term manure research site. Plots shaded dark grey were included in the current study; i.e., only these plots were sampled for greenhouse gas emissions during the period from spring 2011 through spring 2014; plots shaded light grey were not sampled. .... 28

Fig. 3.2. Plot map (not drawn to scale) for the solid cattle manure (SCM) trial at the Dixon long-term manure research site. Plots shaded dark grey were included in the current study; i.e., only those plots were sampled for the greenhouse gas emissions during the period from spring 2011 through spring 2014; plots shaded light grey were not sampled. .... 29

Fig. 3.3. Vented, non-steady state chamber. Note: prior to deployment, each chamber was wrapped with insulation and reflective foil to minimize heating effects..... 30

Fig. 3.4. Mean daily temperature and daily total precipitation for the study period. (Environment Canada, 2015a). Days are based on Julian days (Day 1 = January 1<sup>st</sup>). The black arrows indicate the yearly seeding date. The blue shaded region is the gas sampling period after snowmelt, but prior to seeding in the spring; while the tan shaded region is the gas sampling period from immediately after seeding until snowfall and soil freeze-up in the fall..... 38

Fig. 3.5. Denitrification enzyme activity (DEA) measured in the plots with a history of LSM applications. Note: the plots were sampled once in 2011; three times in 2012 and 2013 (i.e., before fertilization, after fertilization, and harvest); and once in 2014 (before fertilization). The shaded region is the mean  $\pm$  standard deviation for the HUF-A1 (urea) soil amendment, the squares are HLS-C0 [■], circles are HLS-T2 [●], triangles are HLS-A4 [▲]. The error bars represent one standard deviation from the mean. .... 40

Fig. 3.6. Denitrification enzyme activity (DEA) measured in the plots with a history of SCM applications. Note: the plots were sampled once in 2011; three times in 2012 and 2013 (i.e., before fertilization, after fertilization, and harvest); and once in 2014 (before fertilization). The shaded region is the mean  $\pm$  standard deviation for the HUF-A1 (urea) soil amendment, the squares are HLS-C0 [■], circles are HLS-T2 [●], triangles are HLS-A4 [▲]. The error bars represent one standard deviation from the mean. .... 41

Fig. 3.7. Daily N<sub>2</sub>O emissions from the historical control (HLS-C0; top row) plots and plots that had received annual applications of manure at the 4 $\times$  rate (HSC-A4 and HLS-A4; middle and bottom rows, respectively). The daily flux from each plot (rep) is shown as a green circle (●); the mean flux is shown as a red line (—). The x-axis is the day of the year. In cases where one or more fluxes exceeded the y-axis range, these values are listed on the graph. The black arrow represents the seeding/fertilization date (Note: in 2014 sampling ended prior to seeding)..... 44

Fig. 3.8. Cumulative N<sub>2</sub>O emissions during the three years following termination of the historical LSM applications. Manure applications were made either (A) for the historical annual LSM and (B) for the historical annual SCM applications. Error bars represent one standard deviation from the mean total cumulative emissions. .... 47

Fig. 3.9. Cumulative N <sub>2</sub> O emissions during the three years following termination of the historical LSM applications. Manure applications were made either (A) for the historical triennial LSM and (B) for the historical triennial SCM applications. Error bars represent one standard deviation from the mean total cumulative emissions.....	48
Fig. 3.10. Cumulative N <sub>2</sub> O emissions during the three years following termination of the long-term urea applications. Urea applications were made on an annual basis. Error bars represent one standard deviation from the mean total cumulative emissions. ....	49
Figure 3.11. Concentrations of soil nitrate (NO <sub>3</sub> <sup>-</sup> ) in plots with a history of (A) LSM and (B) SCM applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (green), after harvest in fall 2013 (light green), and at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark green). ....	52
Figure 3.12. Concentrations of soil ammonium (NH <sub>4</sub> <sup>+</sup> ) in plots with a history of (A) LSM and (B) SCM applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (blue), after harvest in fall 2013 (light blue), and at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark blue). ....	53
Figure 3.13. Concentrations of soil nitrate (NO <sub>3</sub> <sup>-</sup> ) and ammonium (NH <sub>4</sub> <sup>+</sup> ) in plots with a history of variable urea applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (green), after harvest in fall 2013 (light green), and at the end of snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark green). (A) NO <sub>3</sub> -N: spring 2013 (green), fall 2013 (light green), and spring 2014 (dark green). (B) NH <sub>4</sub> -N: spring 2013 (blue), fall 2013 (light blue), and spring 2014 (dark blue). ....	53
Appendix A1. Range of total precipitation in millimetres between Saskatoon and the Dixon long-term research site based on geographic distance. ....	74
Appendix A2. Range in mean total temperature in degrees Celsius between Saskatoon and the Dixon long-term research site based on geographic distance. ....	75
Appendix B1. Daily fluxes for the LSM and SCM trials for the no N-fertilizer control treatment for all years monitored. ....	76
Appendix B2. Daily fluxes for the SCM trial all SCM N-fertilizer treatments for all years monitored. ....	77
Appendix B3. Daily fluxes for the LSM trial all Urea N-fertilizer treatments for all years monitored. ....	78
Appendix B4. Daily fluxes for the LSM trial all LSM N-fertilizer treatments for all years monitored. ....	79

# 1 INTRODUCTION

## 1.1 Background

Long-term nitrogen (N) fertilizer application can increase crop productivity and impact future management decisions; yet repeated N fertilizer applications can produce an excess of nutrients that can accumulate or be lost into the surrounding environment. Soil amendments such as manures not only supply available nutrients for increased plant growth, but can also alter the activity and structure of the soil microbial community, which in turn can affect nutrient cycling and the production/emission of greenhouse gases. However, very little data exists that relates long-term manure applications to N<sub>2</sub>O emissions in Saskatchewan or western Canada in general.

The Dixon long-term manure research site was established in 1996 to investigate the agronomic and environmental benefits of liquid swine manure (LSM) and solid cattle manure (SCM) applications (Mooleki et al., 2002; Mooleki et al., 2004). Since its establishment, the Dixon site has been managed as a grain-oilseed rotation with manure amendments, as well as equivalent urea fertilizer treatments applied in late fall of each year. Unfertilized control plots also were established in both the LSM and SCM experiments. The 2010 cropping season was the last year in which crop N demand was met by the previous fall's manure/urea application; i.e., starting in spring 2011 there was an N-fertilizer change-over with annual spring applications of inorganic fertilizer-N at rates (based on a spring/fall soil test) designed to meet the needs of that year's crop.

Research conducted at the Dixon site between 2009 and 2011 quantified and compared N<sub>2</sub>O emissions from the plots receiving long-term applications of LSM and SCM to those receiving equivalent rates of urea fertilizer (Farrell et al., 2011). The study's authors concluded that "long-term applications of manure-N can, at high application rates or following frequent applications, produce a 'priming' effect which may exacerbate N<sub>2</sub>O emissions if a more available form of N (e.g., urea fertilizer) is applied to the soil". Thus, the present study was initiated in 2011 to examine the N<sub>2</sub>O priming effects associated with long-term manure applications, and provide insights into the implications of changes to long-term management practices on

greenhouse gas emissions. The costs associated with the use of N fertilizers to increase crop yields can be weighed against the loss through N<sub>2</sub>O emissions or leaching (Chantigny et al., 2001; Malhi et al., 2009). However, with new insights, and a greater understanding of how N-fertilizer applications impact N-cycling and losses from agricultural soils, proper management protocols can be established. For example, Cavigelli et al. (2012) reported that increases in N<sub>2</sub>O emissions in the United States have been slowed by improving N application efficiency and can be further reduced by changing farming practices. Clearly, with increased research in this area proper and sustainable agricultural practices can be developed and implemented.

## **1.2 Research Objectives**

The main objective of my study was to assess the occurrence and duration of a ‘priming effect’ on microbial communities and the resulting increase in N<sub>2</sub>O emissions from an agricultural soil that has received long-term applications of liquid swine manure or solid cattle manure. To address this objective two hypotheses were tested:

- long-term manure applications ‘prime’ the soil for more rapid N transformations that result in greater loss of N<sub>2</sub>O when a more readily available N-source is applied to the soil; and
- the duration of the ‘priming effect’ will be short lived and not expressed over the duration of monitoring;

## **1.3 Organization of the Thesis**

The thesis is organized in manuscript format and contains a detailed literature review followed by a research chapter organized as a complete, publishable paper; and a final synthesis chapter that brings together the research chapter and supplemental information presented in the Appendices, discusses the implications of the research as a whole and presents suggestions for future studies. The Literature Review (Chapter 2) presents a broad outline of relevant topics and research related to my objectives, and is followed by a research chapter dealing with the impact of long-term manure applications on N<sub>2</sub>O emissions and denitrification enzyme activity (Chapter 3).

Chapter 3 focuses on the impacts of long-term manure applications on the production of N<sub>2</sub>O emissions following conversion from a manure-based to an inorganic fertilizer-based management system. This chapter collates the GHG-data (focusing on N<sub>2</sub>O) collected from the Dixon site over three growing seasons (2011-2013) plus the snowmelt period in early spring 2014. Daily N<sub>2</sub>O flux data were used to calculate cumulative emissions for a N-budget year that allows for an assessment of how the different historical manure treatments (which varied in type of manure, rate of application and frequency of application) impacted current N<sub>2</sub>O emissions. Denitrification enzyme activities (DEAs) were used to examine the potential N<sub>2</sub>O production associated with the historical manure treatments.

Chapter 4 presents the Synthesis and Conclusions, which summarize the results of the research chapter and presents conclusions regarding the study as a whole, the implications of this research, and some suggestions for future studies. This chapter is followed by a Reference section (Chapter 5), containing a complete list of the citations used throughout the thesis, and the Appendices, which include supplemental field data, daily N<sub>2</sub>O loss figures, correlation tables, as well as a summary of the crop yield data.

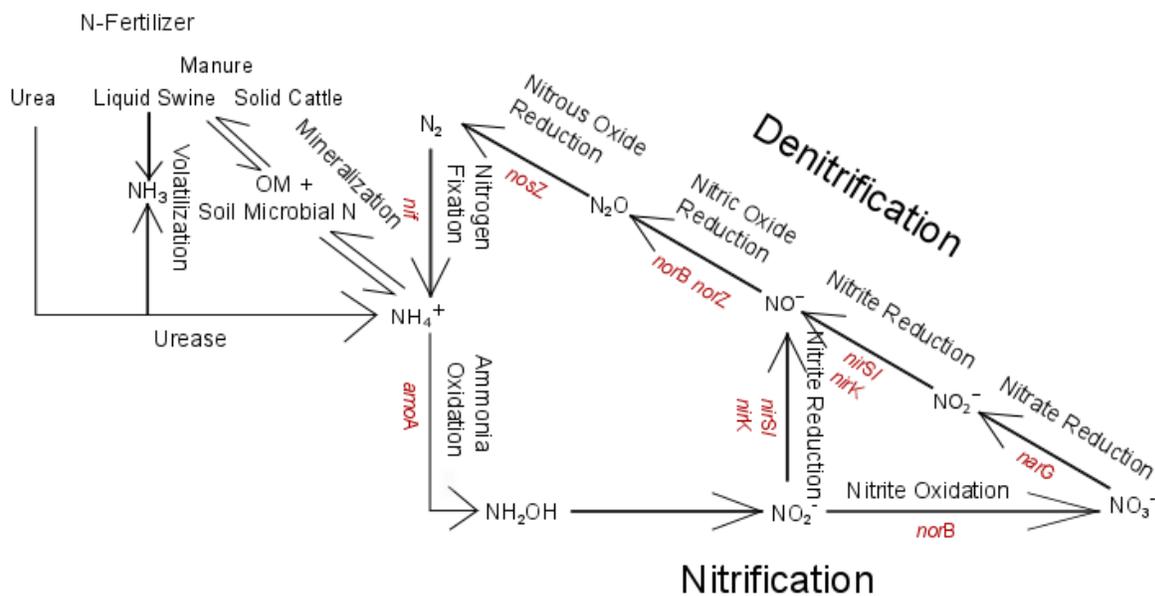
## 2 LITERATURE REVIEW

### 2.1 Nitrogen Cycle

#### 2.1.1 Overview

Nitrogen (N) is essential for all life as it is a building block of amino acids, nucleic acids, enzymes and proteins. Nitrogen is a common constituent in the environment, with the majority of N occurring in the atmosphere as di-nitrogen gas ( $N_2$ ). However, the second largest reserve of N is organically bound N in the soil (Whalen and Sampedro, 2010). In soils, the N cycle involves microbially mediated processes that convert bound or inaccessible N into plant available forms, while producing energy through the transfer of electrons. Nitrogen fixation is the process of converting molecular  $N_2$  gas into ammonium ( $NH_4^+$ ) by N fixing bacteria, or abiotically through lightning strikes (Canfield et al., 2010). The fixed inorganic N that is deposited into the soil can be temporarily stored or taken up by plants or microbes and becomes bound organically. Although this soil organic matter (SOM) pool is a large source of organic N in the surface layers (Olk, 2008), it is not readily available to plants (Whalen and Sampedro, 2010).

Organic forms of N are recalcitrant because they bind to clay particles in the soil, can be bound covalently, and are commonly found in amide and aromatic structures that vary in configuration and limit microbial breakdown (Olk, 2008). In order for this N to become available, it must be converted into inorganic forms through mineralization of the soil organic matter. Organic amendments, such as manures, also contribute N to the soil, though the organic N in manure itself requires the same transformations as soil organic N before it becomes available to plants. Manures consist of a combination of relatively undecomposed plant residues and the products of animal metabolism (Beegel et al., 2008), thus they also provide substantial amounts of C that can affect the transformation of N by microbes. Nitrogen transformations in the soil continue until the N is finally released back into the atmosphere as either nitric oxide (NO), nitrous oxide ( $N_2O$ ), or  $N_2$  gas (Fig. 2.1). These transformations are catalyzed by a number of different enzymes produced by soil microbes that convert the various inorganic N forms to gaseous species along specific pathways (e.g., nitrification and denitrification). Nitrous oxide is of particular concern as it is an important ozone depleting and greenhouse gas.



**Fig. 2.1. Conceptual model of N cycling showing the important transformations of reactive N. Adapted from Prosser (2007).**

### 2.1.2 Mineralization

Mineralization is the transformation of organic N into ammonium ( $\text{NH}_4^+$ ) by soil microbes. This part of the N cycle is facilitated by several extracellular enzymes such as proteinase, aminopeptidase, endonuclease, and urease (Whalen and Sampedro, 2010). Mineralization is a two-way biological process where the organic N is transformed by ammonification into  $\text{NH}_4^+$  ions, or immobilized by soil microbes (Mitsch and Gosselink, 2007; Whalen and Sampedro, 2010). Mineralization of N can be driven by nutrient gradients. High C additions to the soil, such as those which occur during manure application, can lead to limited mineralization (Qian and Schoenau, 2002; Calderón et al., 2005), and, ultimately, to potential immobilization of  $\text{NH}_4^+$  by microbes (Whalen and Sampedro, 2010). The N becomes bound to the organic matter or is taken up by microbes resulting in immobilization of the N. For example, cattle manure, which has a high organic carbon content, can impede mineralization for several weeks (Paul and Beauchamp, 1994; Calderón et al., 2005). Conversely, inorganic N fertilizer addition (e.g., urea) can increase mineralization rates in soils (Zhang et al., 2008). Ammonium is typically only released into the soil matrix from microbes when there is an excess of N in the system, or from microbial cell lysis (Whalen and Sampedro, 2010). The  $\text{NH}_4^+$  can then be taken

up by plants, utilized by microbes, lost to the atmosphere as  $\text{NH}_3$  through volatilization, or transformed during nitrification (Mitsch and Gosselink, 2007).

### 2.1.3 Nitrification

Ammonium can be utilized by plants and microbes, but under conditions of abundant N concentrations in the soil, the  $\text{NH}_4^+$  can be further transformed via the nitrification pathway. Nitrification occurs under aerobic conditions and is very rapid. Transformation of N allows for the exchange of electrons that can be used to generate energy, or facilitate other reactions such as fixing inorganic C (Norton, 2008; Canfield et al., 2010). During nitrification, microbial enzymes facilitate the oxidation of  $\text{NH}_3$  or  $\text{NH}_4^+$  producing  $\text{NO}_3^-$  in two stages (Prosser, 2007). The first stage involves ammonium oxidation by ammonia oxidizing chemolithotrophic microbes (bacteria and archaea) where  $\text{NH}_4^+$  is oxidized and transformed into nitrite ( $\text{NO}_2^-$ ) in two reactions. The first part of this reaction utilizes the enzyme ammonia monooxygenase that converts the  $\text{NH}_3$  into the intermediary product hydroxylamine ( $\text{NH}_2\text{OH}$ ), which is then oxidized to  $\text{NO}_2^-$  by the enzyme hydroxylamine oxidoreductase (Norton, 2008; Canfield et al., 2010). The second phase of the nitrification process is the conversion of  $\text{NO}_2^-$  into nitrate ( $\text{NO}_3^-$ ) through nitrite oxidation catalyzed by the nitrite oxidoreductase (encoded by the *norB* gene) enzyme (Prosser, 2007) (Figure 2.1). These two plant available forms of N ( $\text{NO}_2^-$  and  $\text{NO}_3^-$ ) can be utilized by plants or soil microbes, but are also able to move rapidly through the soil resulting in losses to the environment (Norton, 2008).

The effect of manure application on the rate of nitrification varies with the type of manure. For example, the application of solid cattle manure (in which the majority of the added N is in organic forms) often results in an increase in the C:N ratio of the soil, which can lead to the removal of mobile N by creating conditions conducive for N immobilization and, in turn, can limit or decrease net mineralization (Calderón et al., 2005). This can slow subsequent transformations of N — including the production of  $\text{N}_2\text{O}$ . Liquid swine manure amendments, on the other hand, have been shown to increase  $\text{NO}_3^-$  in soils (Chantigny et al., 2001), especially with larger repeated application rates (Stumborg et al., 2007). This reflects the fact that most of the N in liquid swine manure is present as  $\text{NH}_4^+$  and urea, which can promote higher rates of nitrification and the subsequent build-up of  $\text{NO}_3^-$  in the soil. Inorganic N fertilizers also can create greater potential for N losses as they also generate increased  $\text{NO}_3^-$  (Ding et al., 2010; Tatti

et al., 2012; Cui et al., 2016). Because  $\text{NO}_3^-$  is highly mobile, it is susceptible to leaching below the root zone or into ground water systems. Nitrate accumulation can also promote denitrification — leading to further potential  $\text{N}_2\text{O}$  losses — if soil moisture conditions are sufficient (Norton, 2008).

Nitrous oxide produced during nitrification is generally thought to be the result of chemical decomposition of the intermediates formed between  $\text{NH}_4^+$  and  $\text{NO}_2^-$  (e.g.,  $\text{NH}_2\text{OH}$ ). The amounts of  $\text{N}_2\text{O}$  produced in the semi-arid northern Great Plains via this pathway are generally considered to be small and the major flux events are linked to denitrification type events following increases in moisture at snowmelt and fertilizer application (Dusenbury et al., 2008), and as such *nitrifier denitrification* may represent a more significant source of  $\text{N}_2\text{O}$  production (Wrage et al., 2001; Wrage et al., 2005). Nitrifier denitrification involves sequential reduction of the  $\text{NO}_2^-$  produced during  $\text{NH}_4^+$  oxidation (see Fig. 2.1) and differs from coupled nitrification-denitrification in that it is carried out by a single group of microorganisms (i.e., autotrophic  $\text{NH}_3$ -oxidizers). The enzymes involved in these transformations are believed to be the same as those involved in both  $\text{NH}_3$  oxidation and denitrification (see Section 2.1.4). The rest of this pathway proceeds with the further reduction of  $\text{NO}$  to  $\text{N}_2\text{O}$  and, finally, to  $\text{N}_2$  (Prosser, 2007).

#### **2.1.4 Denitrification**

Denitrification is the principle process of returning inorganic oxidized N back to the atmosphere through the reduction of  $\text{NO}_3^-$  to  $\text{N}_2$  gas under anaerobic conditions in the soil (Tiedje et al., 1984; Coyne, 2008). The denitrification process is beneficial in that it creates energy for soil microbes that can utilize N oxides as terminal electron acceptors (Coyne, 2008; Whalen and Sampedro, 2010). Denitrifiers are heterotrophic microorganisms capable of reducing N-oxides when oxygen becomes limited (Bremner, 1997). Many different types of soil microbes are capable of denitrification, including many bacterial species and some fungi (Canfield et al., 2010). The diversity of denitrifiers in soil microbial populations is large, and bacterial species that contain the genes needed to carry out denitrification are constantly being discovered (Kostka et al., 2012). While many denitrifier soil microbes are present in the soil matrix only a small number actually express the functional genes (Yoshida et al., 2012). The denitrification pathway is closely linked to the supply of available soil organic C (SOC), soil pH, and soil temperature

(Wei-xin et al., 2007; Coyne, 2008). In addition, denitrification requires a supply of N-oxides, a microaerophilic environment, and soil microbes that produce nitrite reductases which are encoded by the *nirK* and *nirS* genes, nitric oxide reductase (encoded by the *norB* gene), and nitrous oxide reductase (encoded by the *nosZ* gene) (Coyne, 2008; Canfield et al., 2010).

The denitrification pathway begins with the reduction of  $\text{NO}_3^-$ , the end product of nitrification, or added directly from an external source into the soil matrix (e.g., as N-fertilizer). Nitrate is reduced to  $\text{NO}_2^-$  by the nitrate reductase enzyme (encoded by the *narG* gene) and is then further reduced by either of the nitrite reductases (encoded by the *nirK* and *nirS* genes), thereby producing nitric oxide (NO). While these two enzymes perform the same N transformation, they are distinct and soil microorganisms may have either but not both; as well, these two enzymes are produced from two separate genes and are differentiated by having either a heme-type (*nirS* gene) or a copper-type (*nirK* gene) reductase (Coyne, 2008). The NO is then reduced to  $\text{N}_2\text{O}$  by nitric oxide reductase (encoded by the *norB* gene). The final phase of denitrification is the conversion of  $\text{N}_2\text{O}$  into  $\text{N}_2$  gas—the end product of the N cycle—through the action of the enzyme nitrous oxide reductase (*nosZ* gene).

Denitrification can also proceed by utilizing the intermediary  $\text{NO}_2^-$  created from nitrification. This allows the microorganisms to directly transform the  $\text{NO}_2^-$  with nitrite reductases into NO, by-passing further N transformations that generate  $\text{NO}_3^-$  (Fig. 2.1). The rest of this pathway proceeds with the further reduction of NO to  $\text{N}_2\text{O}$ , and finally  $\text{N}_2$  gas (Prosser, 2007). While denitrification commonly proceeds to completion releasing  $\text{N}_2$ , some  $\text{N}_2\text{O}$  can escape the soil matrix into the atmosphere increasing the amount of GHGs. Specific environmental conditions (sufficient soil moisture and aeration) can limit the *nosZ* enzyme preventing the pathway from completing the final reducing step which can lead to a greater proportion of  $\text{N}_2\text{O}$  lost to the atmosphere.

Denitrification becomes important in the contribution to  $\text{N}_2\text{O}$  emissions after application of N to soils (i.e. fertilization) as it is dependent on the presence of N oxides. This is potentially even a greater problem with manures as they also add organic C to the soils. Cattle manure applications can be important in denitrified N losses as suggested by high denitrification enzyme activity (DEA) in manure amended soils (Calderón et al., 2004), with especially high  $\text{N}_2\text{O}$  emissions resulting from manures with high initial  $\text{NH}_4^+$  (Calderón et al., 2005). Liquid swine

manures can also lead to increased N<sub>2</sub>O losses from denitrification as liquid swine application can lead to higher rates of nitrification that can lead to an accumulation of nitrate in the soil prompting denitrification. Nitrous oxide losses are further increased when environmental conditions increase soil moisture creating anaerobic conditions.

Denitrification and the microbial rates of denitrification can result in the greatest flux of N<sub>2</sub>O emissions under anaerobic conditions whereby water filled pore spaces go beyond a 60% threshold (Linn and Doran, 1984). However, as the soils become further saturated it can result in complete N reduction into N<sub>2</sub> reducing N<sub>2</sub>O loss to the atmosphere. In agricultural systems that apply inorganic N-fertilizer, increased soil water content can lead to greater N<sub>2</sub>O emissions as the soils begin to become saturated with water (Mulvaney et al., 1997).

## **2.2 Nitrous Oxide and the Environment**

### **2.2.1 N<sub>2</sub>O and the atmosphere**

Greenhouse gases (GHG) are important atmospheric constituents which increase average global temperatures by trapping solar long wave infrared radiation (IPCC, 2013). The major GHG contributors to atmospheric warming potential are: water vapour, carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), and methane (CH<sub>4</sub>). These GHGs are produced naturally in the environment through nutrient cycling and environmental processes. However, among these GHGs, CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub> are of particular interest as increases in the emission of these gases can be linked to human activities. Anthropogenic inputs (i.e. N-fertilizer) can directly influence GHG emissions through their manufacturing, or indirectly by altering the amount of GHG precursor molecules in the environment that can then lead to increases in emissions. Nitrous oxide is also a significant GHG that has a global warming potential that is approximately 298 times more effective than CO<sub>2</sub> at trapping outgoing radiation in the atmosphere (IPCC, 2013). Therefore, these processes can lead to increases of GHGs in the atmosphere that can increase global temperatures and impact the climate. Nitrous oxide emissions are of particular interest because of the high emissions from agriculture which are recognized as being the primary source of N<sub>2</sub>O emissions in Canada (Rochette et al., 2008a) and the U.S. (Cavigelli et al., 2012). Ozone depletion in the stratosphere has been shown to be a negative consequence of N<sub>2</sub>O emissions (Ravishankara et

al., 2009). It is important to understand the anthropogenic sources of these gases so potential mitigation strategies can be implemented.

### **2.2.2 N<sub>2</sub>O emissions and the soil environment**

Soil water content can be a major driver of N<sub>2</sub>O emissions especially when the soils are not nutrient limited. In agricultural soils the highest N<sub>2</sub>O fluxes are generally seen under higher moisture conditions, as increases to soil temperature and water filled pore space (WFPS) result in greater N<sub>2</sub>O losses (Wei-xin et al., 2007). Linn and Doran (1984) found that when WFPS exceeds 60% there is a shift towards denitrification; while aerobic microbial activity increases at water contents between 45-60%, and it is in this range that nitrification is typically the dominant microbial process. Higher moisture conditions reduce the amount of O<sub>2</sub> available and promote N<sub>2</sub>O production through the process of denitrification (Tiedje et al., 1984). Under field conditions higher moisture contents occur following precipitation events. In fact, it is these rainfall events that increase the water content in soils that lead to large seasonal losses of N<sub>2</sub>O (Tiedje et al., 1984). These precipitation events contribute to the greatest fluxes of N<sub>2</sub>O (Malhi et al., 2009). For regions with more intermittent precipitation events, high N<sub>2</sub>O emissions can be linked to the seasonal fluctuations in precipitation (Corre et al., 1996). In addition, higher emissions can be observed following snowmelt (Lemke et al., 1998; Dusenbury et al., 2008). These seasonal increases in soil moisture (precipitation and snowmelt events) accompanied by increases in temperature lead to greater emissions, and these “hot moments” can be responsible for the bulk of N<sub>2</sub>O fluxes (Molodovskaya et al., 2012). The sub-humid prairies in the Black soil zone of Saskatchewan where this research was conducted is a northern climatic region where precipitation events are relatively infrequent and freeze thaw cycles are prominent. In conjunction with these precipitation and freeze thaw driven moisture events, the soils in this agroecosystem under long-term manure application management are not often limited in nutrients and thus able to generate these hot moments becoming sources of significant amounts N loss.

Topographic factors that influence soil temperature or soil moisture content can also affect the production of N<sub>2</sub>O emissions from soils (Corre et al., 1996; Yates et al., 2006a), and can vary within the same landscape (Helgason et al., 2005). Furthermore, soil texture can influence N<sub>2</sub>O emissions as sandier soils can result in lower emissions than finer textured soils

(Corre et al., 1996). Smaller soil pore sizes commonly associated with soils with a higher clay content can have higher water filled pore space and result in greater N<sub>2</sub>O emissions (Skiba and Ball, 2002). The micro-environments within soil aggregates have been shown to be favorable sites for nitrifier and denitrifier communities because of their ability to capture available C and N, as such, may induce higher N<sub>2</sub>O losses (Kong et al., 2010). These soil micro-environments can therefore be important factors in estimating N<sub>2</sub>O losses in agricultural soils. Soil texture can also influence N<sub>2</sub>O losses. Upon comparing soils with a greater clay content to loamy textured soils, Rochette et al. (2008b) observed that despite lower reactive N in the clay soils they resulted in higher N<sub>2</sub>O emissions and attributed the increased N<sub>2</sub>O emission to a higher water content.

Soil moisture and available oxygen content have also been linked to N<sub>2</sub>O losses. As aerated and drier soil conditions can also generate N<sub>2</sub>O emissions by the nitrification pathway. Both denitrification and nitrification produce N<sub>2</sub>O as an intermediary part of their pathways and are large contributors to the production of atmospheric N<sub>2</sub>O (Bremner, 1997), depending on the soil moisture content and aeration conditions (Skiba et al., 1992). Nonetheless, depending on the amount of precipitation over the year, soil type may influence emissions and nitrogen transformations may become significant.

### **2.2.3 N<sub>2</sub>O emissions and N-fertilizer application**

Fertilizer N can increase crop productivity by providing available N and other important nutrients to the system (Qian and Schoenau, 2000; Wen et al., 2003). While both mineral fertilizers and animal manure applications can increase C and N in soils, manures can promote larger increases (Christensen, 1988). While other studies have noted a decrease in organic C (OC) and N in mineral fertilized soils due to a decrease in pH, in contrast, animal manure amendments tend to raise the pH (Heinze et al., 2010). Inorganic fertilizers provide readily available reactive N that can be immediately used by the soil microorganisms and plants. In contrast, for soils amended with organic fertilizers (e.g. manure), the N is in organic forms and must first be mineralized before it is readily available for plant uptake. In manure amended soils, less reactive N is available because there is a higher C:N ratio that can slow and impede organic-N mineralization (Qian and Schoenau, 2002). The rate and degree of mineralization depends on the C:N ratio of the manure, which can vary between liquid and solid manures. Solid cattle manure has a much higher C:N ratio and OC content than liquid swine manure (Wen et al.,

2003). Therefore, N is not as readily available and mineralizes more slowly (Qian and Schoenau, 2000; Qian and Schoenau, 2002; Helgason et al., 2007). Wen et al. (2003) reported lower inorganic-N recovery rates from cattle manure than from pig manure on Saskatchewan soils. However, continuous solid cattle manure application over several years can result in increased N availability through a reduction of the C:N ratio as a result of decomposition (Mooleki et al., 2004). In addition, the N form and content of manures is quite variable, with the N in liquid swine manures mostly in the form of  $\text{NH}_4^+$  in contrast to the N in solid cattle manure that is mostly in the form of organic-N, which can significantly impact  $\text{N}_2\text{O}$  emissions (Velthof et al., 2003). Consequently, the N in liquid swine manures is nitrified (Whalen, 2000), and converted to  $\text{NO}_3^-$  which can be used by the crop, leached from the soils or converted to  $\text{N}_2\text{O}$  (Chantigny et al., 2001). Mooleki et al. (2002) observed increased N-availability from liquid swine manure applications due to lower C:N ratios and high  $\text{NH}_4^+$  levels, and Velthof et al. (2003) saw greater  $\text{N}_2\text{O}$  losses due to the higher available N and available C found in liquid swine manure. The method of manure N-fertilizer application can result in differences in  $\text{N}_2\text{O}$  losses, as slurries applied as an injection have been shown to produce greater  $\text{N}_2\text{O}$  emissions than surface applied manure slurries in a corn system (Velthof and Mosquera, 2011).

Organic fertilizers can increase the available N in the soils, but also add extra OC (Hopkins and Shiel, 1996; Whalen, 2000), as well as P and K, and increase aggregate formation (Wu et al., 2006). This increase in OC can, in turn, affect the use of N, as OC is used to accept electrons during the reduction of  $\text{NO}_3^-$  (Canfield et al., 2010). An increase in available C also can result in an increase in N cycling between organic and inorganic forms with high net N immobilization (Paul and Beauchamp, 1994). Thus, the C:N ratio can influence substrate use by microbial communities. In nutrient depleted environments, adding urea can increase microbial abundances; however, adding too much can decrease the abundance by potentially shifting nutrient limitations (Zhang et al., 2008). These shifts in nutrient abundances can also lead to impacts on nutrient cycling as excess nutrients can impact priming effects (Kuzyakov et al., 2000). For example, a change in available substrates such as OC required for heterotrophic denitrifiers to transform  $\text{NO}_3^-$  into  $\text{N}_2\text{O}$  or  $\text{N}_2$  may in turn effect the ultimate production of  $\text{N}_2\text{O}$  in the soil environment. Truly,  $\text{N}_2\text{O}$  emissions have been strongly linked to the water soluble OC content in soils (Mulvaney et al., 1997). Long-term cattle manure applications have been shown

to increase microbial contribution to residue C in the top soil, and maintain a greater extractable C to SOC ratio at depth than mineral fertilizers (Sradnick et al., 2014).

Excess availability of N as a result of application of manures above crop requirements can lead to  $\text{NO}_3^-$ -N loading (Stumborg et al., 2007) that in turn can lead to increased  $\text{N}_2\text{O}$  emissions (Yates et al., 2006a; Rochette et al., 2008b). Large fluxes of  $\text{N}_2\text{O}$  are a by-product of the extra N introduced in these systems with the greatest fluxes occurring soon after fertilizer applications (Skiba et al., 1992). Using mineral N applications, Lebender et al. (2014) observed linear increases in emissions as application rates were increased even beyond crop demands. Fertilizer additions can impact soil denitrification rates, which have been observed to be high following fertilizer additions that increase N to the system (Mulvaney et al., 1997).

The type of fertilizer applied can also impact mineralization. Cattle manure tends to impede mineralization for multiple weeks after application (Paul and Beauchamp, 1994, Calderón et al., 2005). Inorganic N-fertilizer addition (urea) can increase both mineralization and nitrification rates in soils (Zhang et al., 2008), while denitrification emissions of  $\text{N}_2\text{O}$  have been correlated with inorganic N-fertilizers creating alkaline soil conditions (Mulvaney et al., 1997).

#### **2.2.4 $\text{N}_2\text{O}$ emissions and organic carbon**

Organic C is very important in the production of  $\text{N}_2\text{O}$  emissions from soils during denitrification (Burford and Bremner, 1975) as many of the microbes involved in the N-cycle are heterotrophic and require organic C as an energy source. The links with OC and fertilizer application have been well documented (Christensen, 1988; Mogge et al., 1999; Su et al., 2006; Barrett et al., 2016), where manure soil amendments will add and sustain SOC pools. However, continuous agricultural practices that can lead to less SOC and nutrients may not be sustainable in the long-term. Evidence of resource limitation creating negative impacts on resource acquisition, microbial abundance and activity between long-term organic managed (i.e., no synthetic fertilizer or pesticides) cropping systems vs. conventionally managed (i.e., no-till, synthetic fertilizer) systems (Arcand et al., 2016). Furthermore, establishing nutrient deficits can create deficits in the OM content of the soils as well as negatively impact soil microbial biomass (Chu et al., 2007). Fertilizer applications that add C such as manure amendments can lead to decreases in soil OC loss.

## **2.3 Soil Environment and Microbes**

Soil microbes are important for soil and ecosystem health. They control decomposition and nutrient cycling, and are also a driver of soil formation and aggregation. Soil microsites such as microaggregates are important habitats for microbial growth (Kong et al., 2010). In agricultural soils many natural processes including nutrient cycling are impacted through fertilizer and nutrient additions. Altering the soil pH as a result of N fertilizer applications has been shown to increase microbial denitrification activity (Mulvaney et al., 1997; Nägele and Conrad, 1990). These changes to the pH of soil can impact the success of microbes. Parkin et al. (1985) demonstrated that in low pH soils, the denitrifier populations adapt and select for denitrifiers able to survive and be active under these conditions. Long-term manure and chemical fertilizers applications can impact soil pH, with bacterial communities increasing in abundance when pH increases and fungal communities decreasing in abundance (Parham et al., 2003). This microbial adaptation to low pH conditions can lead to a shift of denitrifier populations and potentially greater N<sub>2</sub>O emission rates (Baggs et al., 2010). Conversely, increasing the soil pH by adding an alkaline substance (e.g., lime) can shift microbial activity from denitrification to NH<sub>4</sub><sup>+</sup> oxidation (Baggs et al., 2010). This ‘priming’ of the soil denitrifiers for low pH conditions suggests the investigations into other priming effects on microbial communities as a result of long-term soil management are warranted. For example, how does the change in soil management following long-term manure applications affect N<sub>2</sub>O emissions?

### **2.3.1 Fertilizer amendments and microbial abundances**

Investigating soil microbial populations in agricultural soils is important to maintaining economically viable and ecologically sustainable agricultural production systems (Kennedy, 1999). Different management approaches can lead to differences in microbial populations. Long-term and short-term agricultural management has been shown to impact enzyme activities and microbial community structure, while microbial biomass has been shown to have a stronger link with short-term management (Stark et al., 2008). The use of N fertilizer improves nutrient uptake by crops, and its use can lead to changes in the abundance, diversity and activity of the microbial communities (Nägele and Conrad, 1990; Mulvaney et al., 1997; Chu et al., 2007a; Zhang et al., 2008; Kong et al., 2010; Chaudhry et al., 2012). Inorganic fertilizers allow plants and microbes in the soil access to specific readily available nutrients making them appealing choices in

agriculture. While organic amendments (i.e. manures) can be more costly to transport and store, they also contain organic compounds (i.e. aromatic compounds, lignins, and microbes) that can increase both C and N to the soil. Manure application benefits can therefore offset the higher associated costs. There is also an energetic cost for the soil microbes, as acquiring the N and C from organic complexes requires an energetic investment. Nonetheless, manure can provide enough C and N to an agroecosystem enabling sustainable management that can maintain soil quality.

Long-term studies on the impact of organic amendments to microbial populations has shown that long-term organic amendments not only increases microbial biomass (Heinze et al., 2010), abundance (Acea and Carballas, 1988) and richness (Zhong et al., 2010), but also microbial activity (Parham et al., 2003; Chu et al., 2007a). Therefore, long-term manure application should alleviate N and C limitations and could create the conditions for increased denitrifier abundance as well as activity in the soil which could lead to potentially greater losses of N<sub>2</sub>O. Specifically it is the denitrifiers that are potentially impacted on a greater scale by this addition of nutrients as they are heterotrophic, and must obtain C through ingesting organic compounds which can then be used to generate energy and to create enzymes to cycle nutrients. Increases in denitrifying microbial activity as a result of N additions perpetuating the loss of N<sub>2</sub>O have indeed been shown to have a strong link to increases in the availability of labile C sources (Bergstrom et al., 1994; Miller et al., 2008), including dissolved organic carbon (DOC) (Barrett et al., 2016). The application of organic amendments can also introduce more than just manure nutrients (C and N) into the soil. This can include straw bedding, as well as some microbes and enzymes from the guts of animals which long-term applications have been shown to increase bacterial species already present in the soil (Chu et al., 2007a).

Investigating the effects of long-term organic and mineral N-fertilizer applications on NH<sub>4</sub><sup>+</sup> oxidizing bacteria, Chu et al. (2007b) observed that N-fertilizer applications shifted the community structure and increased the bacterial diversity with the highest diversity observed in the mineral (combinations of N, P, and K) amendments. Conversely, comparing organic compost and chemical fertilizers, Chaudhry et al. (2012) saw decreases in the diversity of microbial communities and their activities in soils under chemical amendments, similar to the decrease in bacterial abundance observed in Chinese red soils under long-term mineral N fertilizer application (Shen et al., 2010). Shen et al. (2010) observed that manure application alone or in

conjunction with mineral N fertilizer maintained bacterial communities and ecosystem health under long-term management.

These studies illustrate that the impacts on microbial communities from inorganic fertilizer amendment are not clear cut, and can either increase or decrease microbial populations (Hopkins and Shiel, 1996). Hopkins and Shiel (1996) also observed that the increase in microbial abundance from inorganic fertilizer applications had little impact on the microbial activity. The use of inorganic fertilizers that provide a specific nutrient may lead to quick utilization and changes in abundance, but may also create greater deficiencies in the available ratios of other essential nutrients requiring greater costs energetically for their acquisition which may impact activity such as higher rates of nutrient cycling. Decreases in microbial activity have indeed been shown to be greater as inorganic fertilizers create nutrient deficiencies (i.e. use of N, P, or K only) (Chu et al., 2007a). Investigating both organic and inorganic N-fertilizer applications, Kong et al. (2010) did not observe any strong links between rates of nitrification or mineralization of N with any of the observed changes in abundance of the nitrifiers and denitrifiers.

### **2.3.2 Fertilizer amendments and microbial activity**

Interestingly, with the abundances and activities of soil microbes, there are differences in how different fertilizer amendments impact the microbial abundance and gene copy number for enzymes related to the N cycle. Hallin et al. (2009) saw an increase in the gene copy number of some denitrifying enzymes (*narG* and *nosZ*) with cattle manure amendments. However, increases in denitrification activities as a result of fertilizer application with an increase in C availability did not translate into increases in the number of copies of the *nosZ* gene (Miller et al., 2008). Kong et al. (2010) also observed that the greatest *nosZ* gene copies occurred with inorganic amendments; however, it was the *amoA* gene copies that saw the largest increases with organic fertilizer amendments. Organic manure (pea residues) have been shown to increase microbial population growth and activity leading to improved soil conditions (Stark et al., 2008). The difficulty in accurately predicting microbial changes in activity, abundances and diversity caused by long-term manure applications makes developing N-fertilizer management strategies to maximize efficiency and minimize N<sub>2</sub>O losses difficult.

### 2.3.3 Priming effects

Continuous fertilizer amendments such as manures can lead to a buildup of nutrients altering the soil environment. These nutrients can fuel microbial communities leading to changes in activities. Kuzyakov et al. (2000) have suggested that ‘priming effects’ are “strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil”. While priming effects are typically focused on the turnover of C stores in the soil resulting in changes in CO<sub>2</sub> fluxes (Kuzyakov and Bol, 2006), these concepts can be applied to N pools as well. For example, long-term manure applications can lead to an abundance of N and OC in the soil, which in turn could “prime” the soil microbial community — including N cycling microbes — resulting in an increased loss of N (as N<sub>2</sub>O) when a more readily available form of N (e.g., urea fertilizer) is applied to the soil. Jenkinson et al. (1985) referred to this as “added nitrogen interactions (ANI)” that could result in either a ‘real’ or ‘apparent’ priming effect. “Real” priming occurs when N addition to the system results in a direct N-movement (i.e., loss or gain of soil organic N), “apparent” priming occurs when, there is simply pool substitution of the added N.

Organic amendments can be higher in organically bound N, especially solid manure applications (i.e. solid cattle manure (Wen et al., 2003)) which may provide the conditions conducive for a priming effect for increased N<sub>2</sub>O emissions. The length and duration of manure additions, and the subsequent build-up of soil SOM, might produce a priming effect that may persist for several years. While liquid swine manure application can lead to NO<sub>3</sub><sup>-</sup> build-up in agricultural soils which when environmental conditions are suitable (increased moisture), may also lead to higher rates of N<sub>2</sub>O loss. Manure applications provide higher quantities of available C and NO<sub>3</sub><sup>-</sup> that can sustain denitrifiers for periods of time well beyond the application (Calderón et al., 2005). Repeated applications can therefore result in a build-up in nutrients conducive to sustaining microbial activity. With a change in management on agricultural soils from a manure-based N application to a more readily available inorganic fertilizer N source, this carry-over of nutrients and microbial activity could lead to greater N losses, or a reduction of N use efficiency from the agroecosystem. Higher losses of N<sub>2</sub>O have been attributed to adaptations to ammonia oxidizing bacteria (AOB) after receiving long-term synthetic and organic (composted straw) fertilizer applications (Ding et al., 2010). The abundant N pools from continuous manure fertilization at or above recommended rates can induce N mineralization as a result of N

additions as suggested by Jenkinson et al. (1985), which can induce higher rates of denitrification resulting in increased N<sub>2</sub>O emissions. Positive priming occurs with additive effects to the environment (Kuzyakov et al., 2000). Nutrient mineralization as a result of microbial priming has been linked with positive priming effects where urea fertilization has been observed to increase mineralization of the OM. This positive priming effect is further illustrated as a shift in bacterial communities from gram positive to gram negative bacteria, as well as increases in the fungal abundance (Hammer et al., 2009). It is these positive priming effects that may result in negative impacts in the environment as they may increase losses from cycles that produce intermediate products (e.g., increased N<sub>2</sub>O loss to the atmosphere).

### **3 LONG-TERM MANURE FERTILIZER AMENDMENTS PRODUCE A “LEGACY” EFFECT OF INCREASED NITROUS OXIDE EMISSIONS**

#### **3.1 Preface**

The Dixon fertilizer research site was established in 1996 and included a Liquid Swine Manure (LSM) research trial and a Solid Cattle Manure (SCM) trial that were set up in adjacent blocks at the same site (Mooleki et al., 2002; Mooleki et al., 2004). The manure nitrogen (N) and urea fertilizer amendments were applied in the fall over the course of this study, until the final manure application in the fall of 2009. With two exceptions, the 1996 and 2002 fall applications which due to an early fall freeze-up were applied the following May. A no-fertilizer control treatment was included. The 2009 fall application was the final manure application and provided nutrients for the 2010 growing season. Beginning in the spring of 2011, an annual urea application was applied to the whole site including the former LSM and SCM blocks.

An initial greenhouse gas (GHG) investigation was conducted during the spring of 1998 through to seeding in the spring of 2000 quantifying nitrous oxide (N<sub>2</sub>O) emissions during the early stages of the manure trial at the Dixon long-term research site. A follow up N<sub>2</sub>O investigation was conducted during the 2009 and 2010 growing seasons, into the spring of 2011 (Farrell et al., 2011). During the second period of GHG monitoring, denitrification enzyme assays (DEA's) were conducted which showed a potential “priming” for higher N<sub>2</sub>O emissions in plots that had received the largest application rate of manure compared to the urea or control plots (Farrell et al., 2011). The potential for greater emissions as a result of long-term manure applications (1996-2009), as well as the introduction of a readily available reactive N source on an annual basis (beginning in 2011) provided an opportunity to increase our understanding of how N-fertilizer management may impact GHG emissions, namely N<sub>2</sub>O emissions post long-term manure application of both LSM and SCM in the sub-humid prairies in Saskatchewan, Canada.

### **3.2 Abstract**

Long-term manure applications [liquid swine (LSM) and solid cattle (SCM)] at the Dixon long-term manure research site were used to assess a “priming” effect on increased N<sub>2</sub>O emissions and their production as a result of a management regime change from manure to urea fertilizer. The Dixon site had a 14-year history of manure (LSM and SCM) at different rates (i.e., 1×, 2×, and 4× the recommended rate) and frequency (annually or triennially) of application, which also included urea plots and a no N-fertilizer control. The soils under a history of long-term manure applications had greater denitrification enzyme activities (DEA), which lasted from the first year after manure termination in 2011 — the first year of a blanket application of urea across the whole site including the former manure/N-fertilizer plots — until the final year of monitoring in 2014. This was especially apparent in the soils receiving repeated application of manure at high rates — four times greater than 50 kg plant available N ha<sup>-1</sup> — which resulted in the largest DEAs. The long-term plots with a history of annual LSM applications also showed a legacy effect as these plots had a significantly greater cumulative N<sub>2</sub>O-N loss with the greatest influence observed from the annual 4× LSM plots. Interestingly, the historically applied SCM did not show any significant differences in cumulative N<sub>2</sub>O losses from the historical control (0N) plots. No differences in cumulative N<sub>2</sub>O-N loss were observed between any of the manure treatments from the triennial application plots. Daily emission patterns were similar for all years of monitoring with snowmelt and N-fertilizer application contributing to most of the N<sub>2</sub>O loss. The greatest N<sub>2</sub>O emissions occurred during the spring of 2013 — three years after manure application termination — pointing to a sustained effect and indicating that there can be a priming effect from long-term manure applications that may only be expressed under field conditions when environmental conditions are favorable.

### **3.3 Introduction**

Fertilizer N (both inorganic and organic) is an important resource in agriculture, leading to greater productivity and increased crop yields. However, because of various inefficiencies, the use of fertilizer in agriculture can lead to an accumulation of excess N in the environment. Manure is an organic N fertilizer commonly used in agriculture in the Canadian prairies to supplement crop N demands. Solid cattle and liquid swine manure are two of the most common

manure forms, and are often applied if they are available within close proximity to the crop production operations that require them. In Canada solid manure is applied to approximately 1.75 million ha of land, while approximately 1.12 million ha receive liquid manures (Statistics Canada, 2011). Synthetic fertilizers (e.g. urea) are by far the main source of N-fertilizer applied in Canadian agriculture, and provide a readily plant-available N source. Both organic and inorganic (synthetic) fertilizer N, can increase crop yield by providing available N and other important nutrients to the system (Qian and Schoenau, 2000; Wen et al., 2003). However, the use of N fertilizers in agriculture also is a major source of N<sub>2</sub>O emissions to the atmosphere. Nitrous oxide is a potent greenhouse gas (GHG) and its increased concentration in the atmosphere increases trapping of solar radiation (IPCC, 2013), and stratosphere ozone depletion (Ravishankara et al., 2009). While N<sub>2</sub>O emissions are an unavoidable consequence of soil N cycling, anthropogenic activities such as fertilizer-N usage have greatly increased their magnitude and as such are an important symptom of N use inefficiency — increased N loss from the soil environment.

Losses of N<sub>2</sub>O may be further impacted by the predicted change in regional variability and extremes in temperature and precipitation due to a changing climate (IPCC, 2013). The production and movement of soil-emitted N<sub>2</sub>O is complex, being influenced by changes to external N additions, as well as many biotic (i.e. soil microbes) and abiotic soil factors (i.e. OC, water content, pH, etc.). At present, crop production throughout the Canadian prairies is thought to yield below its potential (Tilman et al., 2011). As food demand increases, the need for enhanced efficiencies and more intensive use of agricultural land will also increase. This may create conditions that augment N<sub>2</sub>O losses by increasing the use of N fertilizers. While the Canadian prairies are believed to be a low N<sub>2</sub>O producing region because they are drier than the more humid eastern agricultural lands (Rochette et al., 2008a), changes to fertility management could negate the benefits of reduced N<sub>2</sub>O loss. Therefore, increasing our understanding of how changes in management (e.g., switching from manure to a more readily available fertilizer N form) impact N<sub>2</sub>O emissions is necessary if we are to develop strategies that maximize N use efficiency and mitigate N<sub>2</sub>O losses.

Solid organic fertilizers (e.g. cattle manure) typically contain N bound in organic forms which must first be mineralized by the soil microbial community before it is available for plant uptake. These solid manure amended soils also have high C:N ratios that can slow and impede

organic-N mineralization (Qian and Schoenau, 2002). The rate and degree of mineralization will not only depend on the C:N ratio of the manure, but also the form and source of manure (i.e. liquid or solid manures). Solid cattle manure tends to have a higher C:N ratio and OC content than liquid swine manure (Table 3.1); therefore, solid cattle manure N is not as readily available and mineralizes more slowly (Qian and Schoenau, 2000; Qian and Schoenau, 2002; Helgason et al., 2007). For example, Wen et al. (2003) reported lower inorganic-N recovery rates from cattle manure than from swine manure on Saskatchewan soils. These studies suggest that solid cattle manures will not produce N<sub>2</sub>O emissions as large in magnitude as liquid manures — particularly liquid swine manure. However, consecutive solid cattle manure applications over several years can increase N availability by reducing the C:N ratio as a result of organic matter (N-containing) decomposition (Mooleki et al., 2004). In addition to the C:N ratio of manures, the N form and content of manures is quite variable, with the N in liquid swine manures mostly in the NH<sub>4</sub><sup>+</sup> form (Mooleki et al., 2002) while the N in solid cattle manure is mostly in an organic form. As a result, the N in liquid swine manures is more easily nitrified (Whalen, 2000) providing a rapid conversion of NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup>. The NO<sub>3</sub><sup>-</sup> can then be used by the crop, leached from the soil profile, or lost as N<sub>2</sub>O during denitrification (Chantigny et al., 2001). Liquid manures also tend to have C that is more readily available, and a higher water content that can lead to a more rapid conversion of N into N<sub>2</sub>O. Rapid utilization of liquid manure C and N accompanied by enhanced denitrification losses was reported by Loro et al. (1997) while examining the effects of liquid and solid cattle manures on denitrification. Interestingly, it was the solid cattle manure applications that resulted in larger N<sub>2</sub>O losses and peak emissions occurred later than those associated with the liquid manure (Loro et al., 1997).

Inorganic fertilizers provide readily available reactive N that can be used immediately by soil microorganisms and plants. In the Canadian prairies, synthetic fertilizer applications are typically applied at seeding or in the fall — before they are required for crop uptake. This can lead to inefficiencies in N utilization. These inefficiencies can be further exacerbated if N is applied in quantities above what is required to ensure maximum yields. Large fluxes of N<sub>2</sub>O are a by-product of the extra N introduced to soils, with the greatest fluxes occurring soon after fertilizer applications (Skiba et al., 1992). Using mineral N applications, Lebender et al. (2014) observed linear increases in emissions as application rates were increased beyond crop demands. However, non-linear (exponential) patterns for fertilized-induced N<sub>2</sub>O losses have also been

reported, and have been proposed to be more appropriate descriptors of fertilizer induced emissions (Shcherbak et al., 2014).

**Table 3.1 Manure fertilizer properties from Canadian studies including the Dixon site from selected literature**

Manure Fertilizers	Total N (g kg <sup>-1</sup> )	Available N (NH <sub>4</sub> <sup>+</sup> )(g kg <sup>-1</sup> )	Total C (g kg <sup>-1</sup> )	C:N	Source
Liquid Swine <sup>‡</sup>	86.00	59.86	411.80	4.79	Chantigny et al., 2001
Liquid Swine <sup>‡‡</sup>	2.55	1.94	20.66	8.10	Mooleki et al., 2002
Liquid Swine <sup>‡</sup>	3.94	2.00	31.91	8.10	Mooleki et al., 2002
Liquid Cattle <sup>‡‡</sup>	2.54	1.33	22.73	8.95	Rochette et al., 2008b
Solid Cattle <sup>†</sup>	12.75	1.88	255.00	20.00	Mooleki et al., 2004
Solid Cattle	16.25	2.25	325.00	20.00	Mooleki et al., 2004
Solid Cattle	3.75	1.10	52.13	13.90	Paul and Beauchamp, 1994
Solid Cattle	20.00	1.78	316.00	15.80	Helgason et al., 2007
Solid Cattle	14.00	1.60	417.00	29.80	Helgason et al., 2007
Solid Cattle <sup>§</sup>	5.45	0.75	71.67	13.15	Rochette et al., 2008b
Composted Cattle <sup>§</sup>	17.38	0.53	197.88	11.73	Helgason et al., 2007
Composted Cattle	5.96	0.02	103.70	17.40	Paul and Beauchamp, 1994

<sup>‡</sup>Units are g L<sup>-1</sup>

<sup>§</sup>Values averaged from source

<sup>†</sup>Manure applied at the Dixon long-term research site

Organic fertilizers add OC as well as nutrients to soils (Hopkins and Shiel, 1996; Whalen, 2000), which is important because it not only alleviates nutrient deficiencies but also provides an abundant energy source for the microbial nutrient transformations. In the case of denitrification, the potential production of N<sub>2</sub>O by heterotrophs requires OC as the primary energy source and electron acceptor during the reduction of NO<sub>3</sub><sup>-</sup> (Canfield et al., 2010). An increase in C can also result in an increase in N cycling between organic and inorganic forms with higher net N immobilization (Paul and Beauchamp, 1994). Therefore, manures with different C:N ratios can have varied influence on substrate use by microbial communities. Higher N additions to soils can promote microbial uptake and immobilization. Residues (i.e., barley residues) contained in the manure or added upon application can also lead to increased mineralization or immobilization of N (Chantigny et al., 2001). The long-term application of cattle manure has been shown to increase microbial C in the topsoil, and maintain a greater extractable-C to SOC ratio at depth,

through additions from microbial C (e.g., amino sugars) (Sradnick et al., 2014). Changes to denitrification also have been observed from long-term manure applications. Denitrification rates in soils are generally high following fertilizer additions that increase N in the system or adjust the soil pH (Mulvaney et al., 1997).

Excess nutrients from any N-fertilizer application that can lead to greater nutrient losses from the system can also impact priming effects (Kuzyakov et al., 2000). In this case, priming effects are defined as long-term manure applications that remove nutrient (N and C) limitations resulting in greater N loss as N<sub>2</sub>O. Therefore, the build-up or soil storage of N as a result of long-term application of manures could lead to future issues of N loss. Nitrogen application rates that exceed crop requirements can lead to NO<sub>3</sub><sup>-</sup>-N loading (Stumborg et al., 2007) that in turn can lead to increased N<sub>2</sub>O emissions (Yates et al., 2006a; Rochette et al., 2008b). The leaching of NO<sub>3</sub><sup>-</sup>-N deeper into the soil profile is a concern with swine manures, as the NO<sub>3</sub><sup>-</sup> can build up rapidly after application if not used by crops (Chantigny et al., 2001). Labile N sources are a concern as they are prone to downward movement in soils, which in the case of long-term liquid swine manure applications can lead to large stores of NO<sub>3</sub><sup>-</sup> at depth. This, in turn, raises the concern that long-term liquid swine manure applications may lead to greater N<sub>2</sub>O losses, particularly in view of the added C that may become available after termination of the application. Because of the capacity of manures to increase OC and N, long-term applications could therefore create conditions conducive to a priming effect for N<sub>2</sub>O emissions. This priming effect could be of great importance as 2.5% of synthetic fertilizer-N produced globally has been estimated to have been converted to N<sub>2</sub>O between 1860 to 2005 (Davidson 2009) and a priming effect could result in even greater losses by increasing the proportion of N lost as N<sub>2</sub>O. While also resulting in economic costs such as lost yields for producers.

This study examined how different N-fertilizers (solid cattle manure, liquid swine manure, or urea) applied at different rates and frequencies over 14 years at the Dixon long-term research site contributed to conditions conducive for increased N<sub>2</sub>O emissions when fertility management was changed to an annual application of chemical N-fertilizer. Soil-emitted N<sub>2</sub>O was measured from snowmelt until snowfall at the Dixon site during the 2011/12, 2012/13, and 2013/14 seasons. Cumulative N<sub>2</sub>O losses were derived from an N-budget years (e.g., 2011/12) incorporating all N<sub>2</sub>O losses from the soil during from fertilizer application until the following years fertilizer application incorporating spring snowmelt into the previous growing season's

budget. Cumulative emissions were used to identify differences in N loss as a result of the historical N-source applications. The potential for greater gaseous N loss was further examined using the denitrification enzyme activity (DEA) assay. The DEA's were conducted on a subset of the soil amendments on three occasions during both the 2012 and 2013 growing seasons. These analyses were used as they allow a “worst case” potential of N<sub>2</sub>O loss alleviating any potential variation due to environmental differences thus enabling the determination of whether the long-term manure applications had “primed” the soil for increased N<sub>2</sub>O emissions and, if so, to assess the duration of the priming effect at the Dixon site. My hypotheses for this study were: (i) long-term manure applications ‘prime’ the soil for increased N<sub>2</sub>O emissions when a more readily available N-source is applied to the same field, and (ii) the duration of this priming will be short-lived and will not impact emissions for a timeframe longer than the three post-manure applications.

### **3.4 Methods and Materials**

#### **3.4.1 Field site description**

Field sampling and monitoring was conducted at the Dixon long-term agricultural research site which is situated on privately owned and operated agricultural land located west of Humboldt, Saskatchewan (U.T.I. Coordinates NW21-37-23-W2). The soil is classified as a Black Chernozem of the Cudworth association that developed on calcareous, lacustrine parent material with a loam texture (31% sand, 45% silt, 24% clay), an electrical conductivity of 1.3 dS m<sup>-1</sup>, and a pH of 7.5 (Wen et al., 2003; Stumborg et al., 2007). The landscape is undulating but the study site is gently sloping (King, 2007). There is a wetland located on the southern edge of the site, and during wet years, small water-filled depressions often develop in the low lying areas.

The research site at Dixon was established in 1996 to investigate the agronomic and environmental benefits of manure applications. Liquid Swine Manure (LSM) and Solid Cattle Manure (SCM) trials were established in adjacent blocks at the same site (Mooleki et al., 2002, 2004). Manure amendments (with application rates based on N content) were applied in the fall — with two exceptions, the fall of 1996 and 2002, when an early freeze prevented fall application and resulted in the manure being applied in the spring of 1997 and 2003 respectively

(Mooleki et al., 2002, 2004; King, 2007). The soil amendments included the manures as well as a set of urea fertilizer treatments and a no-fertilizer control; treatments were applied starting in 1996 and continued through the fall of 2009. The 2010 growing season was the final year in which crop nutrient demands were met by the addition of manure or urea applied the previous fall. Starting in 2011, the entire site received an annual application of urea-N (plus any P, K, and/or S as required) at seeding. Seeding and fertilizer operations were applied at the same time (May 16, 2011, May 27, 2012, and May 22, 2013). Fertilizer rates were based on the results of a fall soil test and the target yield of the coming year's crop.

### **3.4.2 Experimental design**

The Dixon long-term manure research site included two manure trials: one using LSM and the other using SCM. Due to differences in methods of application and the size of the equipment involved, it was not possible to integrate both manure types into a single design. Consequently, the manure trials were established side-by-side, with each trial set up in a randomized complete block design. The LSM trial included 15 treatments replicated four times; the plots (3.05-m wide  $\times$  30.5-m long) were arranged running east to west, with Blocks 1 and 2 located to the north of Blocks 3 and 4 (Fig. 3.1). The LSM treatments were applied using a Prairie Agriculture Machinery Institute (PAMI; Humboldt, SK) manifold/cutter with a 30-cm spacing, and were applied by sub-surface injection at a depth of 10-cm (Mooleki et al., 2002). The SCM trial included 12 treatments replicated 4 times; the plots (3.05-m wide  $\times$  3.05m long) were arranged running east to west in four parallel blocks that were arranged from north to south (Fig. 3.2). The SCM treatments consisted of a feedlot mixture (i.e., fecal matter and straw bedding) that had been stockpiled for less than one year and was mixed prior to being manually broadcast and incorporated into the soil using a rototiller (Mooleki et al., 2004).

For logistical and budgetary reasons, not all of the treatments present at the Dixon site were included in this study. The treatments included are listed in Table 3.2, and varied in N-source (LSM, SCM, and urea), N-rate (1 $\times$ , 2 $\times$ , 4 $\times$  the agronomic rate), and frequency of application (applied annually or triennially). To describe these treatments a unique treatment code was utilized; beginning with an H indicating that these are historical N-treatments; followed by the type of N-treatment (i.e. liquid swine (LS), solid cattle (SC), and urea fertilizer (UF)); the historical timing of application (annually (A) or triennially (T)) ; and the historical rate of

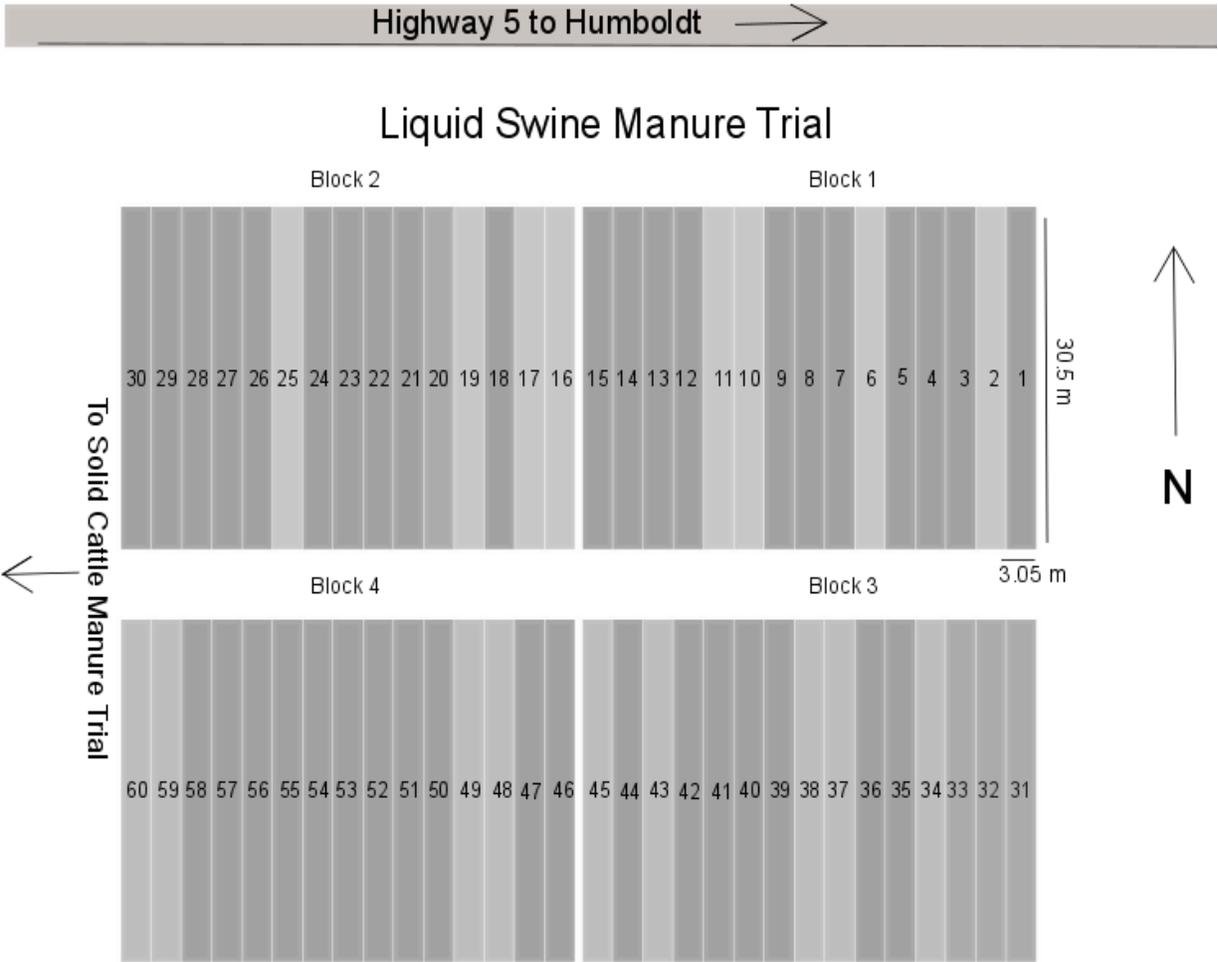
application (i.e., 1×, 2×, or 4×) (e.g., for the historical annual LSM 1× application would be HLS-A1; the historical control plots within the LSM trial or the SCM trial would be HLS-C0 or HSC-C0 respectively). Triennial application means that manure was applied once every three-years. The subset of treatments utilized in this study were selected to provide a range of historical conditions and included (i) the unfertilized (0N) control; (ii) the 1× annual application of urea (i.e., the “standard” BMP); (iii) the 2×, triennial application of manure (i.e., the recommended manure BMP); and (iv) the 4×, annual application of manure (i.e., the “worst case scenario”).

**Table 3.2 Historical N treatments, timings, and application rates for liquid swine manure (LSM) and solid cattle manure (SCM) trials. All amendments applied from fall 1996 until fall 2009. Only the treatments included in the GHG sampling are listed.**

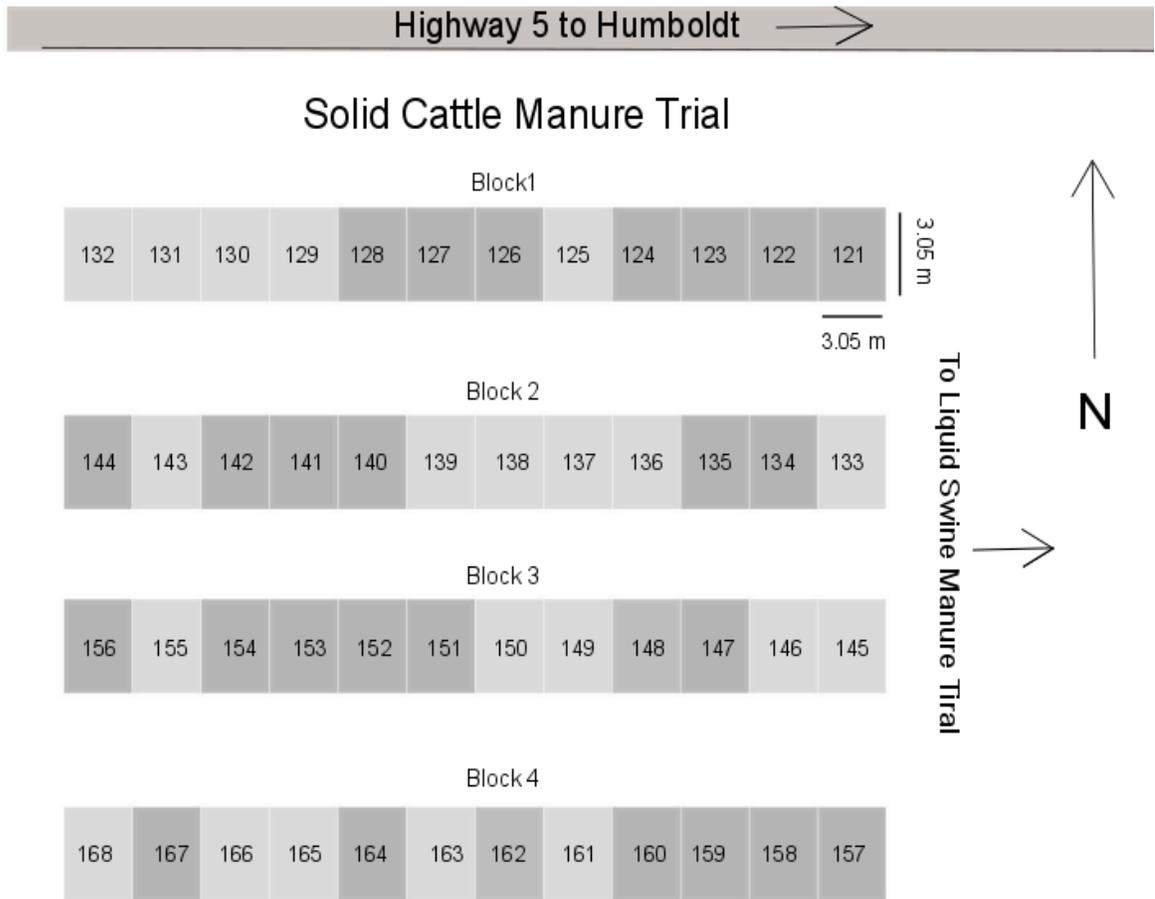
Manure Trial	Plot no.	N source	Application Rate <sup>†</sup>	Application Frequency	Treatment ID
<b>Liquid Swine Manure (LSM)</b>	1, 24, 44, 57	None	0×	— — —	HLS-C0
	13, 20, 33, 47	Urea	1×	Annual	HUF-A1
	14, 22, 39, 46	Urea	2×	Annual	HUF-A2
	15, 26, 36, 51	Urea	4×	Annual	HUF-A4
	4, 29, 38 <sup>‡</sup> , 55	LSM	1×	Annual	HLS-A1
	7, 27, 35, 54	LSM	2×	Annual	HLS-A2
	9, 28, 31, 50	LSM	4×	Annual	HLS-A4
	3, 21, 42, 58	LSM	1×	Triennial	HLS-T1
	5, 30, 32, 52	LSM	2×	Triennial	HLS-T2
	8, 18, 41, 56	LSM	4×	Triennial	HLS-T4
<b>Solid Cattle Manure (SCM)</b>	121, 134, 148, 160	None	0×	— — —	HSC-C0
	123, 140, 156, 159	SCM	1×	Annual	HSC-A1
	126, 141, 149, 158	SCM	2×	Annual	HSC-A2
	128, 142, 154, 157	SCM	4×	Annual	HSC-A4
	122, 144, 152, 162	SCM	1×	Triennial	HSC-T1
	124, 138 <sup>‡</sup> , 153, 167	SCM	2×	Triennial	HSC-T2
	127, 139 <sup>‡</sup> , 147, 164	SCM	4×	Triennial	HSC-T4

<sup>†</sup>Plant available N; 0×, 1×, 2×, 4× rates = 0, 50, 100 & 200 kg N yr<sup>-1</sup> respectively.

<sup>‡</sup>Plots removed from gas sampling due to wrong application during historical applications.



**Fig. 3.1. Plot map (map is not to scale) for the liquid swine manure (LSM) trial at the Dixon long-term manure research site. Plots shaded dark grey were included in the current study; i.e., only these plots were sampled for greenhouse gas emissions during the period from spring 2011 through spring 2014; plots shaded light grey were not sampled.**



**Fig. 3.2. Plot map (not drawn to scale) for the solid cattle manure (SCM) trial at the Dixon long-term manure research site. Plots shaded dark grey were included in the current study; i.e., only those plots were sampled for the greenhouse gas emissions during the period from spring 2011 through spring 2014; plots shaded light grey were not sampled.**

Manure and urea application rates were based on meeting the crop nutrient requirements for the year (agronomic rate), with the base (1×) rate being equivalent to 50 kg plant available-N ha<sup>-1</sup>. Thus, urea was applied at a rate of 50 kg of N year<sup>-1</sup>, but manure was applied at a rate equivalent of 100 kg total-N ha<sup>-1</sup>, assuming only 50% of the N was plant available (Mooleki et al., 2002, 2004; Stumborg, 2006; King, 2007). The research site was managed by PAMI and the area surrounding the site was farmed and maintained by the land owner (Collin Ford). Site management followed a 4-year grain-oilseed rotation of barley, canola, wheat, and flax; and included herbicide applications as necessary.

### 3.4.3 Greenhouse gas monitoring

Ambient N<sub>2</sub>O fluxes were determined using non-steady state, vented chambers (Fig. 3.3) adapted from previous studies (Corre et al., 1996; Farrell et al., 1999; Yates et al., 2006a). The chambers were wrapped in reflective bubble insulation to mitigate against temperature fluctuations during sampling. Collar rings were placed in the experimental plots following the fall manure/urea application and remained in place until just before seeding operations in the spring; the collars were then removed until seeding operations were completed and then placed back in the plots where they remained until agricultural practices (i.e., heavy harrowing) in the fall required that they be removed. Plants were removed from inside the collars to ensure that gas fluxes were measured from the bare soil only.

Chambers were installed at 65 locations (see Table 3.2), with collars (15-cm × 20.3-cm i.d.) for the chambers manually driven into the soil to a depth of 5-cm. Each chamber had an internal headspace volume (including the above-ground portion of the collar) of 1766 cm<sup>3</sup> and a surface area of 176 cm<sup>2</sup>, yielding a volume-to-surface area ratio of *ca.* 10:1. The chambers were attached to the collars using two flip clamps located on opposite sides of the base. Once a chamber was sealed to the collar, a total of four gas samples per chamber were collected at 15-min intervals (i.e., at t<sub>0</sub>, t<sub>15</sub>, t<sub>30</sub>, and t<sub>45</sub>).



**Fig. 3.3. Vented, non-steady state chamber.** Note: prior to deployment, each chamber was wrapped with insulation and reflective foil to minimize heating effects.

Gas samples were collected from the enclosed headspace using a disposable, 25-mL syringe equipped with a 25-gauge,  $\frac{5}{8}$ -inch needle. The syringe was flushed with ambient air 3-times prior to sample collection to eliminate carry-over, and gas samples withdrawn through a Swagelok™ sampling port (sealed with a gray butyl rubber septum) in the top of each chamber. The gas samples were then injected into pre-evacuated (*ca.*  $5 \times 10^{-3}$  atm), 12-mL Exetainer® vials (Labco, Canada) where they were analyzed using gas chromatography (Farrell and Elliott, 2008). The gas samples were stored under a slight positive pressure (*ca.* 2-atm) and returned to the Department of Soil Science, University of Saskatchewan for N<sub>2</sub>O analysis (usually within 36- to 48-h). The CO<sub>2</sub>, N<sub>2</sub>O, and CH<sub>4</sub> greenhouse gas concentrations were determined using a Bruker 450 gas chromatograph equipped with electron capture, thermal conductivity and flame ionization detectors for N<sub>2</sub>O, CO<sub>2</sub>, and CH<sub>4</sub>, respectively (Bruker Biosciences Corporation, Billerica, MA, USA) (Farrell and Elliott, 2008).

Ambient air samples were collected at regular intervals during the sampling period and used to detect the minimum detectable concentration difference (MDCD) between samples collected at  $t_0$  and each subsequent time step. The MDCD was calculated using the average of the differences between paired air samples ( $\mu_{\text{Pair Difference}}$ ) and the standard deviation of the paired air samples ( $\sigma_{\text{Pair Difference}}$ ) (Eqn. 3.1; Yates et al., 2006a).

$$MDCD = \mu_{\text{Pair Difference}} + (2\sigma_{\text{Pair Difference}}) \quad [\text{Eqn. 3.1}]$$

The MDCD was used to identify potentially problematic data points and determine the flux calculation method to be used by the HMR model (Pedersen, 2015) — a modification of the Hutchinson-Mosier method (Hutchinson and Mosier, 1981) — implemented as an add-on package for the R statistical software program (3.1.3; R Development Core Team, 2015). Daily fluxes were calculated using the HMR package and fitted either a non-linear (exponential) regression or a linear regression (LR) to the concentration vs. time data. Linear regression was used to calculate the daily flux whenever the change in concentration was less than the MDCD, allowing for all GHG data to be used; when the change in concentration was greater than the MDCD, the daily flux was calculated using the method suggested by the HMR package. No

outlier data were removed and negative values were not forced to a zero value allowing for any micro-conditions or hot spots caught by the chambers to be assessed.

Gas sampling was conducted from snowmelt in early spring until freeze-up in late fall. Sampling frequency was greatest in the spring (2–3 times per week) in order to catch the large emission events associated with spring thaw and seeding. Sampling frequency decreased as the crop matured and the soil N pool was depleted, with sampling occurring only once per week during June and July, and once every two weeks from August until freeze-up. Cumulative gaseous N<sub>2</sub>O losses were determined by plotting the daily flux vs. time (day of the year) curve for each complete sampling season and calculating the area under the curve using Eqn. 3.2:

$$t - N_2O = f_I + \sum[(DOY_{n+x} - DOY_n) * (\frac{f_{n+x} + f_n}{2})] + f_L \quad [\text{Eqn. 3.2}]$$

where  $t-N_2O$  is the cumulative (total) N<sub>2</sub>O loss for the sampling period (kg N<sub>2</sub>O-N ha<sup>-1</sup> yr<sup>-1</sup>);  $f_I$  and  $f_L$  are the initial and final daily fluxes measured during the sampling period;  $f_n$  is the flux measured on a particular day of the year ( $DOY_n$ ); and  $f_{n+x}$  is the flux measured  $x$  days later ( $DOY_{n+x}$ ).

#### 3.4.4 Soil sampling and processing

Soil samples (0-10 cm) were collected for determination of the available N (NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) in the spring and fall of 2013, and the spring of 2014. Duplicate soil cores (4.5-cm i.d.) were collected from inside the flux chamber collar in each plot; the samples were composited and bagged in the field, and then returned to the Department of Soil Science in Saskatoon where they were stored in a walk-in cooler at 4°C until analyzed. Available N analyses were performed following the method of Maynard et al. (2008); briefly the soils were removed from the cooler, a 5.00 g field moist soil subsample was extracted with 50-mL of 2M KCl solution, shaken for 1-hr at 142 rpm on a rotary shaker, then filtered into 16-dram vials using VWR® 454 filter paper (VWR International Edmonton, AB, Canada). Gravimetric soil water content (GSWC) was determined by drying a subsample of the soil at 105°C for 24-hr; the GSWC was then used to determine the dry weight equivalent of the moist soil sample used in the KCL extraction.

### 3.4.5 Denitrification enzyme activity

Denitrification enzyme activity (DEA) were determined for a subset of the historical urea and manure treatment plots. The plots sampled were the control treatment (HLS-C0), standard practice treatments [i.e., annual application of urea fertilizer at the soil test recommendations (HUF-A1) and manure application at twice the recommended N rate, but applied every third year (HLS-T2 and HSC-T2)], and those receiving the highest manure-N rates [i.e., annual applications at 4-times the recommended N rate (HLS-A4 and HSC-A4)]. The HLS-T2 and HSC-T2 plots are standard practice applications comparable to the annual 1× urea application [i.e., 50 kg plant available N ha<sup>-1</sup> (Stumborg, 2006)] allowing for an equivalent base-line to the annual Urea applications that began in 2011 after the termination of manure applications (fall 2009). Soil samples were collected on three occasions in 2012: prior to fertilization/seeding in May (May 16<sup>th</sup>), at 50% flowering in July (July 10<sup>th</sup>), and at harvest in September (September 4<sup>th</sup>). In 2013, the sample collection dates were changed in order to catch the spike in emissions that normally follows fertilizer/seeding operations. The 2013 samples were taken before (May 14<sup>th</sup>) and after fertilization/seeding (May 29<sup>th</sup>) event in May and again immediately prior to harvest in September (September 16<sup>th</sup>). Each soil sample (0-10 cm) was extracted using a 3.14-cm (i.d.) back-saver probe, and five samples were taken from each plot. Because of the difference in the sizes of plots between the LSM and SCM blocks, the sampling method was different. Sampling of the LSM plots was varied to reflect the larger size of the LSM plots compared to the SCM plots. In the long rectangular LSM plots which were orientated in a north south direction the collars were located approximately 20 feet from the northern edge. The first soil sampling core was taken between the north edge and the collar, three subsequent samples were taken around each collar, and one between the collar and the south edge (except the May 2012 samples were taken in a transect along the length of the plot); while in the smaller square SCM plots, the five samples were taken from around the collar throughout the plot. The five soil cores from each plot were bagged together and homogenized in the field and immediately placed into a cooler with ice packs. The soil samples were returned to the Department of Soil Science in Saskatoon and stored in a 4°C walk-in cooler. Within 24-hr of collection, the soil samples were sieved to pass a 4.75-mm screen and a sub-sample of the sieved soil was used immediately for DEA analysis, with a second sub-sample of the sieved soil stored in a 7-oz Whirl-pak<sup>®</sup> (Nasco,

Fort Atkinson, USA) bag at  $-80^{\circ}\text{C}$ , and the remainder stored in a chest freezer at the University of Saskatchewan.

The DEA assays (Alef, 1995; Groffman et al., 1999; Drury et al., 2008) were conducted between June 2011 to May 2014. The assay itself was adapted from Groffman et al. (1999) and involved weighing 6.50 g field moist soil (equivalent to  $5.24 \pm 0.15$  g oven-dry weight; sieved to  $< 4.75\text{-mm}$ ) into an acid-washed 160-mL serum bottle<sup>1</sup>. A 10-mL aliquot of DEA solution [prepared by dissolving 1.44 g potassium nitrate ( $\text{KNO}_3$ ), 1.00 g glucose, and 0.25 g chloramphenicol in 2 L distilled deionized water] was added to each serum bottle, which was then sealed with a gray butyl rubber septum using an aluminum crimp cap. The serum bottles were then connected to a gas manifold and purged of ambient air by flushing with ultra high purity (UHP)  $\text{N}_2$  gas for 2-min. The serum bottles were disconnected from the manifold and acetylene was injected into each bottle (acetylene concentration – 10% v/v) and the headspace mixed by pumping the syringe for 15-s before withdrawing a 10-mL sample of the headspace gas ( $t_0$ ) and injecting it into a pre-evacuated 12-mL Exetainer<sup>®</sup> vial containing 10-mL UHP  $\text{N}_2$ . To maintain a constant pressure in the serum bottles, 10-mL of UHP  $\text{N}_2$  was injected into each serum bottle immediately after the headspace gas sample was collected. The serum bottles were then placed on a rotary shaker and shaken at 125 rpm for a total of 90 minutes, with headspace sampled at 30-min intervals (i.e.,  $t_{30}$ ,  $t_{60}$ , and  $t_{90}$ ). Analytical blanks (i.e., serum bottles that did not contain soil but were treated exactly the same as the bottles containing the soils) were included in each set of analyses. Duplicate analyses for each soil were run in 2011, 2012, and 2013; however, because of time constraints [and considering that between-duplicate coefficients of variation were generally quite small ( $<7\%$ )] duplicates were not included for the spring 2014 analyses.

The Exetainer<sup>®</sup> vials containing the headspace gas samples were placed in a combi-PAL auto-sampler connected to a Bruker 450 gas chromatograph (Bruker Corp., Billerica, MA, USA) equipped with a  $^{63}\text{Ni}$  electron capture detector for the determination of  $\text{N}_2\text{O}$ . Nitrous oxide concentration ( $\mu\text{L N}_2\text{O-N L}^{-1}$ ) vs. time (min) curves were prepared for each sample, and the DEA calculated using Equation 3.3, adapted from Alef (1995) and modified with Drury et al. (2008):

---

<sup>1</sup> Note: 125-ml serum bottles were used for the May and July 2012 DEA assays.

$$DEA = (S_{N_2O}) \left( \frac{28.014}{V_m} \right) \left( \frac{(V_{SB} - V_{Solution} - V_{Soil})}{ODW} \right) \quad [\text{Eqn. 3.3}]$$

where *DEA* is the denitrification enzyme activity ( $\mu\text{g N}_2\text{O-N g}^{-1} \text{ min}^{-1}$ );  $S_{N_2O}$  is the slope of the  $\text{N}_2\text{O}$  concentration vs. time curve for each sample ( $\mu\text{L N}_2\text{O L}^{-1} \text{ min}^{-1}$ ); 28.014 is the molecular weight of  $\text{N}_2$ ;  $V_m$  is the molar volume of a gas at STP ( $22.414 \mu\text{L } \mu\text{mol}^{-1}$ );  $V_{SB}$  is the volume of the serum bottle (125- or 160-mL);  $V_{Solution}$  is the volume of DEA solution (10-mL);  $V_{Soil}$  is the volume of soil ( $ODW/PD$ ;  $PD = \text{particle density} = \text{g}/2.65 \text{ g cm}^{-3}$ ); and  $ODW$  is the oven dry weight of the soil (g). Note 1: the  $ODW$  was determined by drying a subsample of the soil at  $105^\circ\text{C}$  for approximately 48-hr.

### 3.4.6 Weather data

All weather data were compiled by Environment Canada for the Saskatoon area (i.e., the RCS Station Saskatoon) (Environment Canada, 2015a) which, though located roughly 110 km west of the Dixon site, was the closest accessible weather station to the site. A comparison of the climate at the Saskatoon site with that at the Dixon long-term research site was conducted using spatially interpolated climate data as described by Hijmans et al. (2005, 2016). Climate surfaces comparing the mean annual precipitation and temperature at Saskatoon and Dixon (see Appendix A) were developed using WorldClim interpolated climate layers data (1950-2000) and the “raster” package in R (3.1.3; 2015) (Hijmans et al., 2016).

### 3.4.7 Statistical analysis

All statistical analyses were completed using the statistical software R (3.1.3) (R Development Core Team 2015). The cumulative  $\text{N}_2\text{O}$  loss data were not normally distributed and so were transformed using a Johnson transformation in R (Fernandez, 2015) that assesses the values and probabilities to choose whether to use the “SU” (unbounded system), “SB” (bounded system), or “SL” (log-normal system) transformation functions. Exploratory data analysis revealed that a Johnson “SL” transformation was best for the cumulative  $\text{N}_2\text{O}$  data. The Johnson transformation was chosen based on its effectiveness in normalizing chamber-based  $\text{N}_2\text{O}$  measurements (Moulin et al., 2014), and, indeed there was improvement to the data towards normal distributions following the transformation. Moreover, using Levene’s test for equality of variances ( $\alpha < 0.05$ ), it was determined that the transformed data met the requirements of homogeneity of variance for parametric analyses. Similarly, the denitrification enzyme activity

data were log-transformed to normal distributions that met the requirements of homogeneity of variance for parametric analyses ( $\alpha < 0.05$ ). For each manure type (LSM and SCM), treatment effects on cumulative N<sub>2</sub>O loss were assessed using a two-way ANOVA. The DEA's were natural log transformed for statistical analyses and backtransformed for respective tables. The log of the DEA's was assessed using a one-way ANOVA looking at treatment effects on denitrification enzyme activities. Significant differences ( $p \leq 0.10$ ) between treatments for both the two-way and one-way ANOVA's were assessed using a least significant difference (LSD) test, and was performed using the "agricolae" package in R (Mendiburu, 2016).

## 3.5 Results

### 3.5.1 Local weather

The Environment Canada weather station closest to the Dixon site was in Saskatoon, which is about 110 km west of the research site. However, Saskatoon and Dixon have similar climates —based on the climate surfaces (Appendix A1 and A2) the Dixon area tends to be a little wetter and cooler. During the course of this study (i.e., from spring 2011 through spring 2014), weather conditions in the Central Saskatchewan region ranged from very wet to relatively dry (Table 3.3). Indeed, during the 2010 crop year (i.e., from May 27<sup>th</sup> to October 21<sup>st</sup>) cumulative precipitation was 84% (256 mm) greater than the long-term (30-yr) average. Above-average precipitation (+43%; 130 mm) also occurred in 2012. At the other extreme, 2011 and 2013 were drier than normal — with 20% and 32% less cumulative precipitation, respectively, than the 30-yr average. Mean seasonal temperatures during this period were typically cooler (0.5-1.2°C) than the 30-yr average — except during the years when there was greater than normal precipitation (2010 and 2012), when the temperature averaged about 0.5°C higher than normal.

Greater than normal precipitation in 2010 produced flooding throughout the region, particularly in areas of lower depression and in catchment waterways and had a significant impact on the Dixon research site. For example, seeding and harvest were late (June 2<sup>nd</sup> and October 1<sup>st</sup>, respectively), resulting in reduced yields (Jeff Schoenau, 2013 Personal Communications). Cooler than usual temperatures occurred during the spring 2011, resulting in a late spring thaw and, in turn, a late start to the gas sampling program. Conversely, spring 2012

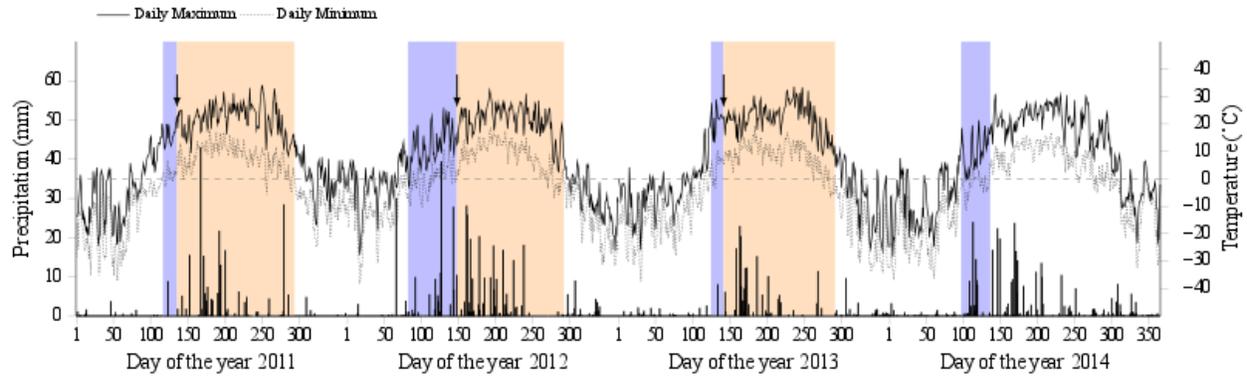
was characterized by a shallower than normal snow cover, with a warmer spring allowing for an early start to the GHG monitoring (see Table 3.3). Greater fall precipitation and deeper snow cover over the 2012/2013 winter — combined with cool temperatures in the spring (Fig. 3.4) — resulted in a late spring melt and delayed start to the 2013 sampling season. In all years, greenhouse gas monitoring was completed in mid to late October when the surface soil became frozen and covered with snow.

**Table 3.3 Total precipitation and mean annual temperature (based on Environment Canada data for Saskatoon, SK RCS Station). The fertilizer applications rates are for that growing season, as well as the crop seeded that year.**

Crop Year	Seasonal Precipitation (mm) ‡	Mean Temperature (°C) ‡	Gas Sampling (start - end)	Fertilizer Application (NPKS) (kg nutrient ha <sup>-1</sup> )	Crop
2010	560.5	10.18	—	LSM & SCM	Canola (Nexera Hybrid)
2011	241.8	9.28	May 27 - Oct 21	81-25 -11-11	Malt Barley (CDC Copeland)
2012	434.3	10.21	Mar 22 – Oct 18	135-29-0-22	Canola (Nexera Hybrid)
2013	208.1	8.54	May 5 - Oct 31	95-25-11-11	Hard Red Spring Wheat
2014	323.7	8.86	Apr 5 - May 16	—	—
<b>Norm<sup>†</sup></b>	<b>304.2</b>	<b>9.75</b>	—	—	—

† 30-yr (1981-2010) average from Environment Canada Weather data Saskatoon, SK.

‡ Sampling season is designated from March 1 – October 31



**Fig. 3.4. Mean daily temperature and daily total precipitation for the study period. (Environment Canada, 2015a). Days are based on Julian days (Day 1 = January 1<sup>st</sup>). The black arrows indicate the yearly seeding date. The blue shaded region is the gas sampling period after snowmelt, but prior to seeding in the spring; while the tan shaded region is the gas sampling period from immediately after seeding until snowfall and soil freeze-up in the fall.**

### 3.5.2 Denitrification enzyme activity

The historical N-treatment had a significant impact on present day (post-manure) DEA activities; e.g., DEA's associated with the long-term application of LSM were generally greater than those associated with the non-fertilized control (HLS-C0) or historical 1× urea treatment (HUF-A1) (Table 3.4). This was especially true for the treatment receiving annual applications of LSM at the 4× rate (HLS-A4). Moreover, although by 2014 all the plots had received an annual application of urea for 3 years, the HSL-A4 treatment was still producing more ( $p = 0.018$ ) total N<sub>2</sub>O-N than the historical control (HLS-C0) (Table 3.4). Likewise, the manure BMP treatment (HLS-T2) yielded DEAs that were greater than the historical control and/or the urea BMP treatment (HUF-A1) in three of four sampling years. Interestingly, the HLS-A4 treatment was significantly greater than the HLS-T2 treatment only in the driest years (i.e. 2011 and 2013). Similar results were observed for the plots with a history of SCM applications (Table 3.4). Interestingly, DEAs associated with the historical BMPs (i.e., the HLS-C0 and HUF-A1) did not differ significantly despite the fact the HLS-C0 plots had received no N fertilizer during the 14 years prior to 2011.

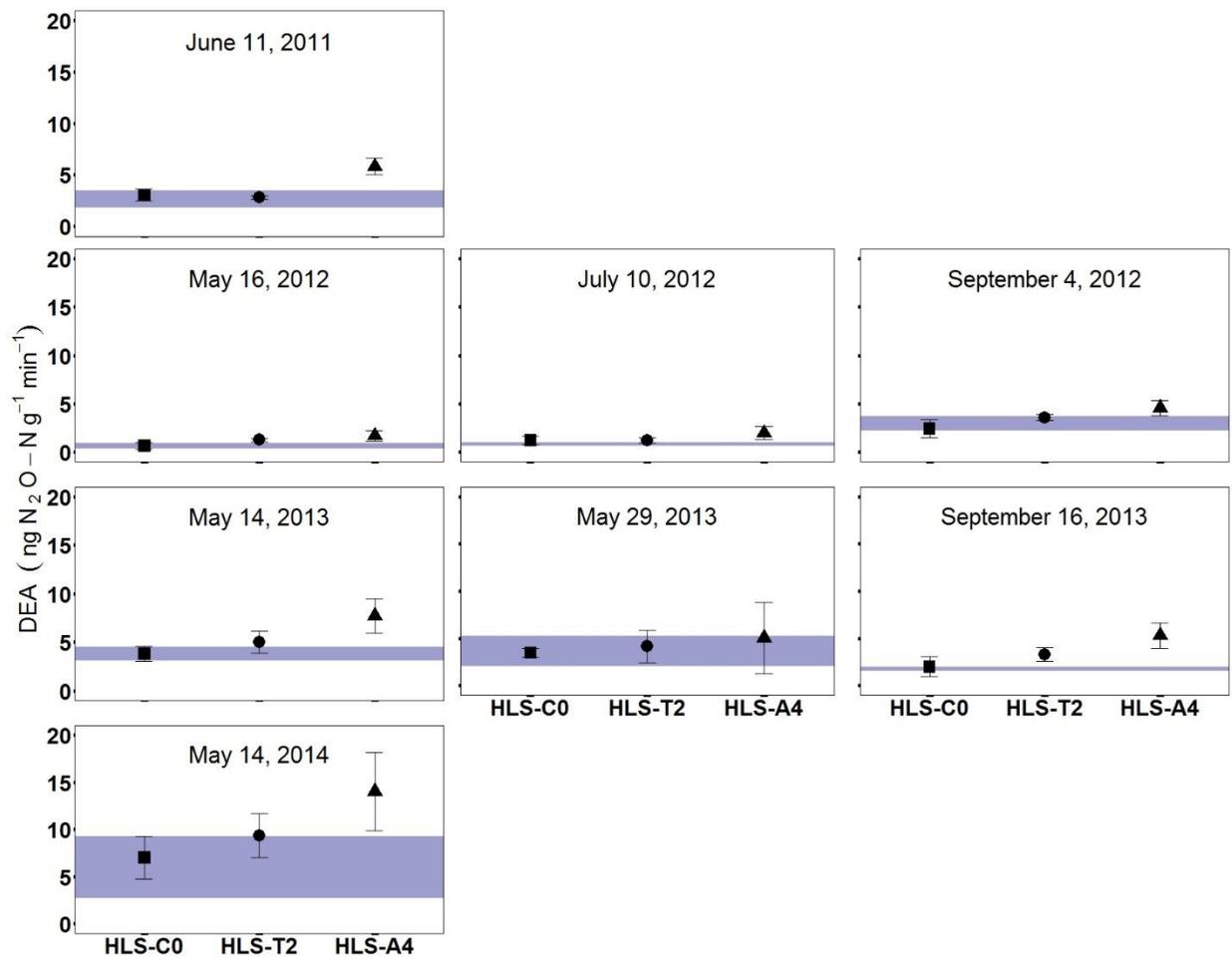
**Table 3.4 Mean DEA activities for the historical liquid swine manure (top table) and solid cattle manure (bottom table) N-fertilizer treatments for each year of analysis. The *p* values are probabilities from One-Way ANOVA performed for each year and trial separately.**

Treatment ID	n	DEA (ng N <sub>2</sub> O-N g <sup>-1</sup> min <sup>-1</sup> )			
		2011 (1)	2012 (3) <sup>‡</sup>	2013 (3) <sup>‡</sup>	2014 (1)
<b>Liquid Swine Manure Trial</b>					
HLS-C0	4	2.99 b	1.18 b	2.85 c	6.78 bc
HUF-A1	4	2.53 b	1.12 b	2.76 c	5.44 c
HLS-T2	4	2.76 b	1.77 a	3.86 b	9.14 ab
HLS-A4	4	5.76 a	2.39 a	5.31 a	13.53 a
<i>p value</i>		<b>0.008</b>	<b>0.000</b>	<b>0.000</b>	<b>0.018</b>
<b>Solid Cattle Manure Trial</b>					
HLS-C0	4	2.99	1.18 b	2.85 b	6.78 b
HUF-A1	4	2.53	1.12 b	2.76 b	5.44 b
HSC-T2	3	2.44	1.71 a	4.21 a	8.42 ab
HSC-A4	4	3.41	2.20 a	4.93 a	10.72 a
<i>p value</i>		<b>0.378</b>	<b>0.001</b>	<b>0.000</b>	<b>0.084</b>

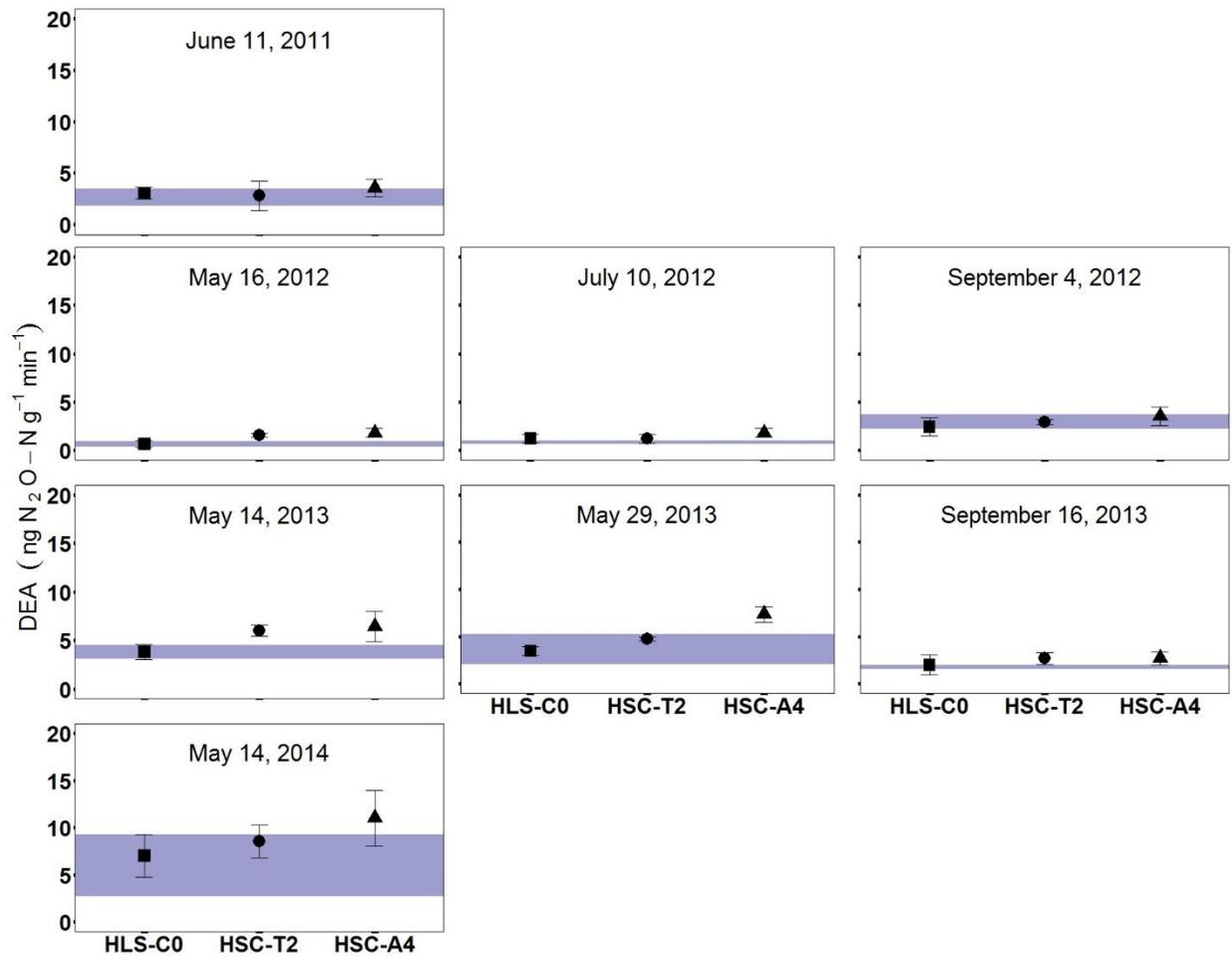
† Within columns and within a trial, means followed by the same letter are not significantly ( $p \leq 0.10$ ) different according to the least significant difference (LSD) test.

‡ Values in parentheses are the number of sampling events in a given year.

In general, DEAs associated with the HLS-A4 and HSC-A4 treatments were greater than those associated with the other treatments and, despite some seasonal variability, this trend remained relatively consistent throughout the 3-yr monitoring period (Fig. 3.5 and Fig. 3.6). As well, significant differences between the fertilizer (HUF-A1) and manure BMPs (HLS-T2 and HSC-T2) were observed on several occasions, with the historical manure treatments yielding higher DEAs than the urea treatment. Differences between the HUF-A1 and historical control treatment (HLS-C0), on the other hand, were not significantly different at any time during the monitoring period.



**Fig. 3.5. Denitrification enzyme activity (DEA) measured in the plots with a history of LSM applications.** Note: the plots were sampled once in 2011; three times in 2012 and 2013 (i.e., before fertilization, after fertilization, and harvest); and once in 2014 (before fertilization). The shaded region is the mean  $\pm$  standard deviation for the HUF-A1 (urea) soil amendment, the squares are HLS-C0 [■], circles are HLS-T2 [●], triangles are HLS-A4 [▲]. The error bars represent one standard deviation from the mean.



**Fig. 3.6. Denitrification enzyme activity (DEA) measured in the plots with a history of SCM applications. Note: the plots were sampled once in 2011; three times in 2012 and 2013 (i.e., before fertilization, after fertilization, and harvest); and once in 2014 (before fertilization). The shaded region is the mean  $\pm$  standard deviation for the HUF-A1 (urea) soil amendment, the squares are HLS-C0 [■], circles are HLS-T2 [●], triangles are HLS-A4 [▲]. The error bars represent one standard deviation from the mean.**

Regardless of amendment history, the highest overall DEA activity occurred during the spring 2014 sampling event. Conversely, DEA activities were generally lowest during the 2012 sampling events. At the same time, however, DEAs at the 2012 harvest sampling event were significantly ( $p < 0.05$ ) greater than those measured earlier in the year (Fig. 3.5 and Fig. 3.6). The opposite was true in 2013 — with DEAs being significantly ( $p < 0.05$ ) greater in the early spring (before fertilizer/seeding operations) than after harvest in the fall. It is also important to note that in spring 2014 — four years after termination of the manure applications — the potential denitrification activities associated with the “worst case” scenarios (i.e., the HLS-A4 and HSC-A4 treatments) remained significantly higher than those associated with the BMPs (Table 3.4).

### **3.5.3 Daily N<sub>2</sub>O emission**

Regardless of amendment history, daily N<sub>2</sub>O emissions at the Dixon long-term manure research site followed typical background/event-based pattern (Yates et al., 2006a, 2006b) during peak sampling season (i.e., from snowmelt to snowfall). This consisted of two large event-based peaks (at snowmelt and fertilization), followed by small precipitation-driven spikes during early growing season (before the crop had depleted the available N supply), and then by “background” emissions during the latter part of monitoring period (Fig. 3.7). Whereas the seasonal patterns reflected local weather and current fertilizer management practices, the magnitude of the daily N<sub>2</sub>O emissions was clearly impacted by amendment history.

As indicated above, the magnitude of the daily N<sub>2</sub>O flux was impacted by the historical N-treatment — which included both the rate at which the manure/urea was applied and the frequency of application (See Appendix B: Figs. B1-B4). Treatment effects were observed both within and between years (Fig. 3.7) and there was a high degree of within treatment (i.e., between reps) variability in the magnitude of emissions. This was most apparent in 2013 — the only year in which spring melt emissions were greater than those induced by the fertilizer application — particularly in HSC-A4 and HLS-A4 plots.

Several distinct trends were observed for the plots with a history of LSM application. For example, the higher the application rate, the larger the magnitude of the peak fluxes and the greater the variability between plots with the same history (i.e., rate and frequency of application). Regardless of the historical N-treatment, the largest daily fluxes observed during

the study occurred during the spring melt, and following the application of urea fertilizer in 2013 (Fig. 3.7). This was also true for the plots with a history of no manure/fertilizer additions (i.e., the non-fertilized controls), though there appeared to be less between-rep variability among the historical controls — and the historical urea treatments — than the LSM treatments (See Appendix B). Likewise, daily N<sub>2</sub>O emissions from the plots with a history of SCM applications were greater in 2013 and the spring of 2014 than in either 2011 or 2012. Unlike N<sub>2</sub>O emissions from the plots with a history of LSM and urea applications, emissions from the SCM amended plots were much more variable across the sampling season.

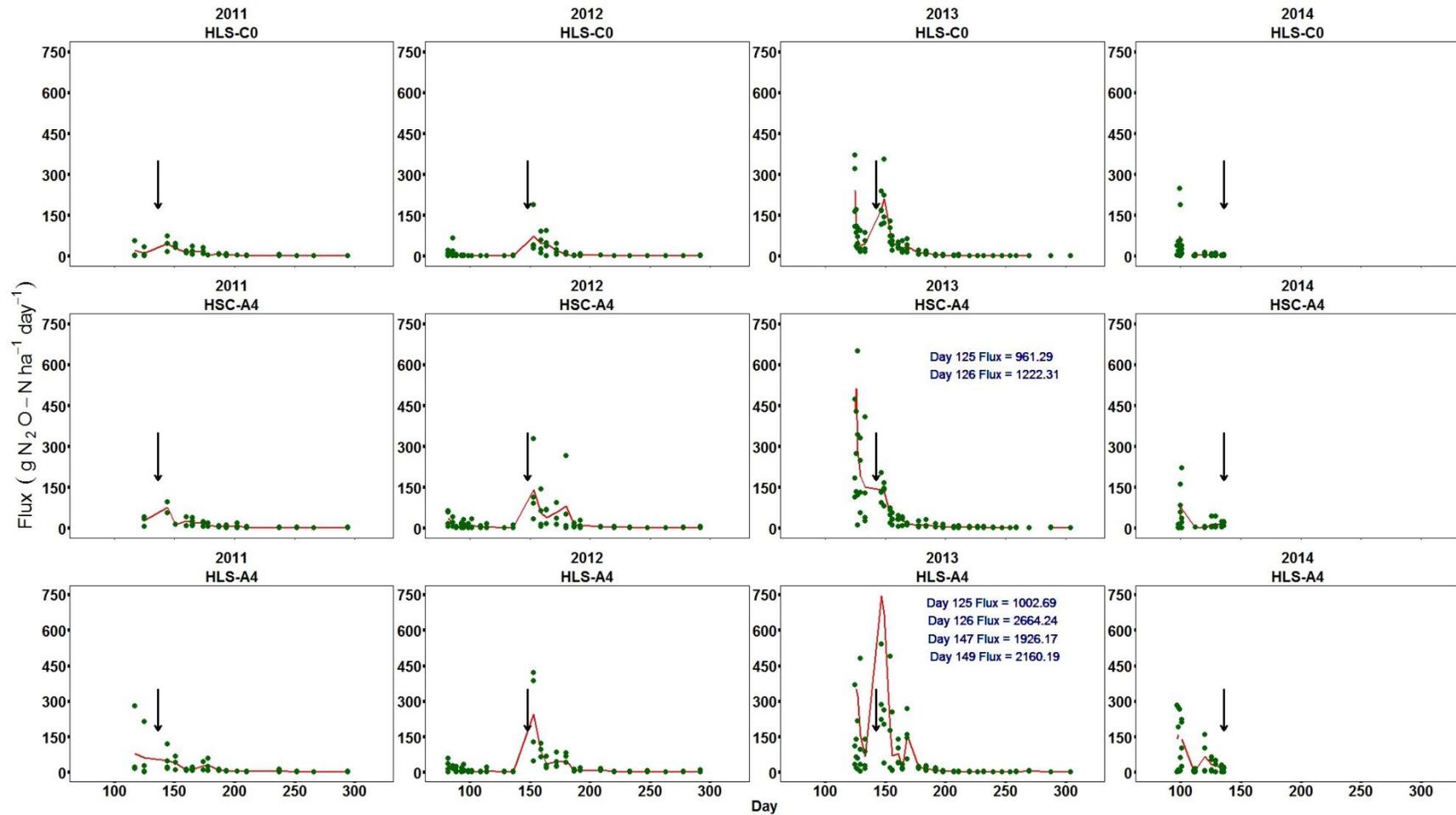


Fig. 3.7. Daily  $N_2O$  emissions from the historical control (HLS-C0; top row) plots and plots that had received annual applications of manure at the 4 $\times$  rate (HSC-A4 and HLS-A4; middle and bottom rows, respectively). The daily flux from each plot (rep) is shown as a green circle ( $\bullet$ ); the mean flux is shown as a red line ( $-$ ). The x-axis is the day of the year. In cases where one or more fluxes exceeded the y-axis range, these values are listed on the graph. The black arrow represents the seeding/fertilization date (Note: in 2014 sampling ended prior to seeding).

Whereas the historical manure treatments affected the peak emissions associated with the events triggered by snow-melt and the spring fertilizer application, no such effect was observed in the “background” emissions (Fig. 3.7). Moreover, whereas the magnitude of the N<sub>2</sub>O emissions was clearly impacted by the rate and frequency of the historical LSM and urea applications, there appeared to be little if any impact of the historical SCM treatments on emissions after the treatments were terminated (see Appendix B).

#### **3.5.4 Cumulative nitrous oxide loss and historical fertilizer amendments**

Cumulative N losses were calculated for an “N budget year”; i.e., from fertilizer application in YEAR 1, through the growing season and spring thaw period in YEAR 2. For example, the cumulative N loss for the 2011/12 N-budget year includes the period from the spring fertilizer application on May 27, 2011 through to the subsequent spring thaw period ending on March 21, 2012. As a result, the spring melt-fluxes were included in the previous year’s N<sub>2</sub>O emission budget. In addition, total cumulative losses were calculated by summing the annual losses over the course of the 3-yr monitoring period. These cumulative N<sub>2</sub>O-N losses were then used to assess the potential of the long-term manure treatments to create conditions conducive for greater N<sub>2</sub>O losses after the treatments were terminated (Table 3.5 and Table 3.6).

In general, cumulative N<sub>2</sub>O-N losses associated with historical treatments involving annual applications of LSM were significantly ( $p < 0.10$ ) greater than those associated with the equivalent SCM treatments, yet were similar to the equivalent urea treatments (Table 3.5). Furthermore, cumulative N<sub>2</sub>O-N losses (averaged across N rate) increased in order: non-fertilized control  $\approx$  SCM < LSM. Not surprisingly, cumulative N<sub>2</sub>O-N losses also increased with increasing N rate, though this effect was only significant at the 4 $\times$  rate.

Conversely, cumulative N<sub>2</sub>O-N losses associated with historical treatments involving triennial applications of LSM were not significantly greater than those associated with the equivalent SCM treatments (Table 3.6). Likewise, N rate had no significant effect on cumulative N<sub>2</sub>O-N emissions when the manures were added every third year as opposed to on an annual basis (Table 3.6).

**Table 3.5. Annual and total cumulative N<sub>2</sub>O-N loss measured between the spring fertilizer application in 2011 (May 27<sup>th</sup>) and the end of snowmelt period in 2014 (May 16<sup>th</sup>). Only data from the plots with a history of annual manure/urea applications were considered; data were analysed using a 2-way ANOVA with N-source and N rate as the main effects.**

N-source	Cumulative N <sub>2</sub> O loss (kg N <sub>2</sub> O-N ha <sup>-1</sup> ) <sup>‡</sup>			
	2011/12	2012/13	2013/14	3-yr Total
Control	2.609	2.371 b	2.649 c	7.629 b
Urea	1.149	4.151 ab	5.986 ab	11.286 ab
SCM	1.824	3.944 ab	3.026 bc	8.794 b
LSM	1.522	5.405 a	7.745 a	14.672 a
<i>p value</i>	<b>0.407</b>	<b>0.094</b>	<b>0.014</b>	<b>0.073</b>

N Rate <sup>†</sup>	2011/12	2012/13	2013/14	3-yr Total
0×	2.609	2.371	2.649 bc	7.629 c
1×	1.595	4.031	2.619 c	8.245 bc
2×	1.618	3.976	4.759 ab	10.353 ab
4×	1.289	5.378	8.951 a	15.618 a
<i>p value</i>	<b>0.337</b>	<b>0.116</b>	<b>0.001</b>	<b>0.025</b>

<sup>†</sup>Plant available N; 0×, 1×, 2×, 4× rates = 0, 50, 100 & 200 kg N year<sup>-1</sup> respectively.

<sup>‡</sup>Within columns and N-fertilizer or N-rate, means followed by the same letter are not significantly ( $p \leq 0.10$ ) different according to the least significant different (LSD) test.

**Table 3.6. Annual and total cumulative N<sub>2</sub>O-N loss measured between the spring fertilizer application in 2011 (May 27<sup>th</sup>) and the end of snowmelt period in 2014 (May 16<sup>th</sup>). Only data from the plots with a history of triennial manure/urea applications were considered; data were analysed using a 2-way ANOVA with N-source and N rate as the main effects.**

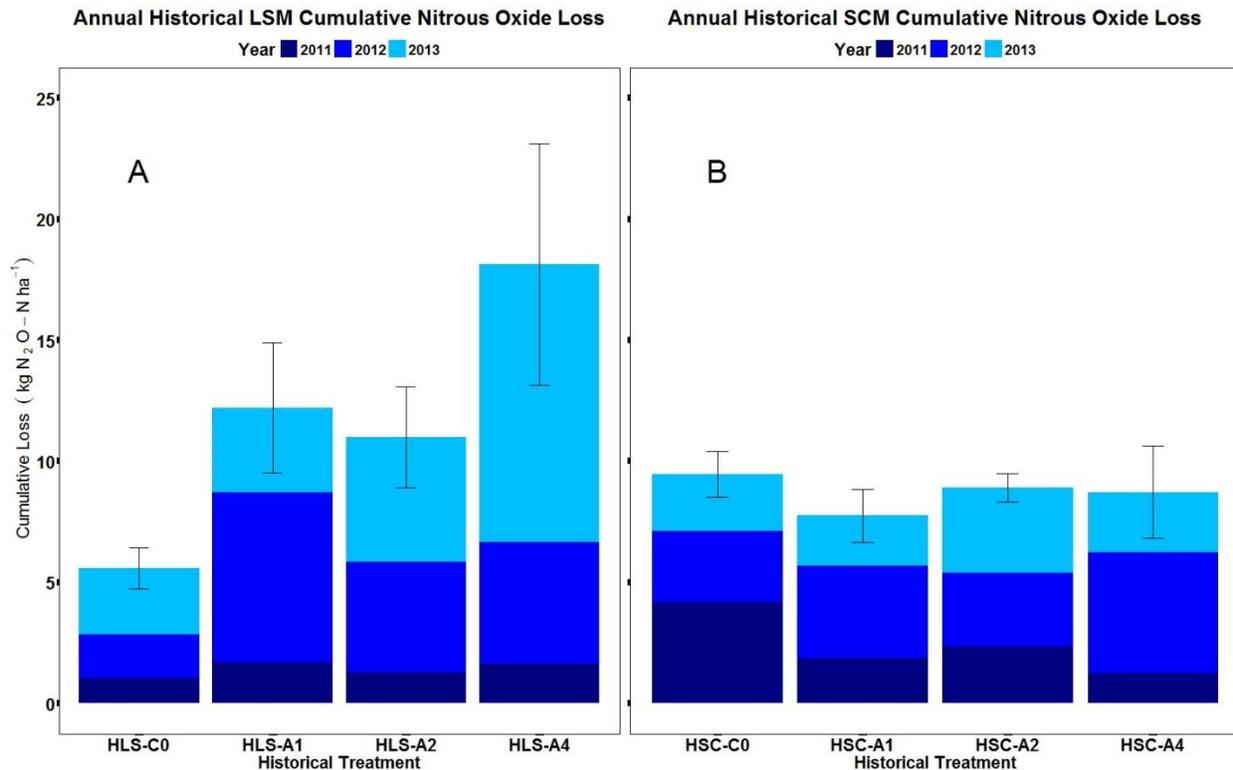
N-source	Cumulative N <sub>2</sub> O loss (kg N <sub>2</sub> O-N ha <sup>-1</sup> )			
	2011/12	2012/13	2013/14	3-yr Total
Control	2.609	2.371	2.649	7.629
SCM	1.783	3.062	3.250	8.096
LSM	1.525	4.283	3.416	9.224
<i>p value</i>	<b>0.876</b>	<b>0.475</b>	<b>0.772</b>	<b>0.702</b>

N-rate <sup>†</sup>	2011/12	2012/13	2013/14	3-yr Total
0×	2.609	2.371	2.649	7.629
1×	1.552	3.448	3.483	8.483
2×	1.865	2.734	2.765	7.364
4×	1.524	5.042	3.754	10.320
<i>p value</i>	<b>0.952</b>	<b>0.849</b>	<b>0.604</b>	<b>0.744</b>

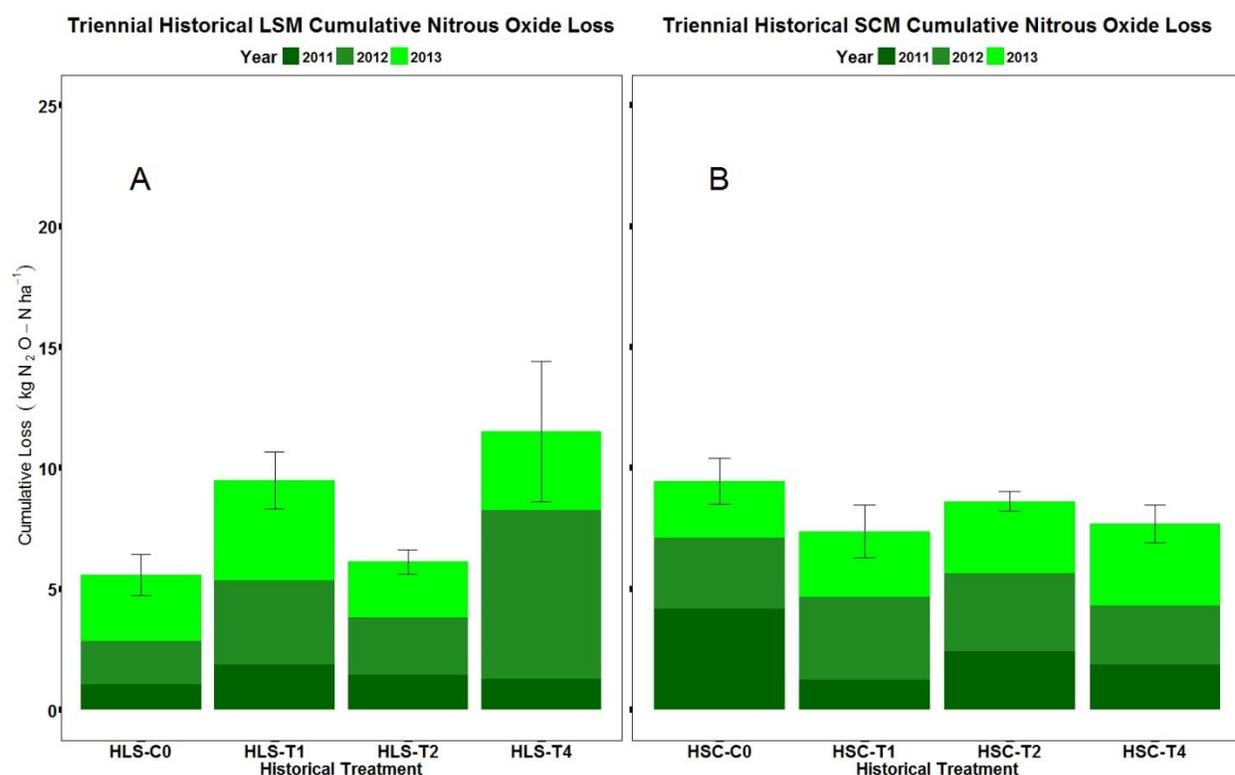
<sup>†</sup>Plant available N; 0×, 1×, 2×, 4× rates = 0, 50, 100 & 200 kg N year<sup>-1</sup> respectively.

Cumulative annual (and total) N<sub>2</sub>O emissions associated with the individual historical treatments are shown in Figures 3.8-3.10. For the sum of all three-years, the plots receiving annual applications of LSM exhibited significant ( $p = 0.017$ ) treatment effects (Fig. 3.8A). Cumulative N<sub>2</sub>O losses from the plots that had received long-term applications of the LSM (HLS-A1, HLS-A2, and HLS-A4) were greater than those from the historical control (HLS-C0) plots. Moreover, plots that had received the highest (4×) rate of LSM (HLS-A4) produced greater cumulative emissions than those receiving the lower (1× and 2×) rates (Fig. 3.8A). Conversely, application rate had no significant ( $p = 0.345$ ) effect on cumulative N<sub>2</sub>O emissions from plots that had received triennial applications of the LSM (Fig. 3.9A).



**Fig. 3.8. Cumulative N<sub>2</sub>O emissions during the three years following termination of the historical LSM applications. Manure applications were made either (A) for the historical annual LSM and (B) for the historical annual SCM applications. Error bars represent one standard deviation from the mean total cumulative emissions.**

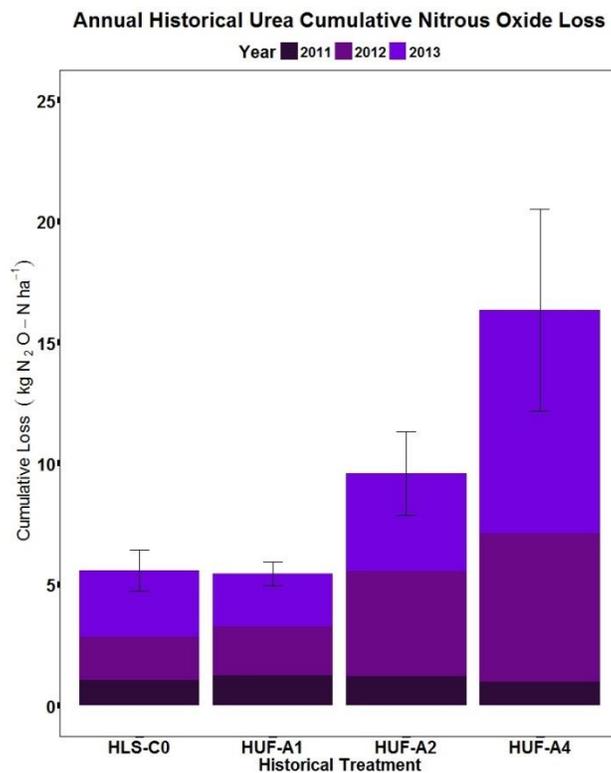
Unlike the LSM, for the sum of all three years long-term annual applications of SCM had no significant ( $p = 0.751$ ) effect on cumulative  $N_2O$  losses (Fig. 3.8B). This was true regardless of application frequency (i.e., annual or triennial). Whereas plots with history of annual applications of SCM yielded cumulative  $N_2O$  emissions that were significantly ( $p = 0.073$ ; Table 3.5) lower than those from equivalent LSM plots. However, there was no significant ( $p = 0.702$ ; Table 3.6) difference in cumulative emissions from plots with a history of triennial applications of SCM compared to LSM.



**Fig. 3.9. Cumulative  $N_2O$  emissions during the three years following termination of the historical LSM applications. Manure applications were made either (A) for the historical triennial LSM and (B) for the historical triennial SCM applications. Error bars represent one standard deviation from the mean total cumulative emissions.**

Much like the historical LSM treatments, the sum of the three-year cumulative loss from plots receiving annual applications of urea fertilizer exhibited a significant treatment (N rate) effect ( $p = 0.004$ ). When looked at annually this treatment effect was significant in two of the three years of the study (Fig. 3.10). The lone exception occurred in 2011/12 — a year

characterized by relatively cool, dry conditions that resulted in the lowest cumulative emissions measured during the four years of the study (i.e., 0.98-1.25 kg N ha<sup>-1</sup> yr<sup>-1</sup>). Moreover, cumulative N<sub>2</sub>O emissions summed over the three-year study period increased in order: HLS-C0 ≈ HUF-A1 < HUF-A2 < HUF-A4. Total cumulative N<sub>2</sub>O emissions from the HUF-A4 plots were *ca.* 3-times greater than those from HLS-C0 and HUF-A1 plots, and almost double those from the HUF-A2 plots. It is also noteworthy that cumulative annual emissions associated with the urea best management practice (i.e., the HUF-A1 treatment) were essentially the same as those associated with the previous control (non-fertilized; HLS-C0) plots.



**Fig. 3.10. Cumulative N<sub>2</sub>O emissions during the three years following termination of the long-term urea applications. Urea applications were made on an annual basis. Error bars represent one standard deviation from the mean total cumulative emissions.**

Annual cumulative N<sub>2</sub>O-N losses associated with the different historical treatments varied depending on crop management and weather — though the trends described in the previous section remained relatively constant across time. In general, cumulative emissions were lowest during the 2011/12 N-budget year and greatest during the 2013/14 N-budget year (Table

3.7). The same trend was observed for the historical urea N-treatments; i.e., cumulative annual emissions increased in the order 2011/12 < 2012/13  $\approx$  2013/14. Regardless of the historical manure/urea treatment, cumulative annual emissions in any N-budget year were driven primarily by two large emission events associated with (i) the fertilizer N application in the spring of that year and (ii) the snowmelt period in the subsequent spring (i.e., preceding the following year's N-fertilizer application) (Table 3.7). Indeed, emission occurring during these two events accounted for 47, 77 and 77% of the cumulative annual N<sub>2</sub>O-N losses (averaged across all treatments) in the 2011/12, 2012/13, and 2013/14 N-budget years, respectively. Cumulative emissions during the snowmelt period associated with the 2011/12 N-budget year were 2- to 3-times lower (averaging about 16%) than those in the 2012/13 and 2013/14 N-budget years (which averaged *ca.* 43 and 31%, respectively).

**Table 3.7. Cumulative annual N<sub>2</sub>O-N loss and the proportion of the cumulative annual N<sub>2</sub>O loss associated with the spring application of N fertilizer (N<sub>f</sub>) and the snowmelt (SM) period preceding the next year's spring fertilizer application. Emissions associated with the spring application of N fertilizer were calculated based on a 20-day period immediately following snowmelt or fertilizer application.**

N source	N rate <sup>†</sup>	F <sup>‡</sup>	Cumulative annual N <sub>2</sub> O-N loss								
			2011/12			2012/13			2013/14		
			CL <sup>§</sup> (kg ha <sup>-1</sup> )	N <sub>f</sub> (%)	SM (%)	CL <sup>§</sup> (kg ha <sup>-1</sup> )	N <sub>f</sub> (%)	SM (%)	CL <sup>§</sup> (kg ha <sup>-1</sup> )	N <sub>f</sub> (%)	SM (%)
—	0×	A	1.0	41	12	1.8	36	46	2.9	63	18
LSM	1×	A	1.7	39	8	7.0	40	39	3.6	47	15
LSM	2×	A	1.3	28	17	4.6	30	40	5.7	40	34
LSM	4×	A	1.6	28	15	5.0	33	38	12.9	34	48
LSM	1×	T	1.9	37	4	3.5	21	62	4.3	52	23
LSM	2×	T	1.5	17	16	2.4	30	38	2.6	36	40
LSM	4×	T	1.3	17	15	7.0	26	55	3.4	45	23
—	0×	A	4.2	50	15	2.9	49	27	2.4	52	27
SCM	1×	A	1.9	32	25	3.8	49	37	2.2	52	23
SCM	2×	A	2.4	29	31	3.0	50	21	4.1	51	32
SCM	4×	A	1.3	29	19	5.0	19	54	2.8	48	30
SCM	1×	T	1.3	42	9	3.4	23	57	2.7	55	12
SCM	2×	T	2.4	28	30	3.2	56	23	3.0	69	14
SCM	4×	T	1.9	29	16	2.5	42	10	4.2	29	53
Urea	1×	A	1.3	25	14	2.0	36	41	2.3	38	38
Urea	2×	A	1.2	27	10	4.3	20	70	4.4	40	42
Urea	4×	A	1.0	33	13	6.1	19	67	11.2	26	61
<b>Overall mean</b>			<b>1.7</b>	<b>31</b>	<b>16</b>	<b>4.0</b>	<b>34</b>	<b>43</b>	<b>4.4</b>	<b>46</b>	<b>31</b>

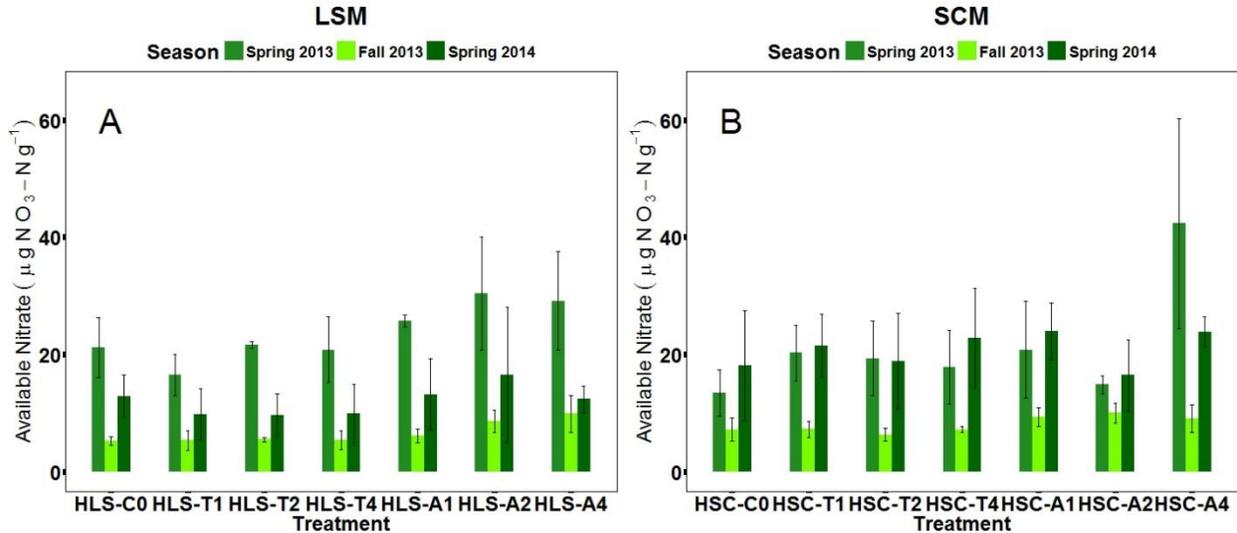
<sup>†</sup>Plant available N; 0×, 1×, 2×, 4× rates = 0, 50, 100 & 200 kg N year<sup>-1</sup> respectively.

<sup>‡</sup>F = Application frequency of the historical manure/urea treatment; A = annual application; T = triennial application

<sup>§</sup>CL = Cumulative N<sub>2</sub>O-N loss (kg N<sub>2</sub>O-N ha<sup>-1</sup>)

The available soil N pool (i.e., NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup>) was measured during 2013 and 2014 — with samples collected prior to fertilizer/seeding operations in 2013, and after fall 2013 harvest, and prior to fertilizer/seeding operations in 2014. Results of these analyses are summarized in Figs. 3.11-3.13. Soil NO<sub>3</sub><sup>-</sup> concentrations were greatest in the spring of 2013 and lowest in the fall of 2013 — though the concentrations then rebounded over winter and were again relatively high in spring 2014. However, there appeared to be less recovery in those plots with a history of

LSM applications (Figure 3.11A) than in those with a history of SCM applications (Figure 3.11B).



**Figure 3.11.** Concentrations of soil nitrate ( $\text{NO}_3^-$ ) in plots with a history of (A) LSM and (B) SCM applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (green), after harvest in fall 2013 (light green), and at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark green).

Concentrations of soil  $\text{NH}_4^+$ , on the other hand, showed no such trend and were greater in both spring and fall 2013 than in spring 2014 (Figure 3.12). As with soil  $\text{NO}_3^-$ , however, soil  $\text{NH}_4^+$  concentrations measured in spring 2014 tended to be greater in the plots with a history of SCM applications (Figure 3.12B) than those with a history of LSM applications (Figure 3.12A). Soil  $\text{NO}_3^-$  and  $\text{NH}_4^+$  concentrations in plots with a history of variable urea applications (Figure 3.13) followed patterns that were similar to those observed for the LSM plots.

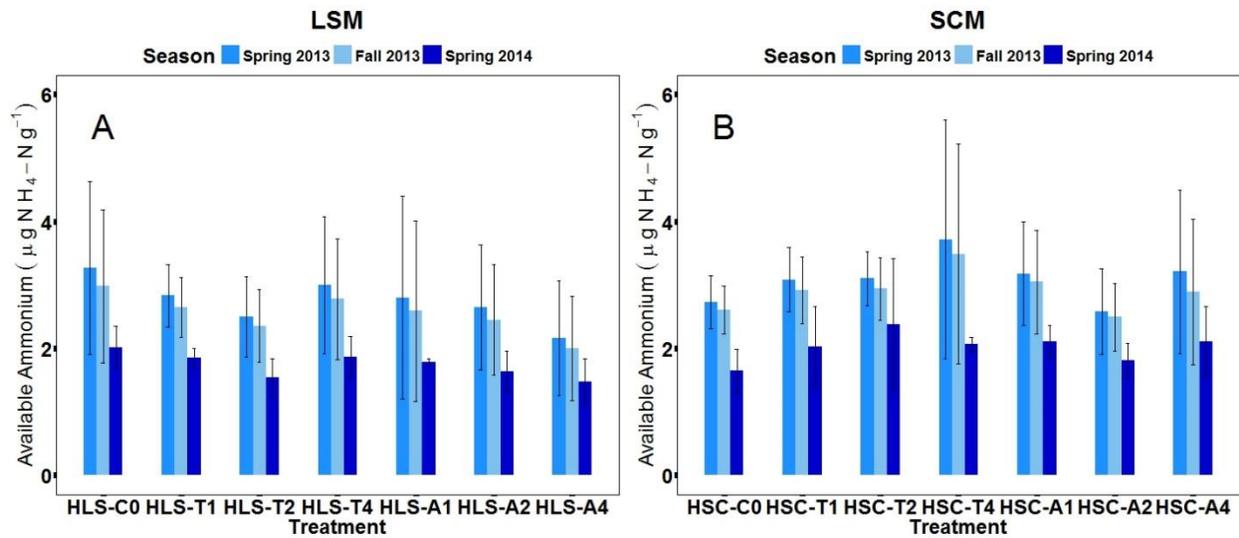


Figure 3.12. Concentrations of soil ammonium ( $\text{NH}_4^+$ ) in plots with a history of (A) LSM and (B) SCM applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (blue), after harvest in fall 2013 (light blue), and at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark blue).

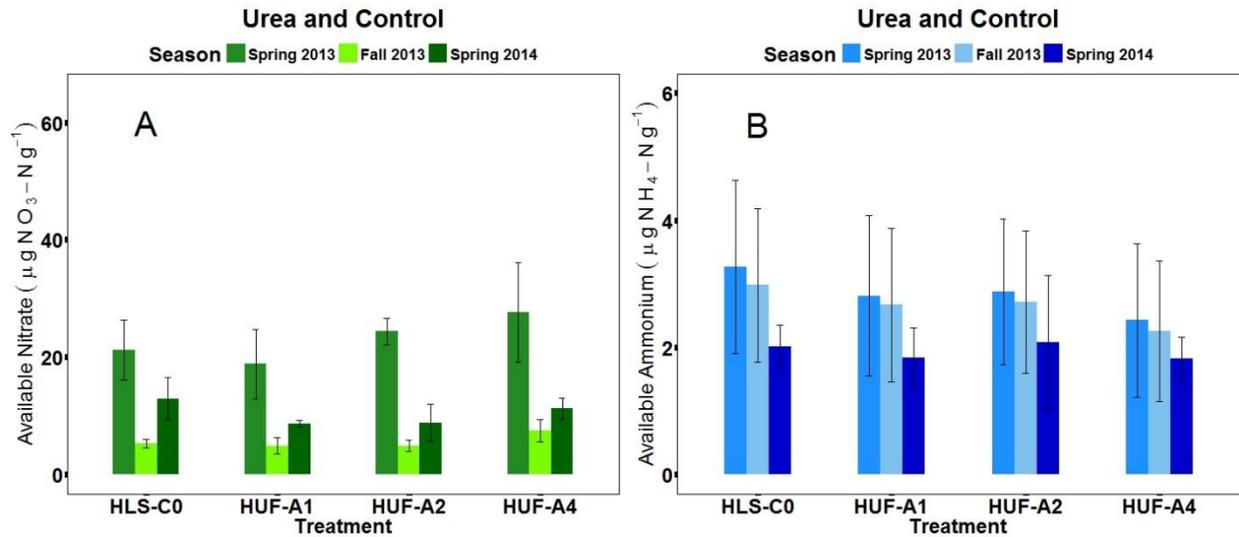


Figure 3.13. Concentrations of soil nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) in plots with a history of variable urea applications. Available N was determined at the end of the snowmelt period (prior to seeding/fertilizer operations) in spring 2013 (green), after harvest in fall 2013 (light green), and at the end of snowmelt period (prior to seeding/fertilizer operations) in spring 2014 (dark green). (A)  $\text{NO}_3^-$ -N: spring 2013 (green), fall 2013 (light green), and spring 2014 (dark green). (B)  $\text{NH}_4^-$ -N: spring 2013 (blue), fall 2013 (light blue), and spring 2014 (dark blue).

### 3.6 Discussion

The Dixon long-term manure research site provided a unique opportunity to examine how N-management history affected N<sub>2</sub>O emissions after manure applications had ceased; i.e., to determine whether there was a “legacy” effect associated with the long-term manure applications. And, if so, to determine whether this could be attributed to a “priming” effect of the manures. The 2010 cropping season was the last in which crop N demands were supplied by the application of manure (either LSM or SCM), and starting in 2011 N was supplied to all the research plots at a single rate (based on a field average soil test recommendations) using granular urea as the N-source. Nitrous oxide emissions from each plot were then measured from spring 2011 through spring 2014 and cumulative annual N<sub>2</sub>O-N losses calculated from each of the historical manure/fertilizer treatments.

Emission patterns for the daily N<sub>2</sub>O fluxes observed at the Dixon site were generally dominated by two large emission events: the first occurring in early spring — with snowmelt, and the second associated with the first precipitation event following the spring fertilizer application. Outside of these events (and a few other smaller emission events associated with early season precipitation), the bulk of the daily N<sub>2</sub>O fluxes were low-level background emissions ( $123 \pm 165$  ng N<sub>2</sub>O-N m<sup>-2</sup> min<sup>-1</sup>). This pattern is common to the semi-arid prairies, including Saskatchewan and the Prairie Pothole Region in general (Yates et al., 2006a; Lemke et al., 2010; Dunmola et al., 2010). Dixon is located in the Black Soil Zone, which is more sub-humid than other areas of the Prairie Pothole Region, and events that increase the soil moisture content (e.g., snowmelt and precipitation) and trigger denitrification are more common in this area. Daily losses of N<sub>2</sub>O at the Dixon site illustrate the importance of N application and environment conditions in driving N<sub>2</sub>O losses. As well showing that N applied in excess above best management recommendations can lead to N use inefficiencies. These trends and patterns in daily emissions can also relate on a broader scale to the rest of the province of Saskatchewan. At Dixon, approximately half to three-quarters of the cumulative annual emissions measured during the 2011/12, 2012/13, and 2013/14 N-budget years were observed to occur during a 20-day period following snowmelt and fertilizer application (see Table 3.7). The largest of these spring flux events occurred in 2013, when the soils were quite wet (volumetric water content = 0.311-0.420). Likewise, DEAs measured during this period were generally greater than those measured

later in the season or previous springs (see Fig. 3.5 and Fig. 3.6). Large N<sub>2</sub>O emissions have been shown to accompany high DEAs when field conditions include high levels of NO<sub>3</sub>-N and WFPS (Dandie et al., 2008).

Following termination of the manure applications (i.e., starting with the 2011 cropping season, including both the 2011/12 and the 2013/14 N-budget year), the spring urea application appeared to be the event driving the majority of the N<sub>2</sub>O-N losses — with over 31% of cumulative N<sub>2</sub>O-N losses occurring after this event. This implies that the microbial communities may be accustomed to higher N levels from long-term fertilizer application and transform it quite quickly. Nonetheless, in the 2012/13 N budget year snowmelt generated N<sub>2</sub>O emissions greater than those associated with fertilization N losses — with 43% of cumulative N<sub>2</sub>O-N occurring during the snowmelt period, and 34% occurring during the 20-day period following the urea-N application. This may imply a change in microbial utilization of N whereby the residual N in the soil was used to a greater extent than that of the added urea-N, resulting in increased N<sub>2</sub>O loss. The high spring-melt losses also illustrated how soil moisture increases can lead to significant N<sub>2</sub>O emissions in the Black soil zone where the build-up of OM (Pennock et al., 2011) likely provides a steady supply of available C (and other nutrients), suggesting minimal nutrient limitations for microbial activity. That the majority of N<sub>2</sub>O emissions occurred in conjunction with spring snowmelt and during the period immediately following N-fertilizer application is a common feature of agricultural ecosystems (Corre et al., 1996; Dobbie et al., 1999, Yates et al., 2006b). Indeed, Dusenbury et al. (2008) observed that snowmelt-based emissions in Montana comprised up to 84% of cumulative annual losses. High emissions during spring thaw also have been reported on agricultural land in the Prairie Pothole region of Manitoba, Canada (Dunmola et al., 2010), and the Prairie Parkland region in Alberta (Lemke et al., 1998). These data indicate that N use inefficiencies associated with the application of N-fertilizers, in combination with precipitation and snowmelt, are likely to lead to higher N losses and an increase in GHG emissions. Rochette et al. (2008a) pointed out that mineral N is generally not limiting in the semi-arid prairies, rather it is a lack of the precipitation needed to drive denitrification that is responsible for the lower cumulative N<sub>2</sub>O production (on a per hectare basis) observed in prairie regions. Therefore, it is the enhanced denitrification resulting from increased soil moisture during snowmelt and following early season rainfall events (when N is not limiting) that is the most important driver of N<sub>2</sub>O production/emission in the semi-arid prairies.

Severe weather occurred throughout central Saskatchewan in 2010, with nearly twice as much rainfall as usual (see Table 3.3). Consequently, the Dixon site in 2010 saw extremely wet conditions that carried over to spring 2011 and left much of the research site inaccessible during the early spring. Moreover, the extremely wet conditions likely restricted the oxygen supply in the soil which may have led to more anaerobic conditions and, in turn, may have caused denitrification to proceed to completion (i.e., promoted the reduction of  $\text{N}_2\text{O}$  to  $\text{N}_2$ ). Additionally, these conditions likely contributed to relatively low cumulative annual  $\text{N}_2\text{O}$ -N emissions measured in 2011.

In general, cumulative annual  $\text{N}_2\text{O}$ -N losses during the 2011/12 N-budget year were relatively low (ranging from 0.98 to 4.18 kg  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$ ), reflecting both the very wet conditions in early spring and the relatively dry conditions that occurred throughout most of the subsequent growing season (see Fig. 3.4). Conversely, cumulative annual  $\text{N}_2\text{O}$ -N losses were generally greatest in 2013/14, especially in the plots with a history of LSM or urea fertilizer application at the highest (4 $\times$ ) N rate (12.89 and 11.18 kg  $\text{N}_2\text{O}$ -N  $\text{ha}^{-1}$  respectively). In part, this most likely reflects the relatively high fertilizer-N input (120 lbs N  $\text{ac}^{-1}$ ) needed to meet the demands of the canola crop planted in 2012, the canola residue left on site following harvest, and the 85 lbs N  $\text{ac}^{-1}$  applied to meet the demands of the wheat seeded in the spring of 2013. That is, precipitation events early in the growing season — before there was a significant crop demand for N — triggered large  $\text{N}_2\text{O}$  emissions that, on average, contributed about 43% of the total annual  $\text{N}_2\text{O}$ -N loss. In general, there is a strong relationship between early-season concentrations of  $\text{NO}_3^-$  and early-season peak  $\text{N}_2\text{O}$  emission in prairie agroecosystems, and there is a very strong positive correlation between  $\text{NO}_3^-$  intensity [i.e., an integrated measure of the accumulated  $\text{NO}_3^-$  in the soil over time ( $\text{mg NO}_3^- \text{ kg}^{-1}$ ); calculated using an AUC analysis of the  $\text{NO}_3^-$  concentration vs. time curve] and cumulative  $\text{N}_2\text{O}$  losses (Burton et al., 2008; Asgedom et al., 2014). Likewise, at the Dixon site, the high spring emissions in 2013 reflected the high spring nitrate ( $\text{NO}_3^-$ ) levels in the soils (Figure 3.11). Although much of the available N was depleted during the growing season, there was a general increase in available N between harvest and the subsequent (2014) spring — which together with the warm, wet conditions that occurred during the 2014 spring thaw likely contributed to the high emissions measured during this period. In fact, there was a significant correlation ( $r = 0.60$ ,  $p = 0.01$ ) between cumulative  $\text{N}_2\text{O}$ -N loss and spring 2014  $\text{NO}_3^-$  concentrations.

Whereas, strong correlations have been made between N<sub>2</sub>O emissions and soil respiration — suggesting a relationship between emissions and microbial activity — (Dandie et al., 2008; Miller et al., 2008; Barrett et al., 2015), links between denitrifying communities and gene expression have been more difficult to demonstrate (Henderson et al., 2010; Tatti et al., 2012). In the current study I found a strong correlation between cumulative N<sub>2</sub>O-N losses and cumulative soil respiration ( $r = 0.60$ ,  $p = 0.01$ ) at the Dixon site, but was unable to demonstrate any significant correlation between N<sub>2</sub>O-N losses and gene copy number for the *nirS*, *nirK*, and *nosZ* gene involved in denitrification (data not shown). At the same time, cumulative N<sub>2</sub>O emissions tended to increase as a function of DEA and there was a strong correlation between the time-averaged DEA and both the cumulative N<sub>2</sub>O emissions (summed over the three-year post-manure period;  $r_s = 0.943$ ,  $p \leq 0.02$ ) and the time-averaged cumulative emissions ( $r_s = 0.860$ ,  $p \leq 0.05$ ). This effect was generally stronger in the plots with a history of LSM applications, and suggest that the long-term applications of manure-N, especially at high application rates, produced a “priming” effect that under the right environmental and management conditions, exacerbated fertilizer induced N<sub>2</sub>O emissions.

Averaged across application rates and frequencies, cumulative N<sub>2</sub>O-N losses associated with the historical LSM and SCM treatments were not significantly different ( $p = 0.338$ ). In addition, though neither the rate nor the frequency of application had a residual or “legacy” effect on post-termination N<sub>2</sub>O-N losses from the plots with a history of SCM applications, plots with a history of LSM application did exhibit a legacy effect. That is, beginning in the spring of 2011, differences between the historical LSM treatment plots could only be attributed to long-term legacy effects from the manure. In general, N<sub>2</sub>O-N losses from plots that had received an annual application of LSM were greater than those from plots that had received triennial applications. Not surprisingly, the HLS-A4 treatment (i.e., annual applications at the 4× rate) had the greatest impact on N<sub>2</sub>O-N loss. Soils from these plots also had the highest DEAs. This suggests that excessive labile C and N from the LSM application generated a priming (legacy) effect that yielded N<sub>2</sub>O-N losses greater than what would have been expected from the spring application of urea fertilizer.

Whereas Farrell et al. (2011) reported that annual applications of SCM at the highest (4×) rate appeared to ‘prime’ the soils for denitrification — as evidenced by the much greater DEAs associated with this treatment — results from the post-manure period (i.e., the 2011/12-2013/14

N-budget years) suggest that actual N<sub>2</sub>O priming in these soils was minimal. In fact, during the first year following termination of the manure treatments, it was the historical control plots (HSC-C0) that generated the greatest N<sub>2</sub>O-N loss. A closer inspection of the data, however, revealed that these higher N<sub>2</sub>O-N losses from the HSC-C0 plots can be attributed mainly to a single plot. This is likely due to some soil redistribution during the flooding that occurred in 2010, as this plot was situated in a low spot along the southern end (Block 4) of the SCM trial nearest a slough. Nevertheless, the lack of a response to the spring application of urea-N in the post-manure period suggests that the lower N<sub>2</sub>O-N loss in the SCM plots may be a result of increased immobilization of N. For example, long-term residue addition (e.g., organic manures as well as pig slurries) have been shown to increase gross N immobilization in soils (Luxhøi et al., 2007). As well, an increase in OM in the SCM plots could have provided the necessary nutrients (including C), and promoted conditions that allowed denitrification to proceed to completion. In turn, this would have contributed to the observation that the historical SCM treatments had no significant effect on the cumulative N<sub>2</sub>O-N losses after they were terminated.

The historical LSM treatments, on the other hand, did produce a legacy effect — with cumulative N<sub>2</sub>O-N losses in the post-manure period being affected by both the rate and frequency of the LSM applications. In the spring of 2013, available soil N (NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>) concentrations in the plots that had received SCM applications were similar to those that had received LSM applications, yet N<sub>2</sub>O-N emissions were greater from the plots with a history of LSM applications. In the spring of 2014, NO<sub>3</sub><sup>-</sup> concentrations in the SCM-amended plots were similar to those measured in 2013, suggesting that the release of immobilized (organic) N in these soils provided a relatively stable source of NO<sub>3</sub><sup>-</sup>. Conversely, NO<sub>3</sub><sup>-</sup> concentrations in the LSM-amended plots were lower in 2014 than 2013; nevertheless, N<sub>2</sub>O-N losses during the 2014 snowmelt period were greater from the LSM-amended plots than the SCM-amended plots. Denitrification enzyme activities also were greater in the LSM-amended soils than the SCM-amended soils during the snowmelt periods, suggesting that either (i) the denitrifier communities in the LSM-amended soils were larger or more active, or (ii) denitrification was more likely to proceed to completion in the SCM-amended soils. That NO<sub>3</sub><sup>-</sup> levels in the LSM-amended soils did not rebound in the spring of 2014 to the same extent as they did in the SCM-amended soils suggests that there may be less long-term storage of N from the LSM applications, possibly

indicating that there could be a decrease in N<sub>2</sub>O losses from the LSM-amended plots in the future.

As indicated by the DEA results, long-term application of manures (especially at high rates) can stimulate denitrification activities when the management regime is changed to supply a more readily available source of N (in this case, urea fertilizer). This is consistent with other reports that denitrification potential was greater with manure applications — specifically solid cattle (Enwall et al., 2005) and pig manure amendments (Yin et al., 2015). Miller et al. (2008) examined the influence of glucose and crop residue on denitrification, and reported that the type of C available when a readily available N source is present is what differentiates the amount of denitrification losses and community abundance in soils. This implies that differences in the quality of organic C in the LSM and SCM is a major reason for the differences in N<sub>2</sub>O-N losses observed at the Dixon site. The magnitude of the DEAs measured at the Dixon site one to three years after the manure applications had been terminated were much lower than those reported following a recent manure application (Šimek et al., 2014).

While manure-induced treatment differences were not observed on all sampling dates, the DEA analyses that did generate treatment differences that seemed to coincide with periods under drier conditions. Typically, an increase in soil aeration and drier conditions are not as favorable for denitrification. Šimek et al. (2014) have also reported that the DEAs coincided with field N<sub>2</sub>O emissions, and suggested that this may be caused by a decrease in NO<sub>3</sub><sup>-</sup> in microsites. This suggests that there is a masking effect of the long-term manure applications on the growing seasonal decreases in nutrient levels, allowing for a potential of high microbial activity even under less-favorable environmental conditions. Comparing the N<sub>2</sub>O potential losses with the DEA's between manure amendments and the no-fertilizer historical control, the manure amendments show a greater potential for larger losses of N<sub>2</sub>O from the same climatic and management regimes. This supports the hypothesis that long-term (14 yr) manure applications altered the capacity of the soils to efficiently cycle inorganic fertilizer-N under the current management regimes. This was particularly true of the LSM long-term applications which generated larger N<sub>2</sub>O-N losses following application of urea fertilizer. Likewise, plots with a history of urea applications at the highest (4×) rate produced larger N<sub>2</sub>O-N losses than the plots that had received the lower (1× & 2×) application rates.

### 3.7 Conclusion

Nitrous oxide emissions and DEA data from the Dixon long-term manure research site indicate that long-term manure applications (especially when applied at excessive rates) can produce a “legacy” effect — i.e., can lead to greater loss of N<sub>2</sub>O-N when the manure is replaced by a more readily available inorganic N source. Daily N<sub>2</sub>O emissions were highly responsive to spring snowmelt and N-fertilizer application, which strongly suggests that denitrification was responsible for the majority of the N<sub>2</sub>O produced at the Dixon site. The data clearly demonstrated that, under the right environmental conditions, a manure-induced priming effect can result in significant N<sub>2</sub>O-N losses, and that this effect can last for at least four years after the manure applications cease. Moreover, manure type had a major impact on whether or not denitrification potential was translated into quantifiable differences in cumulative N<sub>2</sub>O-N losses. For example, cumulative N<sub>2</sub>O-N losses from the plots with a history of SCM applications were generally lower than those from the LSM amended plots. Furthermore, the historical treatments did not appear to exhibit residual manure (legacy) effect; i.e., neither rate nor frequency of application had a significant effect on cumulative N<sub>2</sub>O-N losses. Conversely, plots with a history of LSM applications exhibited a significant legacy effect — with both the rate and frequency of the LSM application affecting cumulative N<sub>2</sub>O-N losses in the post manure period.

## 4 CONCLUSIONS AND FUTURE RESEARCH NEEDS

### 4.1 Summary and Conclusions

Nitrous oxide emissions are known to be impacted by pH, soil moisture (precipitation and snowmelt), fertilizer addition (both manure and synthetic), and C availability all of which impact the denitrification process (Tiedje et al., 1984; Lemke et al., 1998; Chantigny et al., 2001; Rochette et al., 2008a) which is responsible for the majority of soil-derived N<sub>2</sub>O emissions. For the present study, it was surmised that long-term (14 yr) manure applications would impact not only the soil C and N pools, but also the structure and activity of the soil microbial community — which could then condition (or “prime”) the soil for enhanced denitrification. Moreover, it was hypothesized that a change-over from manure to a synthetic urea fertilizer (i.e., a more readily available N source) might result in increased denitrification and, in turn, greater losses of N<sub>2</sub>O-N. Previous research at the Dixon site (Farrell et al., 2011) demonstrated that N<sub>2</sub>O emissions were significantly impacted by both the rate and frequency of long-term applications of liquid swine (LSM) and solid cattle manure (SCM). As a result, this research led the authors to pose the following question: “*do long-term manure applications ‘prime’ the soil resulting in increased N<sub>2</sub>O emissions when a more readily available N-source is applied?*” The manure applications at the Dixon site ceased in 2010 and the entire site was switched over to a blanket application of urea fertilizer in 2011 — thus providing an opportunity to answer this question. My study attempted to do this by measuring N<sub>2</sub>O emissions from a subset of the treatments at the Dixon site during the three-year period following the change in management.

The objective of my study was to examine the potential for a ‘N<sub>2</sub>O priming effect’ at the Dixon site following 14 years of manure application. The treatments chosen for this study were selected to provide a range of historical conditions, and included (i) the unfertilized (0N) control; (ii) the 1×, annual application of urea (i.e., the “standard” BMP); (iii) the 2×, triennial application of manure (i.e., the recommended manure BMP); and (iv) the 4×, annual application of manure (i.e., the “worst case scenario”). In general, my research demonstrated that the historical LSM-treatments did indeed have a “legacy” effect on cumulative N<sub>2</sub>O-N losses following the conversion from a manure-based to a urea-based N management system. A similar legacy effect was associated with the long-term applications of urea fertilizer at high (i.e., 2× and

4×) rates. Conversely, the historical SCM treatments did not produce any measurable legacy effect. This latter result was surprising, given that gene copy numbers for *nirS* gene (i.e., one of the genes responsible for catalyzing nitrite reduction) were greater in soils with a history of long-term SCM amendments, than in those associated with the long-term application of LSM (data not shown). At the same time, copy numbers of the other genes generally associated with denitrification (i.e., *nirK* and *nosZ*) were not significantly affected by either the type or rate and frequency of application of the historical manure/urea treatments (data not shown). As a result, there was no correlation between either N<sub>2</sub>O emission or DEA and denitrifier community abundance, that was similar to the findings reported by Tatti et al. (2014) who examined the impacts of manure application and microbial overwintering on N<sub>2</sub>O production.

The second objective of this study was to assess the duration of the priming effect, if one was detected. Dambreville et al. (2006) reported that 14 months after termination of a medium-term (7 yr) field study, potential denitrification in soils with a history of composted pig manure applications was higher than that in soils with a similar history of synthetic N fertilizer (ammonium nitrate) applications. At the Dixon site, cumulative N<sub>2</sub>O-N loss from the plots associated with the highest annual application of LSM (HLS-A4) was significantly greater than that from the plots associated with the manure best management practices (HLS-T2). This provides strong evidence that N<sub>2</sub>O priming was still in effect more than four years (i.e., 56 months) after the final manure application. That a similar result was also observed for the plots with a history of high (4×) application rates of urea fertilizer (HUF-A4), but not from those with a history of equivalent SCM applications (HSC-A4), also provides a strong indication that this effect is mediated by the quality of the N source.

Finally increases in N<sub>2</sub>O-N loss also were dependent upon weather — in particular, the amount and distribution of precipitation received during the snowmelt period (including antecedent soil moisture) through a 3-wk window following the spring fertilizer application. Taken together, the data obtained from this study demonstrate that (i) long-term applications of manure can result in a N<sub>2</sub>O priming effect; (ii) this effect is influenced by the type of manure — being greater for LSM than for SCM — as well as the rate and frequency of application of the manure; (iii) this effect can be relatively long-lived; and (iv) while the potential for enhanced denitrification is relatively persistent in the soil, whether this potential translates into a quantifiable increase in cumulative N<sub>2</sub>O-N losses in the field depends on local weather

conditions. It is expected that these results will help improve efforts to model the impacts of changing soil/fertilizer management on N<sub>2</sub>O emissions and inform future fertilizer management decisions in the Canadian Prairies as these results clearly demonstrate that it is possible to keep N<sub>2</sub>O emissions in manured agroecosystem to a minimum by employing best management practices. Perhaps just as importantly, poor N management can have negative effects that may persist well beyond a switch to a best management practice.

## 4.2 Future Research

While the Dixon study provided a unique opportunity to explore how fertilizer management can impact N<sub>2</sub>O emissions, extrapolating these results beyond the Black soil zone may require additional study. Moreover, extending this study to different soil zones may provide a more definitive understanding of the link between long-term manure applications, N<sub>2</sub>O emissions, and microbial adaptations throughout the Canadian Prairies. Potentially incorporating phosphorus, and micronutrients in the analysis for long-term manure application may also close some of the knowledge gaps.

It is well known that the denitrification process transforms NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> into N<sub>2</sub>O and finally N<sub>2</sub> and that the process is regulated by multiple genes each coding for the enzymes controlling specific N-transformations. Specifically, *narG*, and the *nirS* and *nirK* genes lead to the production of N<sub>2</sub>O via the transformation of NO<sub>3</sub><sup>-</sup> or NO<sub>2</sub><sup>-</sup>, respectively, while the *nosZ* gene controls N<sub>2</sub>O consumption. Thus, these genes are often used to monitor the contribution of denitrification to the loss of N<sub>2</sub>O. Whereas we have relatively good understanding how specific genes impact the denitrification process, there are gaps in our knowledge of how these genes relate to enzyme activity and interact under field conditions — especially with regards to how they are impacted by soil and crop management practices, such as long-term manure applications. Moreover, a better understanding of how changes in N management impact N<sub>2</sub>O emissions is critical to furthering improvements in agriculture sustainability. Closing these gaps is likely to require employing advanced analytical techniques that combine stable isotope techniques with the molecular techniques used to study soil microbial communities. Not only is this likely to provide a better understanding of how soil/fertilizer management affects N-cycling process and the production/emission of N<sub>2</sub>O in agroecosystems, but combining stable N isotope, gene quantification and microbial activity assessment techniques may help elucidate the link

between microbial gene expression and changes to N<sub>2</sub>O production in soil. Stable isotopes have been successfully utilized to assess N<sub>2</sub>O production from synthetic fertilizer applications in a closed system (Bol et al., 2003) and in field monitoring with manure applications (Snider et al., 2015). The approach of combining stable isotope and molecular analyses was proven beneficial by Snider et al. (2015), who demonstrated that the two approaches yielded similar inferences about manure-induced N<sub>2</sub>O emissions. Including the *narG* gene in microbial analyses also may provide a clearer picture of the N-transformations associated with denitrification, as this enzyme regulates a key transformation (i.e., the transformation of NO<sub>3</sub><sup>-</sup> into NO<sub>2</sub><sup>-</sup>) in the N cycling process. Examining the nitrification genes for both bacterial and archaeal *amoA* also would help identify the primary sources of N<sub>2</sub>O emissions from a manured agricultural soil, as well as help establish the contributions of nitrification or denitrification to N<sub>2</sub>O production/emission. For example, Snider et al. (2015) demonstrated the importance of both processes — which occurred in unison during a rainfall-induced emission event from manured agricultural soils in Ontario.

In terms of the priming effect itself, Kuzyakov and Bol (2006) conducted a study to examine CO<sub>2</sub> priming effects induced by amending grassland soils with either a C<sub>3</sub>- or C<sub>4</sub>-slurry or sugar, and concluded that priming effect occurred in four stages: (1) r-strategist microorganisms rapidly utilize the added, readily available substrate; (2) this triggers an increase in microbial abundance and activity, resulting in enhanced consumption of the more easily utilizable components of the SOM; (3) followed by enhanced utilization of other, more recalcitrant substrates; and (4) a return to an initial equilibrium community structure and activity. However, whether the same stages apply to the N<sub>2</sub>O priming effect is unknown at this time. Combining stable isotope and molecular techniques, may help unravel this issue.

## 5 REFERENCES

- Acea, M.J., and T. Carballas. 1988. The influence of cattle slurry on soil microbial populations and nitrogen cycle microorganisms. *Biol. Waste.* 23: 229-241.
- Alef, K. 1995. Assay of Denitrification. Chapter 6 Anaerobic microbial activities in soil. p. 285-286. In K. Alef, and P. Nannipieri, (eds.), *Methods in applied soil microbiology and biochemistry.* Academic Press Limited, London, U.K.
- Arcand, M.M., B.L. Helgason, and R.L. Lemke. 2016. Microbial crop residue decomposition dynamics in organic and conventionally managed soils. *Appl. Soil. Ecol.* 107: 347-259.
- Asgedom, H., M. Tenuta, D.N. Flaten, X. Gao, and E. Kebreab. 2014. Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. *Agron. J.* 106: 732-744.
- Baggs, E.M., C.L. Smales, and E.J. Bateman. 2010. Changing pH shifts the microbial source as well as the magnitude of N<sub>2</sub>O emissions from soil. *Biol. Fertil. Soils.* 46: 793-805.
- Barrett, M., M.I. Khalil, M.M.R. Jahangir, C. Lee, L.M. Cardenas, G. Collins, K.G. Richards, and V. O'Flaherty. 2016. Carbon amendment and soil depth affect the distribution and abundance of denitrifiers in agricultural soils. *Environ. Sci. Pollut. Res.* 23: 7899-7910.
- Bergstrom, D.W., M. Tenuta, and E.G. Beauchamp. 1994. Increase in nitrous oxide production in soil induced by ammonium and organic carbon. *Bio. Fertil. Soils.* 18: 1-6.
- Bol, R., S. Toyoda, S. Yamulki, J.M.B. Hawkins, L.M. Cardenas, and N. Yoshida. 2003. Dual isotope and isotopomer ratios of N<sub>2</sub>O emitted from a temperate grassland soil after fertilizer application. *Rapid Commun. Mass Spectrom.* 17: 2550-2556.
- Bremner, J.M. 1997. Sources of nitrous oxide in soils. *Nutr. Cycl. Agroecosys.* 49: 7-16.
- Burford, J.R. and J.M. Bremner. 1975. Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Bio. Biochem.* 7: 389-394.
- Burton, D.L., B.J. Zebarth, K.M. Gillam, and J.A. MacLeod. 2008. Effect of split application of fertilizer nitrogen on N<sub>2</sub>O emissions from potatoes. *Can. J. Soil. Sci.* 88: 229-239.
- Calderón, F.J., G. W. McCarty, J.S. Van Kessel, and J. B. Reeves. 2004. Carbon and Nitrogen Dynamics during incubation of manured soil. *Soil Sci. Soc. Am. J.* 68: 1592-1599.
- Calderón, F.J., G.W. McCarty, and J.B. Reeves. 2005. Analysis of manure and soil nitrogen mineralization during incubation. *Biol. Fertil. Soils.* 41: 328-336.
- Canfield, D.E., A.N. Glazer, and P.G. Falkowski. 2010. The evolution and future of earth's nitrogen cycle. *Science* 330:192-196.
- Cavigelli, M.A., S.J. Del Grosso, M.A. Liebig, C.S. Snyder, P.E. Fixen, R.T. Venterea, A.B. Leytem, J.E. McLain, and D.B. Watts. 2012. US agricultural nitrous oxide emissions: context, status, and trends. *Front. Ecol. Environ.* 10: 537-546.

- Chantigny, M.H., P. Rochette, and D.A. Angers. 2001. Short-term C and N dynamics in soil amended with pig slurry and barley straw: a field experiment. *Can. J. Soil Sci.* 81: 131-137.
- Chaudhry, V., A. Rehman, A. Mishra, P.S. Chauhan, and C.S. Nautiyal. 2012. Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. *Microb. Ecol.* 64: 450-460.
- Christensen, B.T. 1988. Effects of animal manure and mineral fertilizer on the total carbon and nitrogen contents of soil size fractions. *Biol. Fertil. Soil.* 5: 304-307.
- Chu, H., X. Lin, T. Fujii, S. Morimoto, K. Yagi, J. Hu, and J. Zhang. 2007a. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. *Soil Biol. Biochem.* 39: 2971-2976.
- Chu, H., T. Fujii, S. Morimoto, X. Lin, K. Yagi, J. Hu, and J. Zhang. 2007b. Community structure of ammonia-oxidizing bacteria under long-term application of mineral fertilizer and organic manure in a sandy loam soil. *Appl. Environ. Microbiol.* 73: 485-491.
- Corre, M.D., C. van Kessel., and D.J. Pennock. 1996. Landscape and seasonal patterns of nitrous oxide emissions in a semiarid region. *Soil Sci. Soc. Am. J.* 60: 1806-1815.
- Coyne, M.S. 2008. Biological Denitrification. p. 201-253. In J.S. Schepers and W.R. Raun (eds), *Nitrogen in agricultural systems*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Madison, Wisconsin U.S.A.
- Cui, P., F. Fan, C. Yin, A. Song, P. Huang, Y. Tang, P. Zhu, C. Peng, T. Li, S.A. Wakelin, and Y. Liang. 2016. Long-term organic and inorganic fertilization alters temperature sensitivity of potential N<sub>2</sub>O emission and associated microbes. *Soil Bio. Biochem.* 93: 131-141.
- Dambreville, C., S. Hallet, C. Nguyen, T. Morvan, J. Germon, and L. Philippot. 2006. Structure and activity of the denitrifying community in a maize-cropped field fertilized with composted pig manure or ammonium nitrate. *FEMS Microbiol. Ecol.* 56: 119-131.
- Dandie, C.E., D.L. Burton, B.J. Zebarth, S.L. Henderson, J.T. Trevors, and C. Goyer. 2008. Changes in bacterial denitrifier community abundance over time in an agricultural field and their relationships with denitrification activity. *Appl. Environ. Microbiol.* 74: 5997-6005.
- Davidson, E.A. 2009. The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature GeoSci.* 2: 659-662.
- Ding, W., K. Yagi, Z. Cai, and F. Han. 2010. Impact of long-term application of fertilizers on N<sub>2</sub>O and NO production in an intensively cultivated sandy loam soil. *Water Air Soil Pollut.* 212: 141-153.
- Dobbie, K.E., I.P. McTaggart, and K.A. Smith. 1999. Nitrous oxide emissions from intensive agricultural systems: Variations between crops and seasons, key driving variables, and mean emission factors. *J. Geophys. Res.* 104: 26891-26899.

- Drury, C.F., D.D. Myrold, E.G. Beauchamp, and W.D. Reynolds. 2008. Denitrification Techniques for Soils. Chapter 37. p. 471-493. In M.R. Carter and E.G. Gregorich (eds), Soil sampling and methods of analysis second edition. CRC Press Taylor and Francis Group, Boca Raton, Florida, U.S.A.
- Dunmola, A.S., M. Tenuata, A.P. Moulin, P. Yapa, and D.A. Lobb. 2010. Pattern of greenhouse gas emissions from a Prairie Pothole agricultural landscape in Manitoba, Canada. *Can. J. Soil Sci.* 90: 243-256.
- Dusenbury, M.P., R.E. Engel, and P.R. Miller. 2008. Nitrous oxide emissions from a northern great plains soil as influence by nitrogen management and cropping systems. *J. Environ. Qual.* 37: 542-550.
- Environment Canada. 2015a. Daily data report for the Saskatoon RCS Station 2010-2014. [Online]. Available at [http://climate.weather.gc.ca/index\\_e.html#access](http://climate.weather.gc.ca/index_e.html#access) (verified 9 February 2015).
- Environment Canada. 2015b. Canadian climate normals, 1981-2010 for the Saskatoon Diefenbaker International Airport Station. [Online]. Available at [http://climate.weather.gc.ca/climate\\_normals/index\\_e.html](http://climate.weather.gc.ca/climate_normals/index_e.html) (verified 9 February 2015).
- Enwall, K., L. Philippot, and S. Hallin. 2005. Activity and composition of the denitrifying bacterial community respond differently to long-term fertilization. *Appl. Environ. Microbiol.* 71: 8335-8343.
- Farrell, R.E., and J.A. Elliot. 2008. Soil Air. Chapter 64. p. 833-850. In M.R. Carter and E. G. Gregorich (eds), Soil sampling and methods of analysis Second Edition. CRC Press Taylor and Francis Group, Boca Raton, Florida, U.S.A.
- Farrell, R.E., J.J. Schoenau, and J.D. Knight. 1999. Nitrous oxide production from soils amended with fall-applied hog manure. Pp. 227-237. In Proceedings of a tri-provincial conference on manure management, Saskatoon, SK.
- Farrell, R.E., R.L. Lemke, and J.J. Schoenau 2011. Nitrous Oxide (N<sub>2</sub>O) emissions from soils receiving long-term applications of liquid and solid manure: A study at the Dixon long-term manure plots. ADF Project No. 20080135 Final Report.
- Fernandez, E.S. 2015. Johnson Transformation. R package version 1.4. <http://CRAN.R-project.org/web/packages/Johnson/Johnson.pdf> (verified 4 September 2016)
- Groffman, P.M., E.A. Holland, D.D. Myrold, G.P. Robertson, and X. Zou. 1999. Denitrification. p 272-288. In G.P. Robertson, D.C. Colemand, C.S. Bledsoe, and P. Sollins (eds.). Standard soil methods for long-term ecological research. Oxford University Press. Oxford, New York, U.S.A.
- Hallin, S., C.M. Jones, M. Schloter, and L. Philippot. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. *The ISME Journal.* 3: 597-605.
- Helgason, B.L., H.H. Jazen, M.H. Chantigny, C.F. Drury, B.H. Ellert, E.G. Gregorich, R.L. Lemke, E. Pattey, P. Rochette, and C. Wagner-Riddle. 2005. Toward improved coefficients for predicting direct N<sub>2</sub>O emissions from soil in Canadian agroecosystems. *Nutr. Cycl. Agroecosyst.* 72: 87-99.

- Helgason, B.L., F.J. Larney, H.H. Janzen, and B.M. Olson. 2007. Nitrogen dynamics in soil amended with composted cattle manure. *Can. J. Soil Sci.* 87: 43-50.
- Henderson, S.L., C.E. Dandie, C.L. Patten, B.J. Zebarth, D.L. Burton, J.T. Trevors, and C. Goyer. 2010. Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions, and denitrification in anoxic soil microcosms amended with glucose and plant residues. *Appl. Environ. Microbiol.* 76: 2155-2164.
- Hijmans, R.J., S.E. Cameron, J.L. Parra, P.G. Jones, and A. Jarvis, 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* 25: 1965-1978.
- Hijmans, R.J., J. van Etten, J. Cheng, M. Mattiuzzi, M. Sumner, J.A. Greenberg, O.P. Lamigueiro, A. Bevan, E.B. Racine, and A. Shortridge. 2016. Package 'raster': Geographic data analysis and modeling. R version 2.5-8. <https://cran.r-project.org/web/packages/raster/raster.pdf>. (verified 4 September 2016)
- Hopkins, D.W., and R.S. Shiel. 1996. Size and activity of soil microbial communities in long-term experimental grassland plots treated with manure and inorganic fertilizers. *Biol Fertil. Soils.* 22: 66-70.
- Hutchinson, G. L., and A.R. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Sci. Soc. Am. J.* 45: 311-316.
- IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, New York, U.S.A., 1535pp.
- Jenkinson, D.S., R.H. Fox, and J.H. Rayner. 1985. Interactions between fertilizer nitrogen and soil nitrogen-the so-called 'priming' effect. *J. Soil Sci.* 36:425-444.
- Kennedy, A.C. 1999. Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* 74: 65-76.
- King, T.N. 2007. Effect of repeated applications of manure on soil organic carbon and enzymes. MS Thesis. University of Saskatchewan. Saskatoon, Saskatchewan, Canada.
- Kong, A.Y.Y., K. Hristova, K.M.Scow, and J. Six. 2010. Impacts of different N management regimes on nitrifier and denitrifier communities and N cycling in soil microenvironments. *Soil Biol. Biochem.* 42: 1523-1533.
- Kostka, J.E., S.J. Green, L. Rishishwar, O. Prakash, L.S. Katz, L. Marino-Ramirez, I.K. Jordan, C. Munk, N. Ivanova, N. Mikhailova, D.B. Watson, S.D. Brown, A.V. Palumbo, and S.C. Brooks. 2012. Genome Sequences for Six *Rhodanobacter* Strains, Isolated from Soils and the Terrestrial Subsurface, with Variable Denitrification Capabilities. *J. Bacteriol.* 194: 4461-4462.
- Kuzyakov, Y., and R. Bol. 2006. Sources and mechanisms of priming effect induced in two grassland soils amended with slurry and sugar. *Soil Biol. Biochem.* 38: 747-758.
- Kuzyakov, Y., J.K. Friedel, and K. Stahr. 2000. Review of mechanisms and quantification of priming effects. *Soil Biol. Biochem.* 32: 1485-1498.

- Lebender, U., M. Senbayram, J. Lammel, and H. Kuhlmann. 2014. Impact of mineral N fertilizer application rates on N<sub>2</sub>O emissions from arable soils under winter wheat. *Nutr. Cycl. Agroecosyst.* 100: 111-120.
- Lemke, R.L., R.F. Farrell, and S.S. Malhi. 2010. Greenhouse gas emissions from crop production systems in the Northern Great Plains of North America. Chapter 14. p. 385-408. In S.S. Malhi, Y. Gan, J.J. Schoenau, R.L. Lemke and M.A. Leibig (eds), *Recent trends in soil science and agronomy research in the Northern Great Plains of North America*. Research Signpost, Kerala, India.
- Lemke, R.L., R.C. Izaurralde, and M. Nyborg. 1998. Seasonal distribution of nitrous oxide emissions from soils in the parkland region. *Soil Sci. Soc. Am. J.* 62: 1320-1326.
- Linn, D. M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil. Sci. Soc. Am. J.* 48: 1267-1272.
- Loro, P. J., D.W. Bergstrom, and E.G. Beauchamp. 1997. Intensity and duration of denitrification following application of manure and fertilizer to soil. *J. Environ. Qual.* 26: 706-713.
- Luxhøi, J., L. Elsgaard, I.K. Thomsen, and L.S. Jensen. 2007. Effects of long-term annual inputs of straw and organic manure on plant N uptake and soil N fluxes. *Soil Use Manage.* 23: 368-373.
- Malhi, S.S., S.A. Brandt, R.L. Lemke, A.P. Moulin, and R.P. Zentner. 2009. Effects of input level and crop diversity on soil nitrate—N, extractable P, aggregation, organic C and N, and nutrient balance in the Canadian Prairie. *Nutr. Cycl. Agroecosyst.* 84: 1-22.
- Maynard, D.G., Y.P. Kalra, and J.A. Crumbaugh. 2008. Nitrate and Exchangeable Ammonium Nitrogen. Chapter 6. p. 71-80. In M.R. Carter and E.G. Gregorich (eds), *Soil sampling and methods of analysis second edition*. CRC Press Taylor and Francis Group, Boca Raton, Florida, U.S.A.
- Mendiburu, F. 2016. *Statistical Procedures for Agricultural Research*. R package version 1.2-1. <http://cran.r-project.org/web/packages/agricolae/agricolae.pdf> (verified 4 September 2016).
- Miller, M.N, B.J. Zebarth, C.E. Dandie, D.L. Burton, C. Goyer, and J.T. Trevors. 2008. Crop residue influence on denitrification, N<sub>2</sub>O emissions and denitrifier community abundance in soil. *Soil Biol. Biochem.* 40: 2553-2562.
- Mitsch, W.J., and J.G. Gosselink. 2007. *Wetlands Fourth Edition*. John Wiley & Sons Inc. Hoboken, New Jersey, U.S.A.
- Molodovskaya, M., O. Singurindy, B.K. Richards, J. Warland, M.S. Johnson, and T.S. Steenhuis. 2012. Temporal variability of nitrous oxide from fertilized croplands: hot moment analysis. *Soil Sci. Soc. Am. J.* 76: 1728-1740.
- Mogge, B., E. Kaiser, and J. Munch. 1999. Nitrous oxide emissions and denitrification N-losses from agricultural soils in Bornhöved Lake region: influence of organic fertilizers and land-use. *Soil Biol. Biochem.* 31: 1245-1252.

- Mooleki, S. P., J.J. Schoenau, G. Hultgreen, G. Wen, and J.L. Charles. 2002. Effect of rate, frequency and method of liquid swine manure application on soil nitrogen availability, crop performance and N use efficiency in east-central Saskatchewan. *Can. J. Soil Sci.* 82: 457-467.
- Mooleki, S.P., J.J. Schoanau, J.L. Charles, and G. Wen. 2004. Effect of rate, frequency and incorporation of feedlot cattle manure on soil nitrogen availability, crop performance and nitrogen use efficiency in east-central Saskatchewan. *Can. J. Soil Sci.* 84: 199-210.
- Moulin, A.P., A. Glenn, M. Tenuta, D.A. Lobb, A.S. Dunmola, and P. Yapa. 2014. Alternative transformation of nitrous oxide soil flux data to normal distributions. *Can. J. Soil Sci.* 94: 105-108.
- Mulvaney, R.L., S.A. Khan, and C.S. Mulvaney. 1997. Nitrogen fertilizers promote denitrification. *Biol. Fertil. Soils.* 24: 211-220.
- Nägele, W., and R. Conrad. 1990. Influence of soil pH on the nitrate-reducing microbial populations and their potential to reduce nitrate to NO and N<sub>2</sub>O. *FEMS Microbiol. Ecol.* 74: 49-58.
- Norton, J.M. 2008. Nitrification in Agricultural Soils. p. 173-199. In J.S. Schepers and W. R. Raun (eds), *Nitrogen in agricultural systems*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Madison, Wisconsin, U.S.A.
- Olk, D.C. 2008. Organic Forms of Soil Nitrogen. p. 57-100. In J.S. Schepers and W. R. Raun (eds), *Nitrogen in agricultural systems*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc. Madison, Wisconsin, U.S.A.
- Parham, J. A., S. P. Deng, H. N. Da, H. Y. Sun, and W. R. Raun. 2003. Long-term cattle manure application in soil. II. Effect on soil microbial populations and community structure. *Biol. Fertil. Soils.* 38: 209-215.
- Paul, J. W., and E. G. Beauchamp. 1994. Short-term nitrogen dynamics in soil amended with fresh and composted cattle manures. *Can. J. Soil. Sci.* 74: 147-155.
- Pedersen, A. R. 2015. HMR: Flux estimation with static chamber data. R package version 0.4.1. <https://cran.r-project.org/web/packages/HMR/HMR.pdf> (verified 5 September 2016)
- Pennock, D.J., A. Bedard-Haughn, and V. Viaud. 2011. Chernozemic soils of Canada: Genesis, distribution, and classification. *Can. J. Soil Sci.* 91: 719-747.
- Prosser, J.I. 2007. Microorganisms Cycling Soil Nutrients and Their Diversity. p. 237-261. In J.D. van Elsas, J. K. Jansson, and J. T. Trevors (eds), *Modern soil microbiology second edition*. CRC Press, Taylor & Francis Group, Boca Raton, Florida, U.S.A.
- Qian, P., and J.J. Schoenau. 2000. Use of ion exchange membrane to assess soil N supply to canola as affected by addition of liquid swine manure and urea. *Can. J. Soil Sci.* 80: 213-218.
- Qian, P., and J.J. Schoenau. 2002. Availability of nitrogen in solid manure amendments with different C:N ratios. *Can. J. Soil Sci.* 82: 219-225.

- R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/> (verified 4 September 2016).
- Ravishankara, A.R., J.S. Daniel, and R.W. Portmann. 2009. Nitrous oxide (N<sub>2</sub>O): the dominant ozone-depleting substance emitted in the 21st century. *Science* (New York, N.Y.) 326:123–125.
- Rochette, P., D.E. Worth, R.L. Lemke, B.G. McConkey, D.J. Pennock, C. Wagner-Riddle, and R.J. Desjardins. 2008a. Estimation of N<sub>2</sub>O emissions from agricultural soils in Canada. I. Development of a country-specific methodology. *Can. J. Soil. Sci.* 88:641–654.
- Rochette, P., D.A. Angers, M.H. Chantigny, B. Gagnon, and N. Bertrand. 2008b. N<sub>2</sub>O fluxes in soils of contrasting textures fertilized with liquid and solid dairy cattle manures. *Can. J. Soil Sci.* 88: 175-187.
- Schoenau, J.J., T. King, and S.S. Malhi. 2013. The impact of long-term manure applications and cessation of application on Saskatchewan soils. ADF Project No. 20090242 Final Report.
- Shcherbak, I., N. Millar, and G.P. Robertson. 2014. Global metaanalysis of the nonlinear response of soil nitrous oxide (N<sub>2</sub>O) emissions to fertilizer nitrogen. *Proc. Natl. Acad. Sci.* 111: 9199-9204.
- Shen, J.-P., L.-M. Zhang, J.-F. Guo, J.L. Ray, and J.-Z. He. 2010. Impact of long-term fertilization practices on the abundance and composition of soil bacterial communities in Northeast China. *App. Soil Ecol.* 46: 119-124.
- Šimek, M., J. Hynšt, and P. Šimek. 2014. Emissions of CH<sub>4</sub>, CO<sub>2</sub>, and N<sub>2</sub>O from soil at a cattle overwintering area as affected by available C and N. *Appl. Soil Ecol.* 75: 52-62.
- Skiba, U., K.J. Hargreaves, D. Fowler, and K.A. Smith. 1992. Fluxes of nitric and nitrous oxides from agricultural soils in a cool temperate climate. *Atmos. Environ.* 26: 2477-2488.
- Skiba, U., and B. Ball. 2002. The effect of soil texture and soil drainage on emissions of nitric oxide and nitrous oxide. *Soil Use Manage.* 18: 56-60.
- Snider, D., K. Thompson, C. Wagner-Riddle, J. Spoelstra, and K. Dunfield. 2015. Molecular techniques and stable isotope ratios at natural abundance give complementary inferences about N<sub>2</sub>O production pathways in an agricultural soil following a rainfall event. *Soil Biol. Biochem.* 88: 197-213.
- Sradnick, A., M. Oltmanns, J. Raupp, and R. G. Joergensen. 2014. Microbial residue indices down the soil profile after long-term addition of farmyard manure and mineral fertilizer to a sandy soil. *Geoderma* 226-227: 79-84.
- Stark, C.H., L.M. Condon, M. O’Callaghan, A. Stewart, H.J. Di. 2008. Differences in soil enzyme activities, microbial community structure and short-term nitrogen mineralization resulting from farm management history and organic matter amendments. *Soil Biol. Biochem.* 40: 1352-1363.
- Statistics Canada. 2011. Farm and Farm Operator Data. Table 004-0207. Census of Agriculture, manure and manure application methods in the year prior to the census. [Online]. Available at <http://www.statcan.gc.ca/pub/95-640-x/95-640-x2011001-eng.htm>. (verified July 9, 2015).

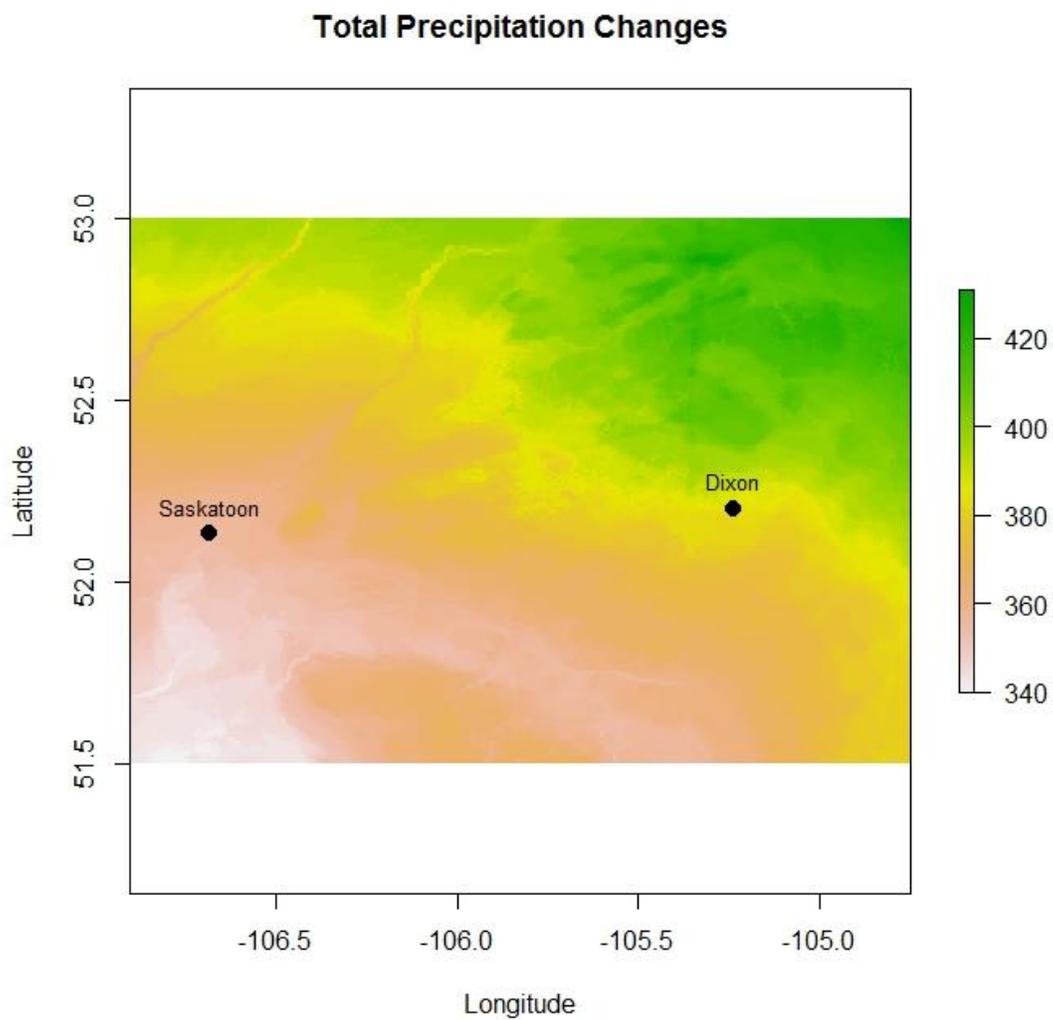
- Stumborg, C.M. 2006. Phosphorus and nitrogen loading in manured Saskatchewan soils. MS Thesis. University of Saskatchewan, Saskatoon, Saskatchewan, Canada.
- Stumborg, C., J.J. Schoenau, and S.S. Malhi. 2007. Nitrogen balance and accumulation pattern in three prairie soils receiving repeated applications of liquid swine and solid cattle manure. *Nutr. Cycl. Agroecosyst.* 78: 15-25.
- Su, Y., F. Wang, D. Suo, Z. Zhange, and M. Du. 2006. Long-term effect of fertilizer and manure application on soil-carbon sequestration and soil fertility under the wheat-wheat-maize cropping system in northwest China. *Nutr. Cycl. Agroecosyst.* 75: 285-295.
- Tatti, E., C. Goyer, M. Chantigny, S. Wertz, B.J. Zebarth, D.L. Burton, and M. Fillion. 2014. Influences of over winter conditions on denitrification and nitrous oxide-producing microorganism abundance and structure in an agricultural soil amended with different nitrogen sources. *Agric. Ecosyst. Environ.* 183: 47-59.
- Tatti, E., C. Goyer, B.J. Zebarth, D.L. Burton, L. Giovannetti, and C. Viti. 2012. Short-term effects of mineral and organic fertilizer on denitrifiers, nitrous oxide emissions and denitrification in long-term amended vineyard soils. *Soil Sci. Soc. Am. J.* 77: 113-122.
- Taylor, R. 1990. Interpretation of the correlation coefficient: A basic review. *JDMS.* 1: 35-39.
- Tiedje, J.M., A.J. Sexstone, T.B. Parkin, N.P. Revsbech, and D.R. Shelton. 1984. Anaerobic processes in soil. *Plant Soil.* 76: 197-212.
- Tilman, D., C. Balzer, J. Hill, and B.L. Befort. 2011. Global food demand and the sustainable intensification of agriculture. *PNAS.* 108: 20260-20264.
- Velthof, G.L., and J. Mosquera. 2011. The impact of slurry application technique on nitrous emissions from agricultural soils. *Agric. Ecosyst. Environ.* 140: 298-308.
- Velthof, G.L., P.J. Kuikman, and O. Oenema. 2003. Nitrous oxide emissions from animal manures applied to soil under controlled conditions. *Biol. Fertil. Soils.* 37: 221-230.
- Wei-xin, D., M. Lei, C. Zu-cong, and H. Feng-xiang. 2007. Effects of long-term amendment of organic manure and nitrogen fertilizer on nitrous oxide emissions in a sandy loam soil. *J. Environ. Sci.* 19: 185-193.
- Wen, G., J. J. Schoenau, J. L. Charles, and S. Inanaga. 2003. Efficiency parameters of nitrogen in hog and cattle manure in the second year following application. *J. Plant Nutr. Soil Sci.* 66: 490-498.
- Whalen, S.C. 2000. Nitrous oxide emission from an agricultural soil fertilized with liquid swine waste or constituents. *Soil Sci. Soc. Am. J.* 64: 781-789.
- Whalen, J.K., and L. Sampedro. 2010. *Soil Ecology & Management.* CABI. Cambridge, Massachusetts, U.S.A.
- Wrage, N., J.W. van Groenigen, O. Oenema, and E.M. Baggs. 2005. A novel dual-isotope labelling method for distinguishing between soil sources of N<sub>2</sub>O. *Rapid Commun. Mass. Spectrom.* 19: 3298-3306.
- Wrage, N., G.L. Velthof, M.L. van Beusichem, and O. Oenema. 2001. Role of nitrifier-denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* 33: 1723-1732.

- Yates, T.T., B.C. Si, R.E. Farrell, and D.J. Pennock. 2006a. Probability distribution and spatial dependence of nitrous oxide emission: temporal change in hummocky terrain. *Soil Sci. Soc. Am. J.* 70: 753-762.
- Yates, T.T., B.C. Si, R.E. Farrell, and D.J. Pennock. 2006b. Wavelet spectra of nitrous oxide emissions from hummocky terrain during spring snowmelt. *Soil Sci. Am. J.* 70: 1110-1120.
- Yin, C., F. Fan, A. Song, P. Cui, T. Li, and Y. Liang. 2015. Denitrification potential under different fertilization regimes is closely couple with changes in the denitrifying community in a black soil. *Appl. Microbiol. Biotechnol.* 99: 5719-5729.
- Yoshida, M., S. Ishii, D. Fujii, S. Otsuka, and K. Senoo. 2012. Identification of active denitrifiers in rice paddy soil by DNA- and RNA-based analyses. *Microbes Environ.* 27: 456-461.
- Zhang, N., S. Wan, L. Li, J. Bi, M. Zhao, and K. Ma. 2008. Impacts of urea N addition on soil microbial community in a semi-arid temperate steppe in northern China. *Plant Soil.* 311: 19-28.
- Zhong, W., T. Gu, W. Wang, B. Zhang, X. Lin, Q. Huang, and W. Shen. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant Soil.* 326: 511-522.

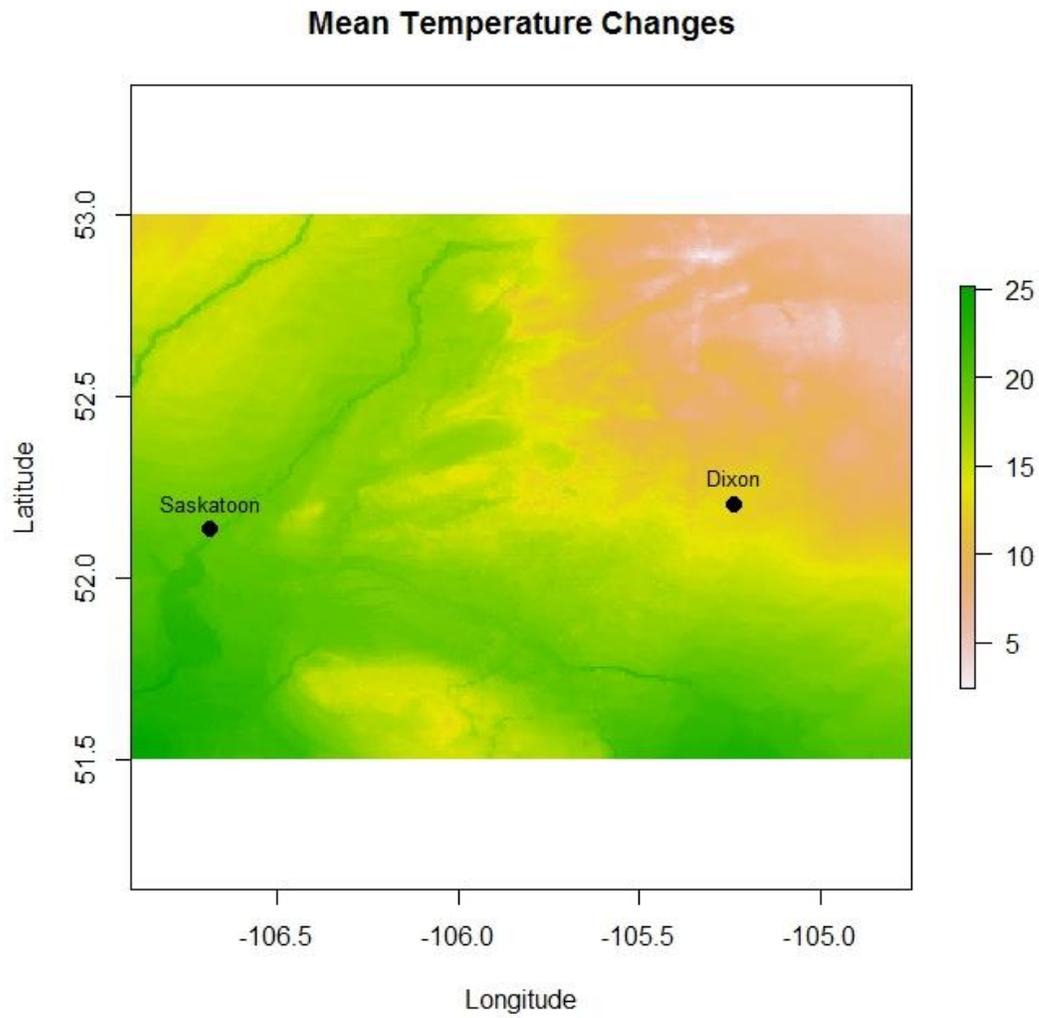
## APPENDIX A

### Precipitation and temperature spatial representations

These figures examine the different grids of historical weather data between Saskatoon and Dixon and displays the average differences in precipitation or temperature.



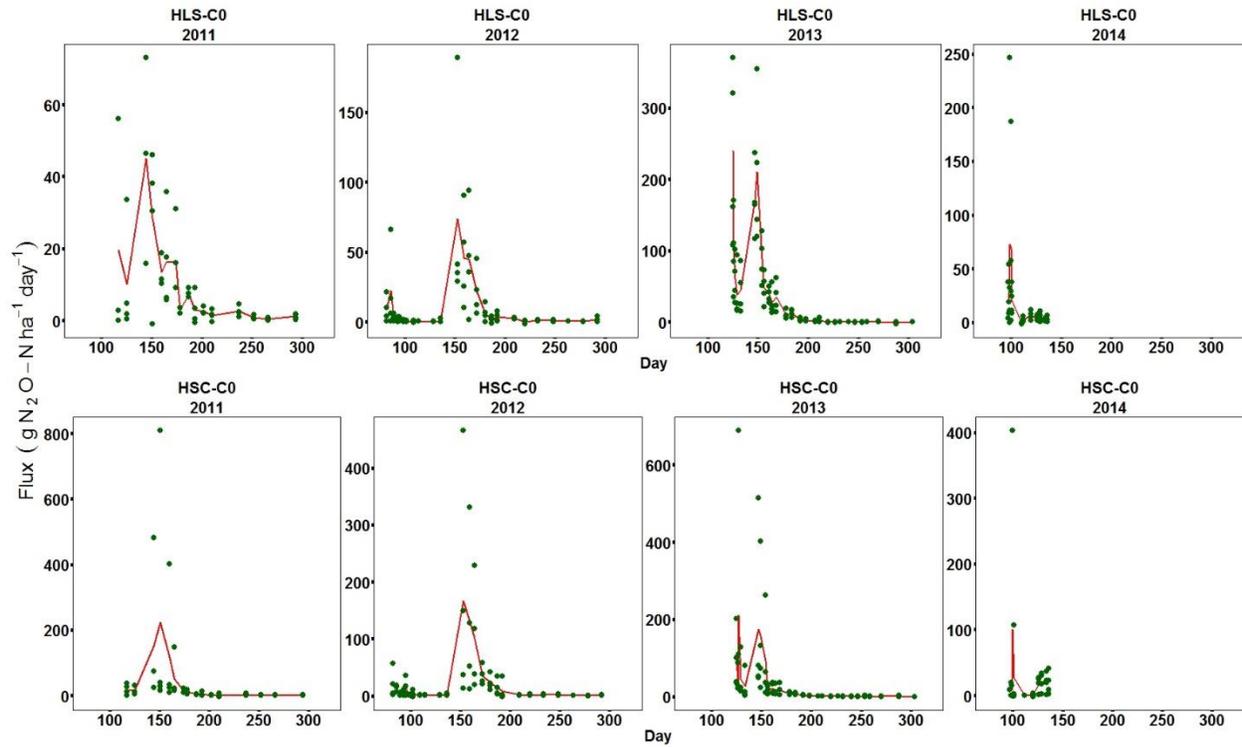
**Appendix A1. Range of total precipitation in millimetres between Saskatoon and the Dixon long-term research site based on geographic distance.**



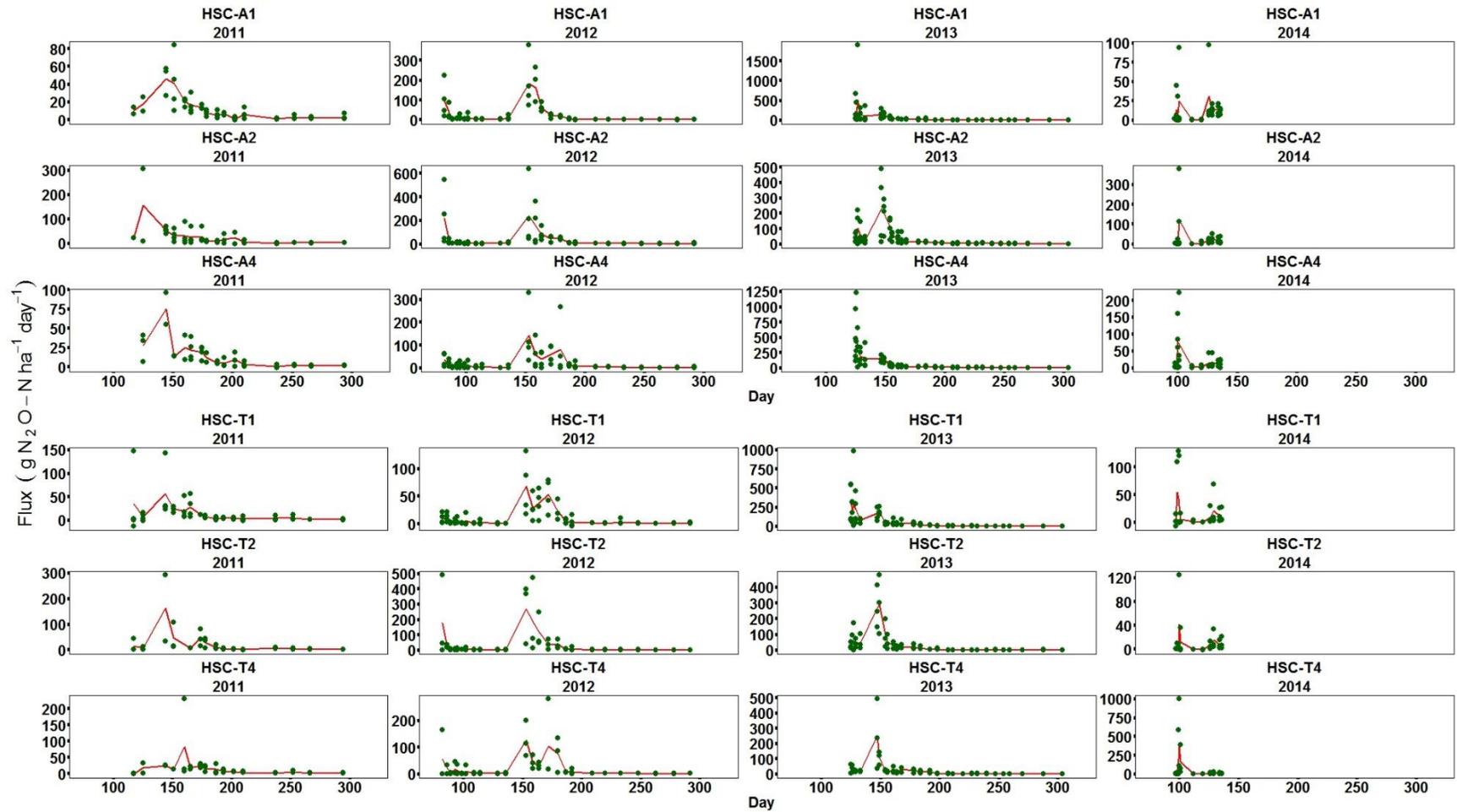
**Appendix A2. Range in mean total temperature in degrees Celsius between Saskatoon and the Dixon long-term research site based on geographic distance.**

## APPENDIX B

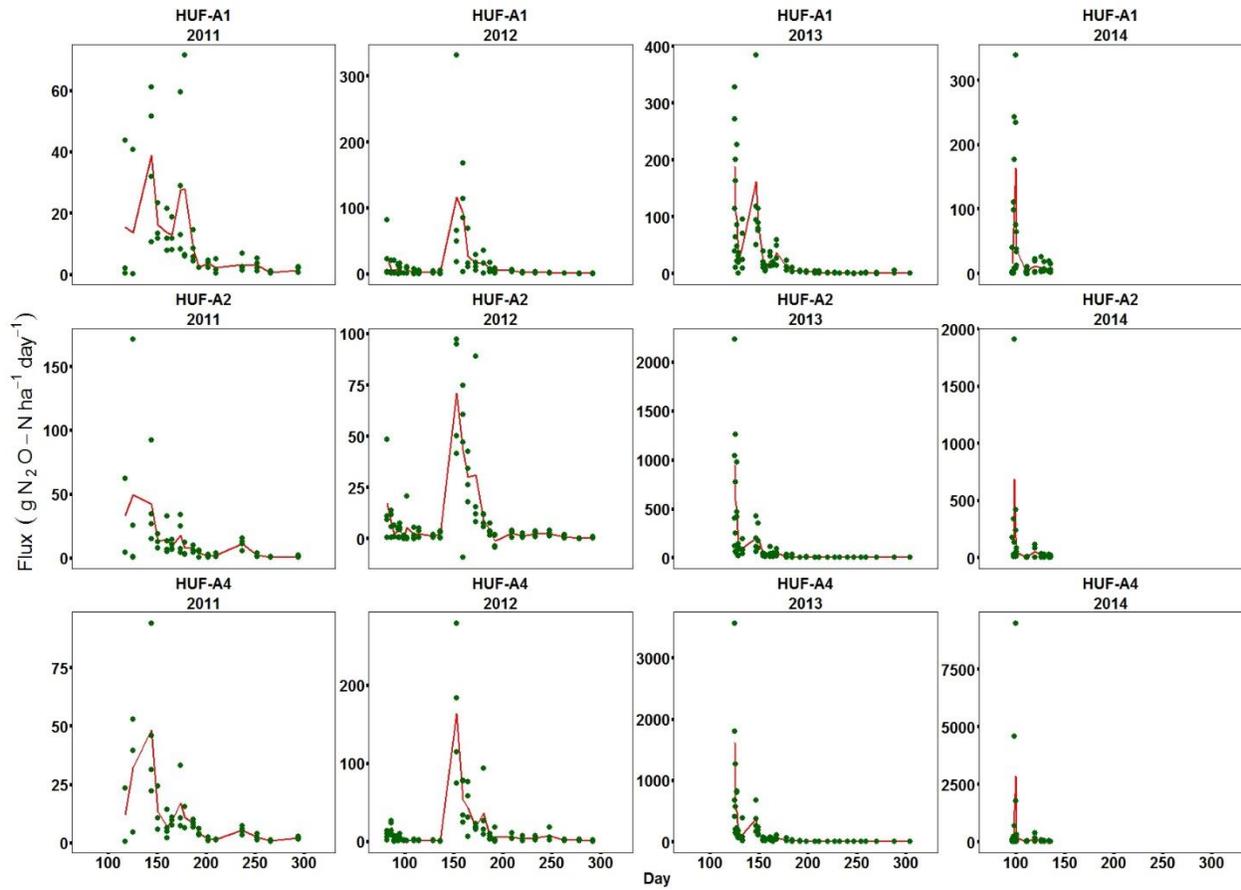
### Daily N<sub>2</sub>O emissions



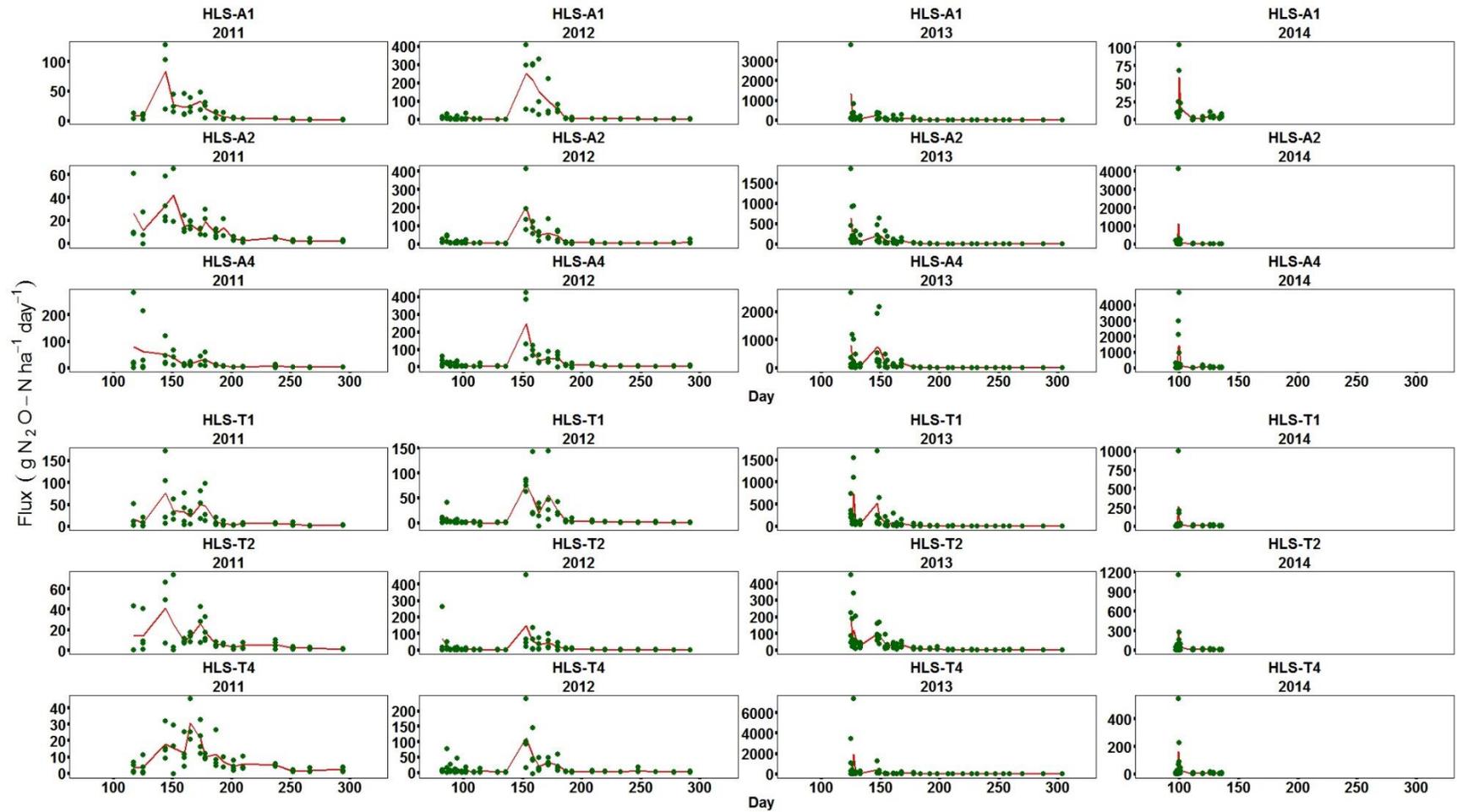
Appendix B1. Daily fluxes for the LSM and SCM trials for the no N-fertilizer control treatment for all years monitored.



Appendix B2. Daily fluxes for the SCM trial all SCM N-fertilizer treatments for all years monitored.



**Appendix B3. Daily fluxes for the LSM trial all Urea N-fertilizer treatments for all years monitored.**



Appendix B4. Daily fluxes for the LSM trial all LSM N-fertilizer treatments for all years monitored.

## APPENDIX C

### Kendall-tau tables for N<sub>2</sub>O losses and abiotic factors

**Table C1. 2012 Kendall-tau correlation table of N<sub>2</sub>O values and abiotic Factors. The values listed are correlation coefficients, and the asterisks represent the probability values.**

	Variable <sup>†‡</sup>												
	GSWC	App. Rate	DEA	pH	EC	OC	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	2013 Spring NO <sub>3</sub> <sup>-</sup>	2013 Spring NH <sub>4</sub> <sup>+</sup>	Daily N <sub>2</sub> O	Cum-N <sub>2</sub> O	Cum-CO <sub>2</sub>
GSWC	—	0.29	0.43	-0.48*	0.05	0.44	0.43	0.09	0.27	0.24	0.16	0.24	0.29
App. Rate		—	0.42	-0.28	0.21	0.79**	0.74**	0.64**	0.64	-0.36	0.14	0.64**	0.79**
DEA			—	-0.10	0.14	0.32	0.29	0.34	0.32	-0.14	0.35	0.26	0.40
pH				—	0.26	-0.60**	-0.62**	0.09	-0.09	-0.26	-0.06	-0.60**	-0.26
EC					—	-0.07	-0.14	0.33	0.47*	0.20	-0.15	-0.07	0.33
OC						—	0.97**	0.33	0.47*	-0.07	0.16	0.73**	0.60**
NH <sub>4</sub> <sup>+</sup>							—	0.28	0.41	0.00	0.14	0.69**	0.55*
NO <sub>3</sub> <sup>-</sup>								—	0.60**	-0.47*	0.03	0.33	0.73**
2013 Spring NO <sub>3</sub> <sup>-</sup>									—	-0.07	-0.01	0.20	0.60**
2013 Spring NH <sub>4</sub> <sup>+</sup>										—	-0.12	-0.33	-0.20
Daily N <sub>2</sub> O											—	0.10	0.01
Cum-N <sub>2</sub> O												—	0.60**
Cum-CO <sub>2</sub>													—

<sup>†</sup> Schoenau, 2013 personal communications

<sup>‡</sup> Significant at the  $p \leq 0.05$  (\*) or  $p \leq 0.01$  (\*\*) level of probability

Note: GSWC = Gravimetric water content; App. Rate = Application rate; EC = Electrical conductivity; OC = Organic Carbon; Cum-N<sub>2</sub>O = Cumulative N<sub>2</sub>O; Cum-CO<sub>2</sub> = Cumulative CO<sub>2</sub>

**Table C2. 2013 Kendall-tau correlation table of N<sub>2</sub>O values and abiotic Factors. The values listed are correlation coefficients, and the asterisks represent the probability values.**

	Variable <sup>†‡</sup>												
	GSWC	App. Rate	DEA	pH	EC	OC	2013 Spring NO <sub>3</sub> <sup>-</sup>	2013 Fall NO <sub>3</sub> <sup>-</sup>	2013 Fall NH <sub>4</sub> <sup>+</sup>	2014 Spring NO <sub>3</sub> <sup>+</sup>	Daily N <sub>2</sub> O	Cum-N <sub>2</sub> O	Cum-CO <sub>2</sub>
GSWC	—	1.00	0.57**	-0.20	0.23	0.23	0.15	0.16	0.10	0.30	0.58**	0.16	0.02
App. Rate		—	0.49**	-0.23	0.59**	0.32	0.64**	0.79**	-0.50*	0.36	0.26	0.64**	0.36
DEA			—	-0.04	0.35	0.16	0.37	0.50*	-0.26	0.25	0.55*	0.41	0.25
pH				—	-0.15	-0.88**	0.07	-0.07	-0.36	-0.21	-0.01	0.07	0.50*
EC					—	0.15	0.41	0.55*	0.00	0.55*	0.27	0.69**	0.28
OC						—	0.15	0.30	0.30	0.45	0.08	0.15	-0.30
2013 Spring NO <sub>3</sub> <sup>-</sup>							—	0.60**	-0.20	0.33	0.20	0.46*	0.60**
2013 Fall NO <sub>3</sub> <sup>-</sup>								—	-0.33	0.47*	0.33	0.86**	0.47*
2013 Fall NH <sub>4</sub> <sup>+</sup>									—	0.20	-0.06	-0.20	-0.33
2014 Spring NO <sub>3</sub> <sup>+</sup>										—	0.16	0.60**	0.20
Daily N <sub>2</sub> O											—	0.34	0.19
Cum-N <sub>2</sub> O												—	0.60**
Cum-CO <sub>2</sub>													—

<sup>†</sup> Schoenau, 2013 personal communications

<sup>‡</sup> Significant at the  $p \leq 0.05$  (\*) or  $p \leq 0.01$  (\*\*) level of probability

Note: GSWC = Gravimetric water content; App. Rate = Application rate; EC = Electrical conductivity; OC = Organic Carbon; Cum-N<sub>2</sub>O = Cumulative N<sub>2</sub>O; Cum-CO<sub>2</sub> = Cumulative CO<sub>2</sub>

## APPENDIX D

**Table D1. Yield data for the 2012 and 2013 cropping seasons at the Dixon long-term research site. Available N data were obtained in spring and fall 2013, and spring 2014. All values are the means for the respective historical N-treatment.**

I.D.	2012 <sup>†‡</sup>		2013 <sup>†‡</sup>				2014 <sup>†</sup>			
	Yield		S13 NO <sub>3</sub> -N	S13 NH <sub>4</sub> -N	F13 NO <sub>3</sub> -N	F13 NH <sub>4</sub> -N	Yield		S14 NO <sub>3</sub> -N	S14 NH <sub>4</sub> -N
	(bu/ac)	(kg/ha)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(bu/ac)	(kg/ha)	(µg/g)	(µg/g)
HLS-A1	22.66	1270	25.76	2.80	6.18	2.59	88.68	5964	13.23	1.78
HLS-A2	28.95	1622	30.42	2.65	8.65	2.45	86.47	5815	16.60	1.63
HLS-A4	26.04	1459	29.18	2.17	9.91	2.00	81.20	5461	12.40	1.48
HLS-C0	19.78	1108	21.19	3.27	5.24	2.98	86.62	5825	12.94	2.01
HLS-T1	28.91	1620	16.57	2.83	5.38	2.65	78.27	5263	9.80	1.85
HLS-T2	26.35	1477	21.61	2.49	5.54	2.36	82.42	5543	9.74	1.54
HLS-T4	24.97	1399	20.82	2.99	5.41	2.78	93.31	6275	10.02	1.86
HSC-A1	30.46	1707	20.81	3.18	9.38	3.05	81.38	5473	23.95	2.10
HSC-A2	28.01	1570	14.91	2.58	10.06	2.49	79.93	5375	16.53	1.80
HSC-A4	28.15	1578	42.34	3.21	9.08	2.89	83.96	5647	23.89	2.11
HSC-C0	28.88	1618	13.51	2.73	7.22	2.61	87.85	5908	18.12	1.65
HSC-T1	25.05	1404	20.33	3.08	7.30	2.92	68.56	4611	21.59	2.03
HSC-T2	29.61	1659	19.39	3.10	6.39	2.94	84.39	5675	21.68	2.55
HSC-T4	32.93	1845	17.90	3.71	7.24	3.49	67.42	4534	22.85	2.06
HUBA-1	30.92	1732	26.79	3.12	7.44	2.89	80.67	5425	15.34	1.85
HUF-A1	30.54	1712	18.82	2.81	4.92	2.67	75.73	5093	8.60	1.84
HUF-A2	29.37	1646	24.40	2.88	4.91	2.71	81.24	5463	8.86	2.08
HUF-A4	31.38	1759	27.66	2.42	7.49	2.26	87.24	5867	11.26	1.82

<sup>†</sup> In 2012, the crop was canola; in 2013, it was hard red spring wheat.

<sup>‡</sup> Soils for available N (NO<sub>3</sub><sup>-</sup> & NH<sub>4</sub><sup>+</sup>) analyses were collected in spring (S) and/or fall (F).