

**SOIL MOISTURE AND TEMPERATURE REGULATION OF THAW-DRIVEN N₂O
EMISSIONS, KINETICS, AND THERMODYNAMICS WITH UREA AND SUPERU IN A
GLEYPED HUMIC VERTISOL**

BY

Kingsford Amoah

A Thesis Submitted to the Faculty of Graduate and Postdoctoral Studies of
The University of Manitoba
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Soil Science,

University of Manitoba,

Winnipeg, Manitoba

Copyright © 2026 by Kingsford Amoah

ABSTRACT

Kingsford Amoah, MSc., The University of Manitoba, August 2026. Soil moisture and temperature regulation of thaw-driven N₂O emissions, kinetics, and thermodynamics with urea and SuperU in a Gleyed Humic Vertisol

Advisor: Dr. Afua Mante

In the Canadian Prairies, freeze-thaw cycles can account for a large share of annual agricultural N₂O emissions. Among the factors controlling these thaw-driven emissions, soil moisture is a major driver, while thawing temperature and fertilizer source may further modify these emissions; their interactions and the processes underlying thaw-induced N₂O productions remain poorly understood. This study evaluated the effects of freezing (-20 °C), thawing temperature (4, 8, 12, and 16°C), soil moisture (32, 50, 75, and 100% water-filled pore space [WFPS]), and fertilizer source (control, urea, and SuperU) on N₂O emissions from a Gleyed Humic Vertisol from southern Manitoba, Canada. Laboratory incubation experiments were conducted following a simulated freeze-thaw event using a freeze-thaw chamber and incubators over a 60-hour thawing period. Results showed that soil moisture was the main factor controlling thaw-induced N₂O emissions. Increasing soil moisture increased mean N₂O flux from 0.14 to 1.19 µg N₂O–N kg⁻¹ h⁻¹, about 8.5 times higher, while cumulative emissions increased from 6.26 to 82.83 µg N₂O–N kg⁻¹, about 13 times higher, across 32% to 100% WFPS. The increase under wetter thaw conditions is associated with higher denitrification rates and greater N₂O production. Soil moisture also affected residual nitrate, whereas residual ammonium was influenced by thawing temperature and fertilizer type. There was no significant difference in N₂O emissions between SuperU and conventional urea. Soil moisture also controlled the kinetics and thermodynamics of thaw-induced N₂O emission. As WFPS increased, the first-order rate constant (k) declined from 0.03 to 0.02h⁻¹, while half-life (t_{1/2}) increased from 24.65 to 34.73 h, indicating slower but more prolonged N₂O accumulation.

Activation energy declined from 38.1 kJ mol⁻¹ to near zero, while Gibbs free energy increased, enthalpy shifted from positive to negative, and entropy became more negative with increasing moisture. Thawing temperature mainly affected the kinetics of N₂O emission when combined with soil moisture, and fertilizer source had little impact on these kinetic or thermodynamic parameters. These results suggest that fall-applied nitrogen fertilizer is prone to thaw-induced N₂O emissions in poorly drained clay soils and that avoiding fall N application under these conditions may reduce emissions on the Canadian Prairies.

ACKNOWLEDGEMENTS

"But those who hope in the Lord will renew their strength. They will soar on wings like eagles; they will run and not grow weary, they will walk and not be faint," Isaiah 40:31 (NIV).

Completing my Master's program showed me how faithful God is through the kindness, encouragement, and support of so many people. I give all glory, honor, and praise to the Almighty God. His grace carried me through every trial, and His strength enabled me to finish this work. I am deeply grateful to my supervisor, Dr. Afua Mante, for her mentorship and thoughtful feedback during my program. I appreciate the guidance and support from my committee members, Dr. Francis Zvomuya and Dr. Hailong He.

I would like to thank Fernando Esposito and Anthony Buckley for their technical assistance. I appreciate the support from the Faculty of Graduate Studies through the International Graduate Student Scholarship, Travel Award, and the Research Completion Scholarship. I am thankful for the funding from the RBC Scholars Award and Dr. Afua Mante's University of Manitoba Start-up grant, which helped support my program.

I am grateful to Susanne Beaudry for being part of my journey. Her kindness toward me specifically and other members of the department deserves special recognition. I extend my heartfelt gratitude to Mr. Roger Kpanpari, Ms. Vivian Gomelesio, Mr. Emmanuel Agyapong, Dr. Clemence Muitire, Taurai Matengu, Taku Nawu, Sarafina Emmanuel each of whom contributed in meaningful ways to the completion of my program.

I would also like to express my sincere gratitude to the following individuals: Ms. Mildred E. Hanson, Rev. Bright Nsiah, Lawyer Brigitte Nsiah, Pastor Temitope Akinsanya, Dr. Charles Bendu, Dr. Ruth T. Boachie, Dr. Stella Owusu-Nketia Mr. Francis Darko, and all members of

Encounter Church AG, the late Mr. Bernard Donkoh and the Hagan Donkoh family, Rev. Samuel Kufour, Mr. Ebenezer Nunoo, Ms. Dora Amoah and the Amoah family.

FOREWORD

This thesis follows the Department of Soil Science, University of Manitoba, manuscript format and is organized into four chapters. Chapter 1 provides a general introduction and literature review, addressing the significance of agricultural nitrous oxide (N₂O) emissions, freeze–thaw processes, soil nitrogen transformations, the interactive effects of thawing temperature and soil moisture, mitigation strategies, the study's contribution and significance, stakeholder benefits, research needs, research objectives, and the overall thesis structure.

Chapters 2 and 3 are presented as independent research manuscripts. Chapter 2 investigates the effects of thawing temperature, soil moisture, and fertilizer source on N₂O flux, cumulative N₂O emissions, and residual soil nitrate and ammonium in a Gleyed Humic Vertisol following a simulated freeze–thaw event. Chapter 3 analyzes the kinetic and thermodynamic responses of cumulative N₂O emissions to thawing temperature, soil moisture, and fertilizer source using first-order kinetic, activation energy, and thermodynamic approaches.

Chapter 4 synthesizes the research findings, discusses the implications for nitrogen management and greenhouse gas mitigation in Prairie clay soils, and offers recommendations for future research and management. Chapters 2 and 3 are being prepared for submission to peer-reviewed journals.

CONTRIBUTIONS OF AUTHORS

The contributions of the authors to this thesis are detailed below:

- Kingsford Amoah: conceptualization, investigation, formal analysis, data curation, and writing the original manuscript draft
- Afua Mante: conceptualization, supervision, funding acquisition, formal analysis, review, and editing of manuscript drafts
- Francis Zvomuya: Advisory committee, formal analysis, review, and editing of manuscript draft
- Hailong He: Advisory committee, review and editing of manuscript draft

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS.....	iv
FOREWORD	v
CONTRIBUTIONS OF AUTHORS.....	vi
LIST OF TABLES	xi
LIST OF FIGURES	xii
1 General Introduction	1
1.1 Agricultural N ₂ O Emissions.....	1
1.2 N ₂ O Emissions from Freeze–Thaw Conditions Need Attention.....	2
1.3 Soil Nitrogen Processes Controlling N ₂ O Emissions	9
1.4 Synergistic effects of thawing temperature and soil moisture	11
1.5 Strategies Used to Reduce Agricultural N ₂ O Emissions.....	12
1.6 Contribution and significance of the study	30
1.7 Advancing research on thaw-induced N ₂ O emissions in the Canadian Prairies.....	32
1.7.1 Expanding FTC studies on clay soils.....	32
1.7.2 More rigorous lab studies on N inhibitors under freeze-thaw conditions.....	33
1.7.3 Testing realistic soil temperature on lab FTC studies	34
1.7.4 Accounting for off-season and early-thaw N ₂ O emissions	34

1.7.5	Interpreting thaw emissions using kinetic and thermodynamic approaches.....	35
1.8	Advancing our understanding of N ₂ O dynamics: Stakeholder benefits.....	35
1.9	Research Objectives.....	37
1.10	Thesis Outline	38
1.11	References.....	39
2	EFFECTS OF THAWING TEMPERATURE, SOIL MOISTURE, AND FERTILIZER SOURCE ON NITROUS OXIDE EMISSIONS FROM A GLEYED HUMIC VERTISOL FOLLOWING A SIMULATED FREEZE–THAW EVENT	55
2.1	Abstract.....	55
2.2	Introduction.....	56
2.3	Materials and Methods.....	60
2.3.1	Study site description.....	60
2.3.2	Soil Sample Collection and Preparation	61
2.3.3	Baseline soil properties	62
2.3.4	Experimental design.....	65
2.3.5	Nitrous oxide emissions.....	68
2.3.6	Statistical Analysis	69
2.4	Results and Discussion	69
2.4.1	N ₂ O flux	69
2.4.2	Cumulative N ₂ O emissions	74

2.4.3	Residual nitrate	77
2.4.4	Residual ammonium	80
2.5	Conclusion	83
2.6	References	84
3	KINETIC AND THERMODYNAMIC RESPONSES OF THAW-INDUCED NITROUS OXIDE EMISSIONS TO THAWING TEMPERATURE, SOIL MOISTURE, AND FERTILIZER SOURCE IN A GLEYED HUMIC VERTISOL.....	94
3.1	Abstract	94
3.2	Introduction.....	95
3.3	Materials and Methods.....	99
3.3.1	Experimental procedures	99
3.3.2	Kinetic modeling.....	99
3.3.3	Activation energy analysis	101
3.3.4	Thermodynamic characterization of cumulative N ₂ O	101
3.3.5	Statistical analysis	102
3.4	Results and Discussion	103
3.4.1	N ₂ O Emission Kinetics and Activation Energy	103
3.4.2	Thermodynamics of N ₂ O Emissions: Gibbs Free Energy, Enthalpy, and Entropy	
	110	
3.5	Conclusion	112

3.6	References	113
4	OVERALL SYNTHESIS	120
4.1	Research Findings	120
4.2	Implication of the study	123
4.3	Study limitations	125
4.4	Recommendations	126
4.5	References	128

LIST OF TABLES

Table 1.1 Summary of freeze-thaw studies on nitrous oxide emissions on the Canadian Prairies .	4
Table 1.2 Summary of Canadian Prairie studies evaluating enhanced efficiency fertilizers and nitrogen inhibitors for reducing nitrogen losses and improving crop performance.....	18
Table 2.1 Baseline properties for soil samples.....	65
Table 2.2 Effect of soil moisture, thawing temperature, and fertilizer source on hourly N ₂ O flux and cumulative emissions	73
Table 2.3 Effect of thawing temperature, moisture, and fertilizer source on residual nitrate.....	78
Table 3.1 Effect of moisture, temperature, and fertilizer source on activation energy (E_a), rate constant (k), and half-life ($t_{1/2}$) of N ₂ O emissions in clay soil following a freeze–thaw event	106
Table 3.2 Effect of moisture and fertilizer source on Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) of N ₂ O emissions in clay soil following a freeze–thaw event.....	111

LIST OF FIGURES

Figure 2.1 Location of the soil sampling site at Glenlea Research Station, Winnipeg, Manitoba, Canada.....	60
Figure 2.2 Treatment layout in Block 1 showing treatment randomization within the incubation chambers	66
Figure 2.3 Effect of thawing temperature, soil moisture, and fertilizer source on N ₂ O flux. Error bars represent the standard errors of the mean.....	74
Figure 2.4 Effect of soil moisture and fertilizer source on residual Nitrate after thawing. Error bars represent the standard errors of the mean.	79
Figure 2.5 Effect of soil moisture and fertilizer source on residual ammonium after thaw. Error bars represent the standard errors of the mean.....	81
Figure 2.6 Effect of thawing temperature on residual ammonium. Error bars represent the standard errors of the mean.	82
Figure 3.1. Interaction between thawing temperature and moisture on the N ₂ O emission rate constant (k) in clay soil following a freeze-thaw event. Error bars are standard errors of the mean. Bars with different letters are significantly different at a probability value of <0.05 (Tukey–Kramer).	107
Figure. 3.2. Interaction between thawing temperature and moisture on the N ₂ O emission half-life (t _{1/2}) in clay soil following a freeze–thaw event. Error bars are standard errors of the mean. Bars with different letters are significantly different at a probability value of <0.05 (Tukey–Kramer).	108

Figure 3.3. First-order kinetic curves fitted to cumulative N₂O flux buildup across the sixteen thawing temperature × soil moisture combinations (0-60 h post-thaw). Flux values are expressed as a fraction of the final cumulative total, and each curve reflects the average rate constant for that combination..... 109

1 General Introduction

1.1 Agricultural N₂O Emissions

Global greenhouse gas (GHG) emissions are increasing, primarily due to fossil fuel combustion, land-use change, and unsustainable consumption and production patterns (IPCC, 2023). Among these gases, nitrous oxide (N₂O) is particularly significant, with a global warming potential approximately 265 times greater than that of carbon dioxide (CO₂) (IPCC, 2014). Its atmospheric concentration continues to rise, increasing by about 0.26% annually and contributing to a 17% increase in radiative forcing between 1998 and 2005 (Forster et al., 2007).

Agriculture is a significant source of N₂O emissions, primarily resulting from the application of synthetic nitrogen fertilizers and soil nitrogen transformations. In Canada, N₂O emissions were estimated at approximately 28 million tonnes of CO₂-equivalent in 2022, accounting for about 4% of total national GHG emissions (Environment and Climate Change Canada, 2024). The agricultural sector remains the predominant source of anthropogenic N₂O emissions in Canada, accounting for nearly 72% of national emissions from fertilizer-derived nitrogen inputs and soil nitrogen processes (Environment Canada, 2014)

This issue is particularly significant in the Northern Great Plains, comprising southern Alberta, Saskatchewan, and Manitoba, where intensive crop production depends on nitrogen fertilizers (Statistics Canada, 2014). In these cold agricultural regions, freeze–thaw cycles (FTCs) strongly affect soil nitrogen transformations and N₂O emissions (Risk et al., 2013; Wagner-Riddle et al., 2024). Thawing events alter soil temperature, moisture, oxygen availability, microbial activity, and mineral nitrogen levels, creating conditions that promote N₂O production (Mørkved et al., 2006;

Risk et al., 2013). Consequently, short-term thawing events can substantially increase seasonal N₂O losses, especially in poorly drained or heavy clay soils.

1.2 N₂O Emissions from Freeze–Thaw Conditions Need Attention

Fall nitrogen fertilizer application is widely practiced in the Canadian Prairies due to its economic and logistical benefits. Producers often apply nitrogen in fall to capitalize on lower fertilizer prices, alleviate spring field operation pressures, and distribute labor more efficiently before the short, intensive growing season (Amiro et al., 2017; Tiessen et al., 2005). The method is designed to retain fertilizer nitrogen in the soil, thereby improving uptake by spring-seeded Prairie crops such as wheat, canola, and barley during the following growing season (Manitoba Agriculture, 2007; Tiessen et al., 2006). However, fall fertilizer application may result in mineral nitrogen remaining in the soil during winter and early spring, periods when crops are not actively absorbing nutrients and soils are subject to freeze–thaw cycles.

A primary environmental concern is that fall-applied nitrogen is susceptible to loss before it is taken up by the crop (Tiessen et al., 2005, 2006). During winter and early spring, freeze–thaw cycles modify soil structure, microbial activity, oxygen availability, and nitrogen transformations. Freezing restricts gas diffusion, whereas thawing releases trapped gases and stimulates microbial activity as liquid water, available carbon, and mineral nitrogen become accessible (Congreves et al., 2018; Risk et al., 2013; Teepe et al., 2001). These processes create conditions that promote nitrification and denitrification, both of which contribute to N₂O production.

Spring-thaw periods are significant because N₂O emissions often occur as brief but intense pulses (Risk et al., 2013; Wagner-Riddle et al., 2017, 2024). These pulses can constitute a substantial proportion of annual emissions despite their short duration (Pelster et al., 2022; Risk et al., 2013).

Research on seasonally frozen croplands (Table 1.1) demonstrates that freeze–thaw events are major contributors to annual N₂O losses, and recent studies in the Prairies underscore the need for year-round N₂O measurements in this region (Wagner-Riddle et al., 2017, 2024). Emissions during the non-growing season have also been shown to account for a considerable share of annual N₂O emissions (35.5%) on the Canadian prairie croplands (Pelster et al., 2022), emphasizing the importance of monitoring emissions outside the main growing season (Ferland et al., 2024).

This issue is particularly relevant in Prairie soils, where thawing conditions interact with soil moisture, texture, drainage, and fertilizer-derived nitrogen (Tenuta et al., 2016; Tiessen et al., 2011b; Wagner-Riddle et al., 2024). Poorly drained or clay soils can develop anaerobic conditions when water-filled pore space is increased, increasing the likelihood of denitrification. Thawing also enhances the availability of substrates required for microbial N₂O production (Risk et al., 2013). In effect, the magnitude of spring-thaw N₂O emissions is determined not only by the presence of nitrogen fertilizer but also by the interaction among thawing temperature, soil moisture, and soil physical properties (Congreves et al., 2018; Risk et al., 2013; Wagner-Riddle et al., 2024).

While fall nitrogen application offers certain advantages for producers, it may decrease nitrogen use efficiency if nitrogen is lost before crop demand rises in spring. Losses can occur via nitrate leaching, runoff, or gaseous emissions, including N₂O. This presents both agronomic and environmental challenges, as producers lose nitrogen that could support crop growth, and agriculture contributes additional greenhouse gas emissions. Therefore, understanding N₂O emissions from fall-applied nitrogen under freeze–thaw conditions is essential for optimizing fertilizer management in cold agricultural regions.

Table 1.1 Summary of freeze-thaw studies on nitrous oxide emissions on the Canadian Prairies

Authors	Objective of Study	Findings	Study site	Study type	Soil type	Location
Lin & Hernandez-Ramirez, (2021)	Assess how fall urea, moisture, and manure history affect soil-derived N ₂ O after freeze–thaw.	Urea caused 24% soil-derived N ₂ O; manure soils and high moisture greatly increased emissions; denitrification dominated (83–97%)	Edmonton Research Farm	Mesocosm experiment	Black Chernozem, clay	Alberta
Lin et al, (2021)	Test ecosys model for fall vs. spring slurry N ₂ O emissions and evaluate if de-watering slurry reduces emissions.	Model captured soil temperature, moisture, and N ₂ O trends; fall slurry caused higher spring-thaw N ₂ O; de-watered slurry reduced emissions by 16–36% (fall) and 23–29% (spring).	Lacombe	Modelling study (ecosys) validated with field data (2014–2017)	Orthic Black Chernozem	Alberta
Hao et al. (2001)	Evaluate how fertilizer timing (fall vs. spring), straw management (retained vs. removed), and tillage (fall plow, spring plow, direct	Fall-applied N resulted in higher N ₂ O emissions than spring-applied N. Straw removal increased N ₂ O when fertilizer was applied, but reduced emissions when	Long-term irrigated rotation at Lethbridge	Field	Dark Brown Chernozemic (Typic Haploboroll)	Alberta

	seeding) affect N ₂ O emissions under irrigation	unfertilized. Fall plowing produced the highest N ₂ O emissions; spring plowing produced the lowest.				
Chang et al. (1998)	Quantify annual N ₂ O emissions after 21 years of cattle manure application and assess environmental controls.	Repeated manure applications increased N ₂ O emissions up to 80-fold by enriching soil nitrate and organic carbon, especially during winter and spring freeze–thaw events	Agriculture & Agri-Food Canada Research Centre, Lethbridge	Field	Dark Brown Chernozemic (Typic Haploboroll)	Alberta
Grant et al. (2020)	Model and validate how nitrification inhibitors reduce N ₂ O emissions from fall- and spring-applied slurry by slowing NH ₄ ⁺ oxidation	NI reduced N ₂ O by 35–53% (spring) and 56–58% (fall); were more effective in wetter conditions; persisted longer in cold soils; reduced NO ₃ ⁻ buildup but slightly increased NH ₃ volatilization	South Campus Farm, Edmonton	Modelling (ecosys) calibrated with field measurements	Black Chernozem	Alberta
Yadav & Wang, (2021)	Improve the UK-DNDC model to more accurately simulate soil temperature, snowmelt, freeze–thaw	Modified UK-DNDC accurately predicted soil temperature, N ₂ O emissions, and snow depth, particularly during freeze–thaw and	Outlook, Saskatoon, Prince Albert, Rich	Modelling	N/A	Alberta and Saskatchewan

	effects, and N ₂ O emissions under Canadian cold-climate conditions and validate it using multi-site field data.	snowmelt but underestimated some early-spring peaks.	Lake, and Breton Plots			
Tenuta & Sparling (2011)	Test how nitrate, soil depth, bulk density, and freeze–thaw cycles affect N ₂ O emissions and N ₂ O: N ₂ ratios, and confirm denitrification as the thaw-emission source	Nitrate increased thaw N ₂ O mainly in the 0–10 cm layer, while deeper, denser soils produced less because of limited carbon and denitrification.	Manitoba Zero-Till Research Farm, near Brandon	Laboratory	Newdale clay loam (Orthic Black Chernozem)	Manitoba
Cui & Wang (2019)	Improve DNDC to better simulate rain–snow partitioning, snow cover, soil temperature, and freeze–thaw dynamics for accurate N ₂ O and	Modified DNDC improved snow and soil temperature simulations and increased N ₂ O prediction accuracy, particularly during the cold season.	Glenlea Research Station (TGAS-Man)	Modelling	clay soil	Manitoba

	CO ₂ predictions in cold regions					
Tremorin et al. (2012)	Determine how fertilizing grassland with hog slurry affects N ₂ O emissions from cattle feces and urine, and evaluate how excreta chemistry (N, NH ₄ ⁺ , NO ₃ ⁻ , moisture) controls emission magnitude	Slurry increased N ₂ O from both feces and urine; urine emitted far more; emissions rose with soil mineral N	La Broquerie Pasture and Hog Manure Research Site	Field	Gleyed Dark Grey Luvisol & Dark Gray Chernozem	Manitoba
Tenuta et al. (2016)	Evaluate whether late-fall anhydrous ammonia application before freeze-up reduces N ₂ O emissions compared with spring pre-plant application.	Late-fall application caused no fall/winter emissions, increased thaw emissions, and reduced early-season emissions, resulting in 35% lower annual N ₂ O than spring application	TGAS-Man site, Glenlea Research Station	Field	clay Vertisol.	Manitoba
Tenuta et al. (2010)	Evaluate how hog slurry timing (split fall–spring vs single	Slurry increased CH ₄ and N ₂ O, but split application reduced N ₂ O; soil moisture and nitrate	La Broquerie Pasture	Field	Gleyed Dark Gray Luvisol	Manitoba

	spring) and soil moisture gradients affect CH ₄ and N ₂ O emissions from coarse-textured grassland soil	determined which gas dominated.			/ Dark Gray Chernozem	
Tiessen et al. (2005)	Assess how application timing, landscape position, and NBPT+DCD affect fall-banded urea efficiency in Manitoba spring wheat.	Spring and late-fall applications improved yield and N recovery in poorly drained positions, while timing had little effect in higher positions, and inhibitors provided minimal benefit.	Kane, Rosser, and Brandon	Field	Red River/Osborne heavy clay; Newdale clay loam	Manitoba
Wagner-Riddle et al. (2024)	Measure Prairie overwinter and spring-thaw N ₂ O emissions and identify key controls related to snow, freezing, and soil nitrogen.	Spring thaw produced the largest N ₂ O pulses, accounting for 40–57% of annual emissions and increasing with snow depth, freeze–thaw severity, and soil nitrate.	University of Saskatchewan North Management area	Field	Dark Brown Chernozem	Saskatchewan

1.3 Soil Nitrogen Processes Controlling N₂O Emissions

The soil nitrogen cycle is governed by interconnected biological and chemical processes, such as nitrogen fixation, mineralization, immobilization, nitrification, and denitrification (Hayatsu et al., 2008; Robertson & Groffman, 2015). These processes regulate the transformation, availability, mobility, and loss of nitrogen in agricultural soils. Following nitrogen fertilizer application, nitrogen can be absorbed by plants, retained in soil organic matter or microbial biomass, converted into other mineral forms, leached as nitrate, volatilized as ammonia, or lost as gaseous nitrogen compounds, including nitric oxide (NO), N₂O, and dinitrogen gas (N₂) (Butterbach-Bahl et al., 2013; Davidson et al., 2000).

Mineralization transforms organic nitrogen into ammonium (NH₄⁺), thereby increasing the pool of mineral nitrogen available for plant uptake and microbial transformation (Schimel & Bennett, 2004). On the other hand, immobilization occurs when soil microorganisms assimilate mineral nitrogen into microbial biomass, which temporarily reduces nitrogen availability to plants and other soil processes (Robertson, & Groffman, 2015; Kuzyakov & Xu, 2013). The balance between mineralization and immobilization is critical, as it determines the availability of NH₄⁺ and nitrate (NO₃⁻), the primary substrates for nitrification and denitrification (Butterbach-Bahl et al., 2013; Schimel & Bennett, 2004).

Nitrification and denitrification represent the primary microbial pathways responsible for N₂O production in soils (Butterbach-Bahl et al., 2013; Davidson et al., 2000; Firestone & Davidson, 1989). Nitrification is an aerobic process in which ammonium is oxidized to nitrate via nitrite as an intermediate (Butterbach-Bahl et al., 2013; Coskun et al., 2017). During nitrification, N₂O can

be produced as a by-product, particularly when oxygen availability is limited or when ammonium and nitrite concentrations are elevated (Kool et al., 2011; Hu et al., 2015; Wrage et al., 2001). Denitrification occurs predominantly under anaerobic conditions, where nitrate is sequentially reduced to nitrite, nitric oxide, nitrous oxide, and ultimately dinitrogen gas (Firestone & Davidson, 1989; Baggs, 2011; Zumft, 1997). Nitrous oxide is emitted when the reduction of N_2O to N_2 is incomplete, which may result from limited activity of N_2O reductase or from denitrification under fluctuating oxygen conditions (Butterbach-Bahl et al., 2013; Giles et al., 2012).

The relative contribution of nitrification and denitrification to N_2O emissions depends strongly on soil moisture, oxygen availability, temperature, carbon availability, soil pH, texture, and mineral nitrogen supply (Butterbach-Bahl et al., 2013; Davidson et al., 2000; Hu et al., 2015). Under relatively dry, well-aerated conditions, nitrification can be an important source of N_2O production because oxygen diffusion is sufficient for ammonium oxidation (Bateman & Baggs, 2005; Kool et al., 2011). Under wetter conditions, oxygen diffusion is restricted, and anaerobic conditions develop, favoring denitrification (Bateman & Baggs, 2005; Butterbach-Bahl et al., 2013; Davidson et al., 2000). Therefore, water-filled pore space is one of the most important controls of N_2O production because it regulates both microbial activity and oxygen availability (Bateman & Baggs, 2005; Davidson et al., 2000; Linn & Doran, 1984).

A comprehensive understanding of these nitrogen transformation processes is essential for this study, as N_2O emissions following thaw events are influenced by multiple factors beyond fertilizer application. These emissions depend on the effects of thawing temperature, soil moisture, and fertilizer source on ammonium availability, nitrate formation, oxygen status, and microbial nitrogen transformations (Butterbach-Bahl et al., 2013; Mørkved et al., 2006; Risk et al., 2013). Accordingly, quantifying N_2O flux, cumulative N_2O emissions, residual $\text{NH}_4^+\text{-N}$, and residual

NO_3^- -N enables linking observed emissions to the underlying soil nitrogen processes that govern N_2O production and loss under freeze–thaw conditions.

1.4 Synergistic effects of thawing temperature and soil moisture

Thawing temperature and soil moisture can interact strongly during freeze–thaw events. Temperature influences the rate at which microbial activity resumes after freezing, while soil moisture controls oxygen diffusion and gas movement through the soil (Linn & Doran, 1984; Teepe et al., 2001; Bateman & Baggs, 2005; Wertz et al., 2013). Therefore, warmer thawing conditions may increase N_2O emissions most strongly when soil moisture is high enough to restrict oxygen availability. Multiple studies provide evidence for this interaction. Sennett et al. (2024) investigated a single freeze–thaw event across two initial WFPS levels and two thawing temperatures, with simulated snowmelt infiltration. The results indicated that N_2O emissions were highest when higher initial WFPS coincided with the warmer thaw temperature. Denitrification rates also increased substantially under the combined condition of higher WFPS and 8.0 °C thaw compared to lower WFPS and 1.5 °C thaw, demonstrating that soil moisture can amplify the effect of thawing temperature.

Evidence from prairie soils reveals a comparable trend. Kpankpari (2025) evaluated 32, 50, and 100% WFPS at thawing temperatures of 4, 8, 12, and 16 °C in a Black Chernozemic sandy loam. The findings showed that soil moisture significantly influenced N_2O emissions across all thawing temperatures, with the highest emissions observed at 100% WFPS and 16°C. These results suggest that wetter soil conditions intensify the effect of warmer thawing on N_2O release.

Additional freeze–thaw studies demonstrate that moisture and temperature influence emission dynamics. Teepe et al. (2004) reported that N_2O emissions during thaw varied with WFPS, with

peak emissions occurring at intermediate to high moisture levels rather than at the driest or wettest extremes. Wertz et al. (2013) also found that temperatures near the freezing point affected both N₂O emissions and nitrogen-transforming microbial communities, confirming that even minor changes in thaw temperature can alter emission responses.

These findings indicate that thaw-induced N₂O emissions are highest when both warmer thawing and elevated soil moisture are present. This interaction is especially important for clay-rich soils because high water retention and slow drainage can maintain wet conditions during thaw, increasing the likelihood that warmer temperatures will lead to greater N₂O release.

1.5 Strategies Used to Reduce Agricultural N₂O Emissions

Efforts to minimize nitrogen (N) losses and improve nitrogen use efficiency (NUE) in agricultural systems have led to the adoption of several nutrient management strategies. One of the most widely recognized is the 4R Nutrient Stewardship framework, which emphasizes applying fertilizer using the right source, at the right rate, at the right time, and in the right place (Liu et al., 2022). This science-based approach ensures that fertilizer applications align with crop nutrient demand, reducing losses through leaching, volatilization, and denitrification. The effectiveness of the 4R strategy can be further enhanced when combined with additional practices that improve fertilizer performance and environmental sustainability (The Fertilizer Institute, 2017), such as the use of inhibitor-treated fertilizers that slow nitrogen transformations and reduce gaseous and leaching losses

Nitrification and urease inhibitors are essential components of enhanced-efficiency fertilizers (EEFs), which are developed to reduce N losses and improve NUE in agricultural systems (Abalos

et al., 2014). These inhibitors function by slowing key microbial and enzymatic reactions involved in soil N transformations, specifically urea hydrolysis and ammonium oxidation, thereby extending the period during which nitrogen remains available to crops while minimizing environmental losses (Trenkel, 2010). Urease inhibitors (UIs) temporarily deactivate the urease enzyme, which catalyzes the rapid hydrolysis of urea into ammonia (NH_3) and carbon dioxide (CO_2), a reaction that can otherwise lead to substantial volatilization losses (Trenkel, 2010; Liu et al., 2019).

Nitrification inhibitors (NIs) target a different stage of the nitrogen cycle by suppressing the activity of ammonia-oxidizing bacteria and archaea, thereby slowing the conversion of NH_4^+ to NO_3^- , a form highly susceptible to leaching and denitrification (Abalos et al., 2014). By stabilizing nitrogen in the ammonium form, NIs help reduce N_2O emissions, limit nitrate losses, and improve synchrony between nitrogen availability and crop uptake. Some fertilizer formulations combine urease and nitrification inhibitors, producing dual-inhibitor products that provide protection against NH_3 volatilization and N_2O emissions (Koch Agronomic Services, 2019). A well-known example is SuperU, a urea fertilizer containing pre-incorporated N-(n-butyl) thiophosphoric triamide (NBPT; UI) and dicyandiamide (DCD; NI) within each granule. In addition to granular products, liquid double-inhibitor formulations such as Agrotain® Plus, ARM U Advanced, and Active Stabilizer Plus (ASP) offer flexibility across a range of fertilizer delivery systems.

NBPT, a urease inhibitor, and dicyandiamide (DCD), a nitrification inhibitor, regulate distinct yet sequential steps in the soil N transformation pathway. In effect, these inhibitors are often assessed both individually and in combination. NBPT functions at the initial stage of urea transformation by inhibiting urease, thereby delaying urea hydrolysis to ammonium and reducing the localized pH increase that facilitates ammonia volatilization following urea application (Cantarella et al., 2018; Abalos et al., 2014). In contrast, DCD acts later in the N cycle by suppressing ammonia-

oxidizing microorganisms and slowing the conversion of ammonium to nitrate. This action reduces nitrate leaching and N₂O emissions associated with nitrification and subsequent denitrification (Di & Cameron, 2002; Akiyama et al., 2010). NBPT is most effective when applied alone in situations where surface-applied urea faces a significant risk of ammonia volatilization, as its primary function is to delay urea hydrolysis and reduce NH₃ loss (Cantarella et al., 2018; Silva et al., 2017). In contrast, DCD is more effective in systems where nitrate leaching and N₂O emissions predominate (Abalos et al., 2014). The combined application of NBPT and DCD aims to achieve broader N stabilization by limiting losses at both the urea hydrolysis and nitrification stages (Abalos et al., 2014; Koch Agronomic Services, 2019). This strategy can extend N retention in plant-available forms and potentially enhance nitrogen use efficiency. However, the extent of these combined effects is influenced by soil properties, temperature, moisture contents, and fertilizer management, and synergistic benefits are not consistently observed across all field conditions (Akiyama et al., 2010; Cantarella et al., 2018).

Research across the Canadian Prairies consistently shows that N inhibitors are effective at reducing nitrous oxide emissions (Degenhardt et al., 2016; Gao et al., 2015; Grant et al., 2020b; Lin et al., 2017, 2023; Lin & Hernandez-Ramirez, 2021; Owens et al., 2019; Pokharel & Chang, 2021; Tenuta et al., 2023; Thilakarathna et al., 2020; Thomas et al., 2017; Wang et al., 2023; Wood et al., 2024). Multiple studies summarized in Table 1.2 show substantial N₂O mitigation when N inhibitors or EEFs were used. For example, Owens et al. 2019, reported that SuperU markedly lowered N₂O emissions without affecting yields, while split applications offered little advantage. Tenuta et al. (2023) also found significant reductions in N₂O emissions and improved crop N uptake with SuperU in Manitoba. Wood et al. (2024) observed reductions of 37–57% depending on fertilizer timing and environmental conditions. Gao et al. (2015) showed that SuperU and ESN

reduced emissions by 30–50%, with greater benefits when fertilizers were banded rather than surface-applied. According to Lin et al. (2017), fall manure produced large spring-thaw N₂O peaks, while inhibitors, especially DMPP, significantly reduced emissions and remained effective through winter; soil moisture controlled most emission patterns. Thomas et al. (2017) reported that Nitrapyrin lowered N₂O by 39–58% and drastically reduced emission factors, while NBPT and NBPT + DCD were ineffective. Lin et al. (2023) found that emissions were moisture-driven and highest following spring manure, with inhibitors reducing N₂O only in wet soils; thaw-related losses were linked to surplus nitrate. Lin & Hernandez-Ramirez, (2021) showed that manure bands increased nitrification and N₂O, while inhibitors cut emissions by about half. In a related study, Lin & Hernandez-Ramirez (2020) found inhibitors were most effective at 60–80% WFPS, reducing N₂O by 56–60%, but were much less effective in drier or very wet clay soils. Thilakarathna et al. (2020) found emissions primarily driven by thawing and rainfall, with spring N sometimes reducing N₂O by half compared to fall applications; EEFs lowered emissions in one year and improved yield and N recovery, but responses varied with weather and background soil N. Pokharel & Chang (2021) showed that Nitrapyrin reduced emissions by 32% at high moisture, but its performance was weakened by biochar; biochar itself reduced N₂O only at moderate moisture. Grant et al. (2020) reported that inhibitors reduced annual N₂O by 26–40%, especially in wet years and after spring slurry, but fall slurry still triggered large thaw-related losses. Wang et al. (2023) further found that side-banded N and EEFs increased NUE and yields under different moisture conditions while lowering N₂O emissions without compromising grain quality. Degenhardt et al. (2016) showed that Nitrapyrin reliably slowed nitrification, reduced N losses, and improved flexibility in timing fertilizer applications.

Research on the prairies shows that EEFs do not always provide consistent benefits. In Manitoba, fertilizer additives offered little advantage compared to changing application timing or considering landscape position (Tiessen et al., 2005). Asgedom et al. (2014) observed that ESN and dairy manure resulted in lower N₂O emissions compared to urea. In contrast, SuperU was ineffective, possibly due to elevated soil organic carbon content. The reduced emissions from ESN and manure were attributed to slower nitrogen release. In southern Alberta, nitrapyrin, SuperU, and polymer-coated urea did not lower N₂O emissions compared to untreated urea in rainfed winter wheat (An et al., 2021). More recently, SuperU reduced emissions at only three of six Manitoba sites, and crop yield and nitrogen uptake were mostly unaffected by the fertilizer used (Wood et al., 2024). These results suggest that SuperU works best as part of the 4R framework rather than as a single solution.

The 4R Nutrient Stewardship framework, centered on selecting the right fertilizer source, applying it at the right rate, at the right time, and in the right place, has gained widespread adoption among farmers across Canada (Liu et al., 2022). This framework is grounded in agronomic and environmental science and is designed to synchronize nutrient supply with crop uptake patterns (Grant & Flaten, 2019). By improving this synchronization, the 4R approach helps minimize N losses to the environment, including nitrate leaching into groundwater, ammonia volatilization, and denitrification, which contribute to N₂O emissions. Tenuta et al. (2023) further demonstrated in Manitoba canola that fertilizer placement and N source influenced N₂O emissions, while Wood et al. (2024) reported that fertilizer source and application timing affected N₂O emissions from spring wheat production in Manitoba. Gao et al. (2017) showed that applying 4R practices in potato production systems in the Canadian Prairies resulted in substantially lower N₂O emissions than

conventional fertilizer management, and their findings highlight how optimizing source, rate, timing, and placement can improve NUE while reducing the greenhouse gas emissions of crop production. Ferland et al. (2024) also showed in a Saskatchewan canola–wheat system that combining two 4R practices, reduced N rate, and an enhanced-efficiency N source, lowered cumulative N₂O emissions substantially without reducing yield. Together, these Prairie studies show that 4R-aligned management can improve NUE and mitigate the greenhouse gas footprint of crop production.

Table 1.2 Summary of Canadian Prairie studies evaluating enhanced efficiency fertilizers and nitrogen inhibitors for reducing nitrogen losses and improving crop performance

Authors	Objective of Study	Type of Inhibitor used	Study type	Key Findings	Location
Owens et al. (2019)	Assess how split application and EEFs affect N ₂ O emissions and efficiency metrics	Nitrapyrin, DCD, NBPT, and polymer-coated urea.	Field	Enhanced efficiency fertilizers, especially SuperU, significantly reduced N ₂ O emissions without affecting yield, whereas split applications offered little additional benefit.	Manitoba
Lasisi et al. (2020)	Assess the effectiveness of urease and double inhibitors in reducing NH ₃ emissions from manures	NBPT commercially available as ARM U TM and NBPT + DMPP marketed as ARM U advanced TM	Field and Controlled study	NBPT and NBPT + DMPP treatments were ineffective at reducing ammonia loss from manure, and the dual inhibitor sometimes exacerbated emissions.	Manitoba
Amiro et al. (2017)	Evaluate agronomists' perspectives on adopting N ₂ O mitigation practices	NBPT and DCD, nitrapyrin, including	Cross-sectional survey of agronomists	Agronomists in Manitoba supported reducing N ₂ O emissions through improved fertilizer management and EEF	Manitoba

	through fertilizer management	polymer-coated urea.	(questionnaire-based)	use, but adoption depended on economic feasibility and equipment availability	
Tenuta et al. (2023)	Evaluate how fertilizer source and placement affect N ₂ O emissions and yield in canola.	<i>SuperU</i>	Field	<i>SuperU</i> consistently reduced N ₂ O emissions (by 30–57%) compared to conventional urea, regardless of placement method, though placement effects varied by rainfall conditions	Manitoba
Gao et al. (2015)	Determine how EEU type and fertilizer placement influence N ₂ O emissions and nitrogen efficiency in crop systems.	<i>SuperU</i> polymer coating (ESN).	Field	Both <i>SuperU</i> and ESN significantly reduced N ₂ O emissions (by 30–50%) compared to conventional urea, with greater reductions observed when fertilizers were banded rather than surface-applied	Manitoba
Grant et al. (2016)	Determine how N source, timing, and placement influence spring wheat yield and grain protein under	<i>SuperU</i> and Agrotain Plus; polymer coating in CRU (ESN)	Field	Enhanced-efficiency fertilizers such as <i>SuperU</i> , Agrotain Plus, and controlled-release urea (ESN) showed limited yield benefits under Prairie conditions, with spring side-banded urea	Manitoba

	varying environmental conditions.			performing equally or better, though ESN occasionally improved grain protein when nitrogen loss potential was higher.	
Lasisi et al. (2021)	Assess NBPT ± DMPP for improving N efficiency and yield of fall vs. spring urea-based fertilizers.	NBPT and NBPT + DMPP.	Field	Inhibitor effectiveness was soil-dependent—beneficial in sandy loam, negligible in clay loam; overall, fall N remained less efficient than spring N.	Manitoba
Asgedom et al. (2014)	Assess how urea source and placement affect N ₂ O emissions in clay soil with and without manure.	SuperU; polymer coating in ESN.	Field	ESN and manure reduced N ₂ O relative to urea, while SuperU was ineffective; lower emissions were associated with slower N release	Manitoba
Wood et al. (2024)	Assess EEFs and timing effects on N ₂ O emissions, yield, and N uptake in spring wheat	Nitrapyrin (eNtrench), NBPT (Limus), NBPT + DCD (SuperU), and polymer coating (ESN)	Field	Nitrification inhibitors significantly reduced N ₂ O emissions without yield penalties, whereas urease inhibitors and polymer coatings were less effective.	Manitoba

Grant (2014)	Assess urease inhibitors and fertilizer placement effects on wheat yield and N efficiency under reduced tillage	NBPT and Ammonium thiosulphate (ATS)	Field	NBPT improved the efficiency of surface-applied UAN, while ATS had limited or inconsistent benefits; spray UAN without inhibitors was least effective	Manitoba
Lasisi et al. (2020)	Compare NH ₃ losses from fall vs. spring N applications and test efficacy of NBPT ± DMPP inhibitors.	NBPT and NBPT + DMPP	Field	NBPT significantly reduced volatilization, especially in fall; double inhibitors were less effective in spring due to interaction effects	Manitoba
Tiessen et al. (2005)	Examine timing, topography, and inhibitors on fall-banded urea efficiency.	SuperU®	Field	Late-fall or spring banding of urea improved yield and nitrogen recovery compared with early-fall applications, while SuperU® provided limited or inconsistent benefits, especially in poorly drained lowland soils.	Manitoba
Lasisi & Akinremi, (2020)	The study aimed to investigate how temperature and inhibitor type (NBPT	NBPT and DMPP	Laboratory	The study found that NBPT significantly delayed urea hydrolysis, but combining it with DMPP reduced its effectiveness,	Manitoba

	and DMPP) influence the rate and energy dynamics of urea hydrolysis in Manitoba soils			indicating a negative interaction between the two inhibitors that can lessen urease inhibition efficiency.	
Beres et al. (2018)	The study aimed to evaluate the effects of enhanced efficiency fertilizers, application methods, and timings on yield, protein content, and nitrogen use efficiency in winter wheat across the Canadian Prairies	NBPT in Agrotain®, SuperU®, and a polymer coating for controlled nitrogen release in ESN®.	Field	The study found that SuperU was the most effective fertilizer for improving nitrogen use efficiency and yield, and that split or spring applications further reduced nitrogen losses and enhanced grain protein in winter wheat	Manitoba, Saskatchewan and Alberta
An et al. (2020)	To test whether split-applied enhanced efficiency fertilizers reduce N ₂ O emissions in semi-arid, rain-fed winter wheat compared	NBPT, nitrapyrin, NBPT + DCD, and a slow-release polymer-coated urea (PCU/ESN).	Field	None of the inhibitor treatments reduced (N ₂ O) emissions, emission factors, or yield-scaled N ₂ O; low soil moisture limited N ₂ O production, making inhibitors ineffective	Alberta

	with conventional fertilizers				
An et al. (2021)	Assess whether EEFs and split-application timing reduce N ₂ O emissions compared with applying all urea at planting in rainfed winter wheat.	NBPT, Nitrapyrin SuperU and Polymer-coated urea (PCU/ESN)	Field	EEFs did not reduce N ₂ O; application timing had a greater effect, while dry soils kept emissions and emission factors very low.	Alberta
Rice & Olsen (1988)	Assess how four nitrification inhibitors affect soil and alfalfa N ₂ fixation, nodulation, and plant growth	Nitrapyrin, Etridiazole, DCD and Potassium methyl xanthate (KMX)	Laboratory and Growth chamber	Nitrification inhibitors affected nitrogenase activity and nodulation mainly at high concentrations, with limited relevance under normal field application rates.	Alberta
Malhi & Nyborg (1988)	Evaluate whether ATC, N-Serve 24E, and thiourea reduce overwinter N losses and improve barley yield and N uptake.	ATC (4-amino-1,2,4-triazole hydrochloride), N-Serve 24E (nitrapyrin; 2-chloro-6-trichloromethyl-	Field	The inhibitors slowed overwinter nitrification and improved spring soil N, barley yield, and N uptake, but were less reliable than spring N application.	Alberta

		pyridine) and Thiourea			
Lin et al. (2017)	To assess how fall vs. spring manure injection and nitrification inhibitors affect N ₂ O emissions, soil N dynamics, and barley performance	Nitrapyrin; DMPP	Field	Fall manure increased spring-thaw N ₂ O emissions, while inhibitors, especially DMPP, reduced emissions but did not consistently improve nitrogen-use efficiency.	Alberta
Thomas et al. (2017)	To quantify N ₂ O emissions from cattle urine patches and evaluate the mitigation potential of nitrapyrin, NBPT, and NBPT + DCD on a semiarid pasture	Nitrapyrin, NBPT and NBPT + DCD	Field	Nitrapyrin reduced N ₂ O emissions by 39–58% and emission factors by 50–68%; NBPT and NBPT + DCD showed no reduction; all emission factors were far below the IPCC 2% default (0.03–0.21%).	Alberta
Malhi & Nyborg (1988)	Determine how nitrification inhibitors affect soil N mineralization and nitrification and reduce	ATC, N-Serve 24E (nitrapyrin) and CS ₂ (carbon disulfide)	Field	ATC and CS ₂ markedly reduced overwinter mineral N losses by inhibiting both ammonification and nitrification, while N-Serve	Alberta

	overwinter mineral N losses.			showed much weaker and inconsistent effects	
Lin et al. (2023)	Quantify fall- and spring-applied manure N ₂ O emissions and assess whether DMPP and nitrapyrin reduce emissions, including legacy spring-thaw effects.	DMPP and Nitrapyrin	Field	N ₂ O emissions were moisture-driven, higher after spring manure, reduced by inhibitors only in wet soils, and greatly amplified by spring-thaw losses linked to residual nitrate	Alberta
Lin & Hernandez-Ramirez (2021)	To evaluate N turnover kinetics and N ₂ O production in manured soils and determine the impact of nitrification inhibitors	DMPP; Nitrapyrin	Laboratory	First-order N turnover kinetics; manure bands caused high nitrification and N ₂ O; N ₂ O rose with NH ₄ ⁺ and nitrification rate; inhibitors reduced N ₂ O by ~50%	Alberta
Lin & Hernandez-Ramirez, (2020)	To determine how different rates of nitrification inhibitors affect N ₂ O emissions from manured soils	Nitrapyrin; DMPSA (3,4-dimethylpyrazole succinic acid)	Laboratory	Inhibitors reduced N ₂ O by 56–60% under moist conditions, but effectiveness declined in very wet clay soils, requiring higher rates as soil moisture increased.	Alberta

	under varying moisture levels				
Thilakarathna et al. (2020)	To evaluate how fertilizer timing (fall vs. spring) and enhanced-efficiency N fertilizers (EENFs) affect N ₂ O emissions, emission factors, and NUE in wheat under variable soil N and weather conditions.	Super U, NBPT and N-(2-nitrophenyl) phosphoric triamide (LIMUS), nitrapyrin (eNtrench) and nitrapyrin (N-Serve)	Field	Soil thaw and rainfall drove N ₂ O emissions; spring N and EENFs reduced emissions and improved yield and N recovery, although benefits varied with weather and background soil N.	Alberta
Pokharel & Chang (2021)	To determine how manure biochar influences the effectiveness of nitrapyrin in controlling nitrification and reducing N ₂ O emissions at different soil moisture levels	Nitrapyrin	Laboratory	Nitrapyrin reduced N ₂ O by 32%, particularly at 80% WFPS, but biochar weakened its effectiveness and altered soil N transformations.	Alberta

Grant, Lin, & Hernandez-Ramirez (2020)	To model how nitrification inhibitors affect N ₂ O emissions from fall- and spring-applied dairy slurry by slowing NH ₄ ⁺ oxidation, and to evaluate direct and indirect impacts on annual N ₂ O emissions	ENTEC and eNtrench nitrogen stabilizer	Modelling and Field	NIs cut annual N ₂ O by 26–40%, most in wet years and spring slurry; reduced NO ₃ ⁻ losses; slightly increased NH ₃ ; fall slurry still caused high spring-thaw N ₂ O peaks	Alberta
Thilakarathna & Hernandez-Ramirez (2021)	Assess how soil moisture affects N ₂ O priming, nitrification vs. denitrification sources, and SOM–N vs. urea–N contributions	Nitrapyrin eNtrench®, NBPT, and LIMUS®	Controlled incubation	Denitrification dominated N ₂ O at all moistures, with N addition causing strong short-lived priming; SOM-derived N ₂ O shifted from negative priming in dry soils to positive priming in wet soils, crossing neutral at ~60% WFPS	Alberta
Malhi et al., (1988)	Compare rapeseed response to NH ₄ ⁺ vs. NO ₃ ⁻ and test effects of nitrification inhibitors	N-Serve and Thiourea	Water culture, pot, greenhouse, + Field	Rapeseed showed no consistent yield benefit from nitrate or nitrification inhibitors, while NBPT-treated UAN improved	Alberta and British Columbia

				yield and N uptake without affecting GHG emissions.	
Owens et al., (2023)	Test how liquid enhanced-efficiency N fertilizers affect wheat yield, N use, and GHG emissions across irrigated and rain-fed Prairie environments.	NBPT; Agrotain Ultra, Nitrpyrin; eNtrench; NBPT + DCD; Agrotain Plus and ESN; Nutrien,	Field	UAN combined with NBPT demonstrated the highest efficacy among EEFs. Adding DCD to NBPT did not increase yield, and EEFs did not reduce greenhouse gas emissions.	Alberta and Manitoba
Fast et al., (2023)	Test whether EEFs and N rates improve Canada Western Red Spring wheat yield, protein, and economics across Prairie sites	NBPT (Agrotain), nitrpyrin (eNtrench), SuperU and polymer-coated urea, ESN	Field	Dual inhibitor slightly increased yields and net return in Dark Brown soils; EEF benefits were small or soil-dependent; 120 kg N ha ⁻¹ was the optimal rate for balancing yield and protein	Alberta and Saskatchewan
Wang et al., (2023)	Evaluate how granular EEFs and fertilizer timing/placement affect winter wheat yield, N use, and GHG emissions.	Nitrpyrin (eNtrench®) and SuperU®	Field	Side-banding N at planting maximized rain-fed NUE, while NBPT+DCD improved irrigated yields and EEFs reduced N ₂ O without compromising yield or grain quality.	Alberta, Manitoba and Saskatchewan

Degenhardt et al. (2016)	Evaluate how nitrapyrin stabilizes soil NH_4^+ and reduces N losses from banded urea, UAN, and ammonia across Canadian field sites.	<i>Nitrapyrin</i> (eNtrench and N- Serve)	Field	Nitrapyrin consistently slowed nitrification, maintained higher NH_4^+ and total mineral N through critical loss periods, reduced N losses, and improved flexibility of N application timing	Alberta, Manitoba, Saskatchewan and Ontario
-----------------------------	--	---	-------	---	--

1.6 Contribution and significance of the study

This research addresses agricultural N₂O emissions by investigating the effects of freeze–thaw conditions, soil moisture, thawing temperature, and fertilizer source on N₂O release from clayey Prairie soil. Freeze–thaw events alter soil structure, microbial activity, oxygen diffusion, and nitrogen substrate availability, thereby stimulating N₂O production during thawing (Congreves et al., 2018; Mørkved et al., 2006; Risk et al., 2013; Teepe et al., 2001). The study evaluates N₂O emissions from a Gleyed Humic Vertisol under soil moisture (32%, 50%, 75%, and 100% WFPS and thawing temperatures (4, 8, 12, and 16 °C). By comparing conventional urea with SuperU, a stabilized urea fertilizer containing urease and nitrification inhibitors, the research assesses whether EEFs can reduce N₂O emissions under freeze–thaw conditions.

Freeze–thaw events can generate short but intense N₂O emission pulses, particularly when mineral nitrogen, elevated soil moisture, and limited oxygen availability coincide (Congreves et al., 2018; Teepe et al., 2001; Wagner-Riddle et al., 2017). Such emissions may contribute significantly to annual N₂O budgets in cold agricultural regions, yet they are often difficult to quantify because they occur only briefly during winter or the early spring thaw (Risk et al., 2013; Wagner-Riddle et al., 2017). Inadequate representation of these emissions can lead to underestimation or mischaracterization of the contribution of seasonally frozen agricultural soils to total N₂O losses in emission factors and greenhouse gas models (Butterbach-Bahl et al., 2013; Wagner-Riddle et al., 2017).

This issue is especially pertinent for clayey Prairie soils, as soil texture and drainage significantly affect water retention, oxygen diffusion, and the development of anaerobic microsites. Fine-textured soils typically retain water for extended periods and may restrict oxygen movement at high WFPS, thereby promoting denitrification and N₂O production (Bateman & Baggs, 2005; Butterbach-Bahl et al., 2013; Davidson et al., 2000; Linn & Doran, 1984). Consequently, N₂O emissions from clay soils may differ from

those of sandy or well-drained soils due to variations in thawing, moisture redistribution, and oxygen limitation across soil textures (Congreves et al., 2018; Risk et al., 2013). These emissions are directly relevant to N management in the Canadian Prairies. Farmers and agronomists require locally relevant information on how fertilizer source, soil moisture, and thawing temperature affect N losses outside the main growing season. Fall or early-season N management can leave mineral nitrogen in the soil during periods when crop uptake is limited, increasing the risk of N losses through leaching, runoff, or gaseous emissions (Amiro et al., 2017; Tenuta et al., 2016; Tiessen et al., 2005). Without region-specific information, fertilizer recommendations may rely on assumptions derived from other soil types, climates, or growing-season conditions that may not adequately represent Prairie freeze–thaw environments.

Another further contribution is the evaluation of stabilized urea under freeze–thaw conditions. Enhanced-efficiency fertilizers, such as those containing urease and nitrification inhibitors, are intended to slow nitrogen transformations, reduce nitrate accumulation, and limit pathways leading to N₂O production (Abalos et al., 2014; Akiyama et al., 2010; Gilsanz et al., 2016). The effectiveness of these inhibitors can vary with soil temperature, moisture, pH, microbial activity, fertilizer timing, and environmental conditions (Akiyama et al., 2010; Lam et al., 2017; Thapa et al., 2016). Testing SuperU across a range of thawing temperatures and moisture levels provides insight into whether inhibitor-based fertilizers can reduce N₂O emissions during thawing or if their performance is constrained under freeze–thaw conditions. The study further supports the 4R Nutrient Stewardship framework, which promotes applying the right fertilizer source at the right rate, at the right time, and at the right place to improve nutrient-use efficiency and reduce environmental losses (Johnston & Bruulsema, 2014; Snyder et al., 2009). By improving understanding of how thawing temperature and soil moisture regulate N₂O emissions from different fertilizer sources, the findings can help refine site-specific nitrogen management practices, improve nitrogen-use efficiency, and reduce unnecessary nitrogen losses from Prairie soils.

The significance of this research extends to climate policy. Canada aims to reduce N₂O emissions from fertilizer application by 30% below 2020 levels by 2030, representing a decrease from 11.82 to approximately 8.27 Mt CO_{2e} yr⁻¹ (Agriculture and Agri-Food Canada, 2023). Data generated by studies of this nature can improve emission estimates, refine greenhouse gas models, and support mitigation strategies that more accurately reflect the conditions of cold-region agricultural systems (Agriculture and Agri-Food Canada, 2023; IPCC, 2019).

Climate change increases the urgency of this research. Warmer winters, variable snow cover, and more frequent freeze–thaw events may alter soil moisture, thawing patterns, microbial activity, and N₂O emissions in seasonally frozen soils (Congreves et al., 2018; Henry, 2007). By investigating the combined effects of moisture, thawing temperature, and fertilizer source, this research provides essential information to support sustainable and climate-resilient N management in the Canadian Prairies.

1.7 Advancing research on thaw-induced N₂O emissions in the Canadian Prairies

1.7.1 Expanding FTC studies on clay soils

Clay soils require explicit consideration in freeze–thaw research due to their fine texture, high water-holding capacity, low permeability, and tendency to remain saturated during snowmelt, all of which can promote oxygen limitation, denitrification, and N₂O production.

To assess the available evidence, a systematic review was conducted on November 3, 2025, using the search string (“soil freeze-thaw” OR “soil freeze-thaw cycl*” OR “soil freezing and thawing” OR “soil column stud*” OR “fall and spring”) AND (N₂O OR “nitrous oxide”) using Web of Science Core Collection and Scopus databases. The review revealed that direct evidence on freeze–thaw-driven N₂O emissions from clay soils in the Canadian Prairies is limited. The only explicit freeze–thaw studies on clay soils identified were van Bochove et al. (2000) and Pelster et al. (2019), both conducted in Quebec. Prairie-based evidence was found in Manitoba, including Tenuta & Sparling (2011), but this study focused on a

Newdale clay loam with a sandy loam surface layer and sandy clay loam subsoil, rather than a uniformly clay soil. Other Manitoba studies, such as Gao et al. (2013) and Asgedom et al. (2014), investigated N₂O dynamics in clay or clay-loam soils but did not specifically isolate the effects of freeze–thaw. Collectively, these findings demonstrate a clear gap in Prairie-specific field and laboratory evidence on freeze–thaw driven N₂O emissions from clay soils.

As a result, most research has focused on sandy loam soils, creating a major knowledge gap about how freeze–thaw cycles affect N₂O emissions and nitrogen transformations in fine-textured soils that dominate Prairie agriculture. This study addresses that gap by investigating how water-filled pore space and thawing temperature interact to regulate N₂O emissions from clay soils, with the goal of improving region-specific nitrogen management and supporting 4R-based greenhouse gas mitigation strategies in cold-region agroecosystems.

1.7.2 More rigorous lab studies on N inhibitors under freeze-thaw conditions

Previous research has demonstrated that N₂O concentrations can increase substantially during early thaw periods. However, these short-term emissions can be overlooked in field studies because wet, snowmelt-affected soils are difficult to access during this time. Consequently, the magnitude, timing, and controlling factors of N₂O release under field conditions remain poorly understood. Laboratory experiments address these challenges by enabling precise control of temperature, moisture, nitrogen sources, and inhibitors, and by allowing frequent monitoring of N₂O emissions during the initial stages of thaw. Such experimental control is particularly valuable for evaluating nitrogen inhibitors, whose effectiveness may vary with thawing conditions, soil properties, and microbial activity. In this study, we use controlled laboratory conditions to examine how thawing temperature, moisture, and two types of inhibitors together affect N₂O emissions from clay soils. Our goal is to better understand these processes to improve nitrogen management in Prairie farming systems.

1.7.3 Testing realistic soil temperature on lab FTC studies

To accurately simulate soil freeze–thaw processes in the lab, temperature treatments must reflect field conditions. This is important because N₂O emissions during freeze–thaw periods depend on factors such as freezing intensity, thaw rate, soil moisture, and the duration of soil below freezing. However, many lab studies still use fixed temperature settings that ignore local climate differences, which limits how well their results apply to real-world conditions (Fouli et al., 2013; Henry, 2007). To address this, the present study uses historical weather data from a nearby Environment and Climate Change Canada station to set realistic freezing and thawing temperatures. This approach makes the experiment more relevant and repeatable and helps us better understand how freeze–thaw cycles affect N₂O emissions and nitrogen changes in Prairie clay soils.

1.7.4 Accounting for off-season and early-thaw N₂O emissions

Nitrous oxide emissions occurring outside the growing season represent a significant source of uncertainty in annual N₂O budgets for cold-region agriculture (Pelster et al., 2022; Wagner-Riddle et al., 2017). During spring thaw, substantial but transient emission pulses can occur when mineral nitrogen is available, crop uptake is limited, and soil water content is elevated (Teepe et al., 2001; Wagner-Riddle et al., 2008, 2017). Field studies may underestimate these losses because wet, snowmelt-affected soils often impede access during the initial thaw, particularly in poorly drained clay landscapes (Pelster et al., 2019; Tenuta & Sparling, 2011; Wood et al., 2024). This limitation increases the likelihood of missing early and potentially important emission pulses. The challenge is especially pronounced in clayey soils. Increased focus on early-thaw emissions is essential for improving annual emission estimates and nitrogen-loss assessments in Prairie agricultural systems. This study addresses the identified research gap by continuously monitoring early-thaw N₂O emissions under controlled thawing temperatures, soil moisture conditions, and fertilizer types.

1.7.5 Interpreting thaw emissions using kinetic and thermodynamic approaches

Numerous FTC studies report cumulative emissions or peak fluxes; however, these metrics do not fully capture the complexity of emission dynamics (Risk et al., 2013; Teepe et al., 2001; Wagner-Riddle et al., 2008; Zhang et al., 2022). Thaw emissions often manifest as brief pulses, leading to treatments with comparable cumulative losses that may nonetheless differ in release rate, half-life, or temperature sensitivity (Pelster et al., 2019; Teepe et al., 2001; Wagner-Riddle et al., 2008). In the present study, first-order kinetic modeling is used to distinguish between emission potential and emission rate, and thermodynamic analysis is used to quantify effective temperature sensitivity (Holtan-Hartwig et al., 2002; Juma et al., 1984; Stanford & Smith, 1972; Zhang et al., 2022). These analytical methods provide a more comprehensive framework for interpreting the influence of thawing temperature, soil moisture, and fertilizer source on N₂O emission dynamics beyond total emissions alone.

1.8 Advancing our understanding of N₂O dynamics: Stakeholder benefits

The outcomes of this research are expected to benefit stakeholders at the farm, policy, industry, and global levels. Since N₂O emissions from agricultural soils are affected by fertilizer management, soil moisture, temperature, and microbial nitrogen transformations, a deeper understanding of these interactions can facilitate more effective nitrogen management in cold-region agricultural systems (Butterbach-Bahl et al., 2013; Congreves et al., 2018; Wagner-Riddle et al., 2017). Such understanding is critical to all parties advancing our efforts to reduce the impact of N₂O on the environment.

Farmers represent a primary beneficiary group of this research. The results offer evidence-based guidance regarding the effects of fertilizer source, soil moisture, and thawing temperature on N₂O emissions and nitrogen retention in clayey Prairie soils. This is especially relevant for fall N applications, during which fertilizer may persist in the soil when crop uptake is limited and is susceptible to loss during FTCs (Amiro et al., 2017; Tenuta et al., 2016; Tiessen et al., 2011). By identifying conditions that increase N₂O emissions, the research enables producers to make informed decisions about fertilizer source, timing, and

soil moisture prior to application. This information can enhance NUE, minimize unnecessary nitrogen losses, and promote stable crop production (Cassman et al., 2002; Raun & Johnson, 1999; Snyder et al., 2009).

Agronomists, crop advisors, and extension specialists will benefit from the research by gaining access to locally relevant data for developing site-specific N recommendations. Soils in the Canadian Prairies vary in texture, drainage, water-holding capacity, and freeze–thaw dynamics. Consequently, fertilizer recommendations based solely on growing-season responses or data from other soil types may not adequately address nitrogen loss risks under Prairie freeze–thaw conditions. The study's findings can reinforce advisory support for the 4R Nutrient Stewardship framework, which promotes the right fertilizer source, rate, timing, and placement to enhance nutrient-use efficiency and reduce environmental losses (Johnston & Bruulsema, 2014; Snyder et al., 2009).

Government agencies and policymakers, such as Agriculture and Agri-Food Canada and Environment and Climate Change Canada, may benefit from enhanced data on N₂O emissions in cold-region soils. Canada has established a national objective to reduce fertilizer-related emissions by 30% from 2020 levels by 2030, alongside broader climate targets, including achieving net-zero emissions by 2050. Official Government of Canada sources confirm both the fertilizer-emission reduction target and the national net-zero commitment. Data from studies of this nature can refine N₂O emission factors, improve agricultural greenhouse gas inventories, and inform mitigation policies that more accurately reflect the environmental conditions of Prairie farming systems (Agriculture and Agri-Food Canada, 2023; IPCC, 2019).

Fertilizer and inhibitor manufacturers may benefit from this research by gaining insights into the performance of enhanced-efficiency fertilizers and nitrogen inhibitors, which are designed to reduce nitrogen losses by slowing urea hydrolysis and nitrification. The effectiveness of these products is influenced by soil temperature, moisture, microbial activity, and application timing (Abalos et al., 2014; Akiyama et al., 2010; Gilsanz et al., 2016; Lam et al., 2017; Thapa et al., 2016). By assessing stabilized

urea under varying thawing temperatures and moisture conditions, the study provides evidence regarding the efficacy of inhibitor-containing fertilizers in freeze–thaw conditions in clayey Prairie soils. This information can inform product development, positioning, and recommendations for environments where inhibitor performance is uncertain.

The broader agricultural sector and society may benefit from improved integration of crop production, environmental protection, and climate action. Enhancing NUE can maintain crop productivity while reducing nitrogen losses to the atmosphere and water. These outcomes support several United Nations Sustainable Development Goals, including SDG 2 (food security), SDG 8 (economic growth), SDG 9 (innovation), SDG 12 (responsible production), SDG 13 (climate action), and SDG 17 (partnerships) (Mangukiya & Sklarew, 2023). Thus, the study's benefits extend beyond individual farms by advancing more sustainable and climate-resilient nitrogen management in the Canadian Prairies.

In summary, this study offers both practical and scientific contributions by evaluating the relationships among freeze–thaw processes, soil moisture, thawing temperature, and fertilizer source, and their effects on N₂O emissions in clayey Prairie soils. The resulting information can assist farmers, agronomists, policymakers, researchers, and the fertilizer industry in formulating nitrogen management strategies that reduce N₂O emissions while sustaining productive, sustainable cropping systems.

1.9 Research Objectives

The literature review identified important research opportunities to advance the understanding of thaw-induced N₂O emissions from Prairie clay soils. In the present study, a laboratory experiment was conducted to evaluate how thawing temperature, soil moisture, and fertilizer source influence N₂O emissions from a Gleyed Humic Vertisol following a simulated freeze–thaw event.

The study had two specific objectives:

- 1) To determine the effects of thawing temperature (4, 8, 12, and 16°C), soil moisture content (32%, 50%, 75%, and 100% water-filled pore space [WFPS]), and fertilizer source (SuperU (Dual-inhibitor) [DI] and urea) on N₂O emissions during thaw.
- 2) To characterize the kinetics and thermodynamics of cumulative N₂O emissions under the same thawing temperature, soil moisture, and fertilizer treatments.

1.10 Thesis Outline

This thesis was prepared in accordance with the thesis guidelines of the Department of Soil Science, University of Manitoba. The thesis consists of four chapters: a general introduction and a comprehensive literature review (Chapter 1), two research chapters prepared in manuscript format (Chapters 2 and 3), and an overall synthesis chapter (Chapter 4).

Chapter 2: Effects of thawing temperature, soil moisture, and fertilizer source on nitrous oxide emissions from a Gleyed Humic Vertisol following a simulated freeze–thaw event.

Chapter 3: Kinetic and thermodynamic response of thaw-induced nitrous oxide emissions to thawing temperature, soil moisture and fertilizer source in Gleyed Humic Vertisol.

Chapter 4: Overall synthesis, conclusions, and recommendations for future research.

1.11 References

- Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., & Vallejo, A. (2014). Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment*, 189, 136–144. <https://doi.org/10.1016/J.AGEE.2014.03.036>
- Agriculture and Agri-Food Canada. (2023). *What we heard report: Fertilizer emissions reduction*. <https://agriculture.canada.ca/en/departement/transparency/public-opinion-research-consultations/share-ideas-fertilizer-emissions-reduction-target>
- Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: Meta-analysis. *Global Change Biology*, 16(6), 1837–1846. <https://doi.org/10.1111/J.1365-2486.2009.02031>
- Amiro, B., Tenuta, M., Hanis-Gervais, K., Gao, X., Flaten, D., & Rawluk, C. (2017). Agronomists' views on the potential to adopt beneficial greenhouse gas nitrogen management practices through fertilizer management. *Canadian Journal of Soil Science*, 97(4), 801–804. <https://doi.org/10.1139/cjss-2017-0062>
- An, H., Owens, J., Beres, B., Li, Y., & Hao, X. (2021). Nitrous oxide emissions with enhanced efficiency and conventional urea fertilizers in winter wheat. *Nutrient Cycling in Agroecosystems* 2021 119:3, 119(3), 307–322. <https://doi.org/10.1007/S10705-021-10118-9>
- An, H., Owens, J., Stoeckli, J., Hao, X., Beres, B., & Li, Y. (2020). Nitrous oxide emissions following split fertilizer application on winter wheat grown on Mollisols of Southern Alberta, Canada. *Geoderma Regional*, 21, e00272. <https://doi.org/10.1016/J.GEODRS.2020.E00272>
- Asgedom, H., Tenuta, M., Flaten, D. N., Gao, X., & Kebreab, E. (2014). Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. *Agronomy Journal*, 106(2), 732–744. <https://doi.org/10.2134/AGRONJ2013.0096>;

- Baggs, E. M. (2011). Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. *Current Opinion in Environmental Sustainability*, 3(5), 321–327. <https://doi.org/10.1016/J.COSUST.2011.08.011>
- Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 2005 41:6, 41(6), 379–388. <https://doi.org/10.1007/S00374-005-0858-3>
- Beres, B. L., Graf, R. J., Irvine, R. B., O'Donovan, J. T., Harker, K. N., Johnson, E. N., Brandt, S., Hao, X., Thomas, B. W., Turkington, T. K., & Stevenson, F. C. (2018). Enhanced nitrogen management strategies for winter wheat production in the Canadian prairies. *Https://Doi.Org/10.1139/Cjps-2017-0319*, 98(3), 683–702. <https://doi.org/10.1139/CJPS-2017-0319>
- Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621). <https://doi.org/10.1098/RSTB.2013.0122/22151>
- Cantarella, H., Otto, R., Soares, J. R., & Silva, A. G. de B. (2018). Agronomic efficiency of NBPT as a urease inhibitor: A review. *Journal of Advanced Research*, 13, 19–27. <https://doi.org/10.1016/J.JARE.2018.05.008>
- Cassman, K. G., Dobermann, A., & Walters, D. T. (2002). Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. *Https://Doi.Org/10.1579/0044-7447-31.2.132*, 31(2), 132–140. <https://doi.org/10.1579/0044-7447-31.2.132>

- Chang, C., Janzen, H. H., & Cho, C. M. (1998). Nitrous Oxide Emission From Long-Term Manured Soils. *Soil Science Society of America Journal*, 62(3), 677–682.
<https://doi.org/10.2136/SSSAJ1998.03615995006200030019X>
- Congreves, K. A., Wagner-Riddle, C., Si, B. C., & Clough, T. J. (2018). Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. *Soil Biology and Biochemistry*, 117, 5–15. <https://doi.org/10.1016/J.SOILBIO.2017.10.040>
- Coskun, D., Britto, D. T., Shi, W., & Kronzucker, H. J. (2017). Nitrogen transformations in modern agriculture and the role of biological nitrification inhibition. *Nature Plants* 2017 3:6, 3(6), 17074-.
<https://doi.org/10.1038/nplants.2017.74>
- Cui, G., & Wang, J. (2019). Improving the DNDC biogeochemistry model to simulate soil temperature and emissions of nitrous oxide and carbon dioxide in cold regions. *Science of The Total Environment*, 687, 61–70. <https://doi.org/10.1016/J.SCITOTENV.2019.06.054>
- Davidson, E. A. ;, Keller, M. ;, Erickson, H. E. ;, Verchot, L. V. ;, & Veldkamp, E. (2000). Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, 50(8), 667–680.
- Degenhardt, R. F., Juras, L. T., Smith, L. R. A., MacRae, A. W., Ashigh, J., & McGregor, W. R. (2016). Application of Nitrapyrin with Banded Urea, Urea Ammonium Nitrate, and Ammonia Delays Nitrification and Reduces Nitrogen Loss in Canadian Soils. *Crop, Forage & Turfgrass Management*, 2(1), 1–11.
<https://doi.org/10.2134/CFTM2016.03.0027;PAGE:STRING:ARTICLE/CHAPTER>
- Di, H. J., & Cameron, K. C. (2002). The use of a nitrification inhibitor, dicyandiamide (DCD), to decrease nitrate leaching and nitrous oxide emissions in a simulated grazed and irrigated grassland. *Soil Use and Management*, 18(4), 395–403. <https://doi.org/10.1111/J.1475-2743.2002.TB00258.X>

- Environment and Climate Change Canada. (2024). *National inventory report 1990–2022: Greenhouse gas sources and sinks in Canada*. https://publications.gc.ca/collections/collection_2024/eccc/En81-4-2022-1-eng.pdf
- Environment Canada. (2014). *National inventory report 1990–2012: Greenhouse gas sources and sinks in Canada*. https://publications.gc.ca/collections/collection_2018/eccc/En81-4-2014-3-eng.pdf
- Fast, A., Strydhorst, S., Wang, Z., Hernandez-Ramirez, G., Hao, X., Semach, G., Thompson, L., Holzapfel, C., Enns, J., Spaner, D., & Beres, B. L. (2023). Integrating enhanced efficiency fertilizers and nitrogen rates to improve Canada Western Red Spring wheat. *https://doi.org/10.1139/Cjps-2023-0095*, 104(2), 144–160. <https://doi.org/10.1139/CJPS-2023-0095>
- Ferland, D., Wagner-Riddle, C., Brown, S. E., Bourgault, M., Helgason, W., Farrell, R. E., & Congreves, K. A. (2024). Improved nitrogen fertilizer management reduces nitrous oxide emissions in a northern Prairie cropland. *Science of The Total Environment*, 956, 177211. <https://doi.org/10.1016/J.SCITOTENV.2024.177211>
- Forster, P. ;, Ramaswamy, V. ;, Artaxo, P. ;, Berntsen, T. ;, Betts, R. ;, Fahey, D. W. ;, Haywood, J. ;, Lean, J. ;, Lowe, D. C. ;, Myhre, G. ;, Nganga, J. ;, Prinn, R. ;, Raga, G. ;, Schulz, M. ;, & Van Dorland, R. (2007). Changes in atmospheric constituents and in radiative forcing. In S. ; Q. D. ; M. M. ; C. Z. ; M. M. ; A. K. B. ; T. M. ; M. H. L. Solomon (Ed.), *Climate Change 2007: The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 129–234). Cambridge University Press. <https://www.ipcc.ch/report/ar4/wg1/changes-in-atmospheric-constituents-and-radiative-forcing/>
- Fouli, Y., Cade-Menun, B. J., & Cutforth, H. W. (2013). Freeze–thaw cycles and soil water content effects on infiltration rate of three Saskatchewan soils. *https://doi.org/10.4141/Cjss2012-060*, 93(4), 485–496. <https://doi.org/10.4141/CJSS2012-060>

- Gao, X., Asgedom, H., Tenuta, M., & Flaten, D. N. (2015). Enhanced efficiency urea sources and placement effects on nitrous oxide emissions. *Agronomy Journal*, 107(1), 265–277. <https://doi.org/10.2134/AGRONJ14.0213>;
- Gao, X., Parsonage, S., Tenuta, M., Baron, K., Hanis-Gervais, K., Nelson, A., Tomasiewicz, D., & Mohr, R. (2017). Nitrogen Fertilizer Management Practices to Reduce N₂O Emissions from Irrigated Processing Potato in Manitoba. *American Journal of Potato Research* 2017 94:4, 94(4), 390–402. <https://doi.org/10.1007/S12230-017-9574-4>
- Gao, X., Tenuta, M., Nelson, A., Sparling, B., Tomasiewicz, D., Mohr, R. M., & Bizimungu, B. (2013). Effect of nitrogen fertilizer rate on nitrous oxide emission from irrigated potato on a clay loam soil in Manitoba, Canada. <https://doi.org/10.4141/Cjss2012-057>, 93(1), 1–11. <https://doi.org/10.4141/CJSS2012-057>
- Giles, M., Morley, N., Baggs, E. M., & Daniell, T. J. (2012). Soil nitrate reducing processes - Drivers, mechanisms for spatial variation, and significance for nitrous oxide production. *Frontiers in Microbiology*, 3(DEC), 30024. <https://doi.org/10.3389/FMICB.2012.00407/TEXT>
- Gilsanz, C., Báez, D., Misselbrook, T. H., Dhanoa, M. S., & Cárdenas, L. M. (2016). Development of emission factors and efficiency of two nitrification inhibitors, DCD and DMPP. *Agriculture, Ecosystems & Environment*, 216, 1–8. <https://doi.org/10.1016/J.AGEE.2015.09.030>
- Grant, C. A. (2014). Use of NBPT and ammonium thiosulphate as urease inhibitors with varying surface placement of urea and urea ammonium nitrate in production of hard red spring wheat under reduced tillage management. <https://doi.org/10.4141/Cjps2013-289>, 94(2), 329–335. <https://doi.org/10.4141/CJPS2013-289>
- Grant, C. A., & Flaten, D. N. (2019). Background of 4R nutrient stewardship. In *4R Management of Phosphorus Fertilizer in the Northern Great Plains: A Review of the Scientific Literature* (pp. 1–6). Fertilizer Canada.

<https://fertilizercanada.ca/wp-content/uploads/2019/07/4R-P-fertilizer-mgmt-Chapter-1-Background-of-4R-nutrient-stewardship-final.pdf>

Grant, C. A., Moulin, A. P., & Tremblay, N. (2016). Nitrogen management effects on spring wheat yield and protein concentration vary with seeding date and slope position. *Agronomy Journal*, 108(3), 1246–1256. <https://doi.org/10.2134/AGRONJ2015.0510>;

Grant, R. F., Lin, S., & Hernandez-Ramirez, G. (2020). Modelling nitrification inhibitor effects on N₂O emissions after fall-and spring-Applied slurry by reducing nitrifier NH₄⁺ oxidation rate. *Biogeosciences*, 17(7), 2021–2039. <https://doi.org/10.5194/BG-17-2021-2020>

Hao, X., Chang, C., Carefoot, J. M., Janzen, H. H., & Ellert, B. H. (2001). Nitrous oxide emissions from an irrigated soil as affected by fertilizer and straw management. *Nutrient Cycling in Agroecosystems* 2001 60:1, 60(1), 1–8. <https://doi.org/10.1023/A:1012603732435>

Hayatsu, M., Tago, K., & Saito, M. (2008). Various players in the nitrogen cycle: Diversity and functions of the microorganisms involved in nitrification and denitrification. *Soil Science and Plant Nutrition*, 54(1), 33–45. <https://doi.org/10.1111/J.1747-0765.2007.00195.X>

Henry, H. A. L. (2007). Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry*, 39(5), 977–986. <https://doi.org/10.1016/J.SOILBIO.2006.11.017>

Holtan-Hartwig, L., Dörsch, P., & Bakken, L. R. (2002). Low temperature control of soil denitrifying communities: kinetics of N₂O production and reduction. *Soil Biology and Biochemistry*, 34(11), 1797–1806. [https://doi.org/10.1016/S0038-0717\(02\)00169-4](https://doi.org/10.1016/S0038-0717(02)00169-4)

- Hu, H. W., Chen, D., & He, J. Z. (2015). Microbial regulation of terrestrial nitrous oxide formation: understanding the biological pathways for prediction of emission rates. *FEMS Microbiology Reviews*, 39(5), 729–749. <https://doi.org/10.1093/FEMSRE/FUV021>
- IPCC. (2014). *Climate Change 2013 – The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (T. F. ; Q. D. ; P. G.-K. ; T. M. ; A. S. K. ; B. J. ; Stocker & A. ; X. Y. ; B. V. ; M. P. M. Nauels, Eds.). Cambridge University Press. <https://doi.org/10.1017/CBO9781107415324>
- IPCC. (2019). 2019 Refinement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories. <https://www.ipcc.ch/report/2019-refinement-to-the-2006-ipcc-guidelines-for-national-greenhouse-gas-inventories>
- IPCC. (2023). Summary for Policymakers. In Core Writing Team, H. ; Lee, & J. Romero (Eds.), *Climate Change 2023: Synthesis Report: Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1–34). IPCC. <https://doi.org/10.59327/IPCC/AR6-9789291691647.001>
- Johnston, A. M., & Bruulsema, T. W. (2014). 4R Nutrient Stewardship for Improved Nutrient Use Efficiency. *Procedia Engineering*, 83, 365–370. <https://doi.org/10.1016/J.PROENG.2014.09.029>
- Juma, N. G., Paul, E. A., & Mary, B. (1984). Kinetic Analysis of Net Nitrogen Mineralization in Soil. *Soil Science Society of America Journal*, 48(4), 753–757. <https://doi.org/10.2136/SSSAJ1984.03615995004800040011>
- Koch Agronomic Services. (2019). *How SuperU works*. Koch Agronomic Services. <https://kochagronomicservices.com/superu>

- Kool, D. M., Dolfing, J., Wrage, N., & Van Groenigen, J. W. (2011). Nitrifier denitrification as a distinct and significant source of nitrous oxide from soil. *Soil Biology and Biochemistry*, 43(1), 174–178.
<https://doi.org/10.1016/J.SOILBIO.2010.09.030>
- Kpankpari, R. (2025). *Freeze-thaw Cycle, Soil Moisture, and Thawing Temperature Effects on Nitrogen Dynamics in a Black Chernozemic Sandy Loam*. <https://mspace.lib.umanitoba.ca/bitstreams/a77a3d49-0fa9-4d10-ba6f-78e0adcee4a8/download>
- Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, 198(3), 656–669.
<https://doi.org/10.1111/NPH.12235>;ISSUE:ISSUE:DOI
- Lam, S. K., Suter, H., Mosier, A. R., & Chen, D. (2017). Using nitrification inhibitors to mitigate agricultural N₂O emission: a double-edged sword? *Global Change Biology*, 23(2), 485–489.
<https://doi.org/10.1111/GCB.13338>;
- Lasisi, A. A., Akinremi, O. O., & Kumaragamage, D. (2021). Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors II. Crop yield and nitrogen use efficiency. *Soil Science Society of America Journal*, 85(2), 299–313.
<https://doi.org/10.1002/SAJ2.20126>;REQUESTEDJOURNAL:JOURNAL:14350661;
- Lasisi, A. A. & Akinremi, O. O. (2020). Kinetics and thermodynamics of urea hydrolysis in the presence of urease and nitrification inhibitors. *Https://Doi.Org/10.1139/Cjss-2020-0044*, 101(2), 192–202.
<https://doi.org/10.1139/CJSS-2020-0044>
- Lasisi, A. A., Akinremi, O. O., Zhang, Q., & Kumaragamage, D. (2020). Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. *Soil Science Society of America Journal*, 84(3), 949–962.
<https://doi.org/10.1002/SAJ2.20062>

- Lin, S., & Hernandez-Ramirez, G. (2020). Nitrous oxide emissions from manured soils as a function of various nitrification inhibitor rates and soil moisture contents. *Science of The Total Environment*, 738, 139669. <https://doi.org/10.1016/J.SCITOTENV.2020.139669>
- Lin, S., & Hernandez-Ramirez, G. (2021). Nitrogen turnover and n₂o production in incubated soils after receiving field applications of liquid manure and nitrification inhibitors. *Canadian Journal of Soil Science*, 101(2), 290–304. <https://doi.org/10.1139/cjss-2020-0102>
- Lin, S., Hernandez-Ramirez, G., Kryzanowski, L., Lohstraeter, G., & Wallace, T. (2023). Adding inhibitors to manure injections can mitigate nitrous oxide emissions from barley croplands. *Nutrient Cycling in Agroecosystems* 2023 126:1, 126(1), 81–100. <https://doi.org/10.1007/S10705-023-10277-X>
- Lin, S., Hernandez-Ramirez, G., Kryzanowski, L., Wallace, T., Grant, R., Degenhardt, R., Berger, N., Lohstraeter, G., & Powers, L.-A. (2017). Timing of Manure Injection and Nitrification Inhibitors Impacts on Nitrous Oxide Emissions and Nitrogen Transformations in a Barley Crop. *Soil Science Society of America Journal*, 81(6), 1595–1605. <https://doi.org/10.2136/sssaj2017.03.0093>
- Lin, S., Mezbahuddin, S., Grant, R., & Hernandez-Ramirez, G. (2021). How could simulated dewatering of slurry mitigate nitrous oxide emissions from fall and spring injections? – A modelling study in a Chernozem soil in Western Canada. *Science of The Total Environment*, 796, 148758. <https://doi.org/10.1016/J.SCITOTENV.2021.148758>
- Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal*, 48(6), 1267–1272. <https://doi.org/10.2136/SSSAJ1984.03615995004800060013X>;
- Liu, M., Xu, W., Zhang, H., Chen, H., Han, G., & Yu, X. (2022). Livestock Production, Greenhouse Gas Emissions, Air Pollution, and Grassland Conservation: Quasi-Natural Experimental Evidence. *SSRN Electronic Journal*. <https://doi.org/10.2139/SSRN.4306904>

- Liu, X., Zhang, M., Li, Z., Zhang, C., Wan, C., Zhang, Y., & Lee, D. J. (2019). Inhibition of urease activity by humic acid extracted from sludge fermentation liquid. *Bioresource Technology*, 290, 121767. <https://doi.org/10.1016/J.BIORTECH.2019.121767>
- Malhi, S. S., & Nyborg, M. (1988). Effect of ATC, N-Serve 24 E and thiourea nitrification inhibitors on yield and N uptake of barley fertilized with fall-applied N1. *Plant and Soil* 1988 105:2, 105(2), 223–229. <https://doi.org/10.1007/BF02376786>
- Malhi, S. S., Nyborg, M., Jahn, H. G., & Penney, D. C. (1988). Yield and nitrogen uptake of rapessed (Brassica campestris L.) with ammonium and nitrate. *Plant and Soil*, 105(2), 231–239. <https://doi.org/10.1007/BF02376787/METRICS>
- Mangukiya, R. D., & Sklarew, D. M. (2023). Analyzing three pillars of sustainable development goals at sub-national scales within the USA. *World Development Sustainability*, 2, 100058. <https://doi.org/10.1016/J.WDS.2023.100058>
- Manitoba Agriculture. (2007). *Manitoba soil fertility guide*. https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/pubs/soil_fertility_guide.pdf
- M.E. Trenkel, T. (2021). *Slow- and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture*.
- Mørkved, P. T., Dörsch, P., Henriksen, T. M., & Bakken, L. R. (2006). N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biology and Biochemistry*, 38(12), 3411–3420. <https://doi.org/10.1016/J.SOILBIO.2006.05.015>
- Owens, J., An, H., Beres, B., Mohr, R., Hao, X., Owens, J., Beres, B., Hao Agriculture, X., Canada, A.-F., An, H., & Mohr, R. (2019). The effects of split application of enhanced efficiency fertilizers on non-winter

- nitrous oxide emissions from winter wheat. *Https://Doi.Org/10.1139/Cjss-2019-0048*, 100(1), 26–43.
<https://doi.org/10.1139/CJSS-2019-0048>
- Owens, J. L., Wang, Z., Thomas, B. W., Hao, X., Coles, K., Rahmani, E., Karimi, R., Gill, K., & Beres, B. L. (2023). Winter wheat responses to enhanced efficiency liquid nitrogen fertilizers in the Canadian Prairies. *Https://Doi.Org/10.1139/Cjps-2022-0208*, 103(4), 353–367. <https://doi.org/10.1139/CJPS-2022-0208>
- Pelster, D. E., Chantigny, M. H., Rochette, P., Bertrand, N., Angers, D. A., Zebarth, B. J., & Goyer, C. (2019). Rates and intensity of freeze–thaw cycles affect nitrous oxide and carbon dioxide emissions from agricultural soils. *Https://Doi.Org/10.1139/Cjss-2019-0058*, 99(4), 472–484.
<https://doi.org/10.1139/CJSS-2019-0058>
- Pelster, D. E., Thiagarajan, A., Liang, C., Chantigny, M. H., Wagner-Riddle, C., Congreves, K., Lemke, R., Glenn, A., Tenuta, M., Hernandez-Ramirez, G., Bittman, S., Hunt, D., Owens, J., & MacDonald, D. (2022). Ratio of non-growing season to growing season N₂O emissions in Canadian croplands: an update to national inventory methodology. *Https://Doi.Org/10.1139/Cjss-2022-0101*, 103(2), 344–352.
<https://doi.org/10.1139/CJSS-2022-0101>
- Pokharel, P., & Chang, S. X. (2021). Biochar decreases the efficacy of the nitrification inhibitor nitrapyrin in mitigating nitrous oxide emissions at different soil moisture levels. *Journal of Environmental Management*, 295, 113080. <https://doi.org/10.1016/J.JENVMAN.2021.113080>
- Raun, W. R., & Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agronomy Journal*, 91(3), 357–363. <https://doi.org/10.2134/AGRONJ1999.00021962009100030001X>;
- Rice, W. A., & Olsen, P. E. (1988). Dinitrogen fixation in soil and alfalfa nodules in the presence of nitrification inhibitors. *Soil Biology and Biochemistry*, 20(2), 245–249. [https://doi.org/10.1016/0038-0717\(88\)90044-](https://doi.org/10.1016/0038-0717(88)90044-)

- Risk, N., Snider, D., & Wagner-Riddle, C. (2013). Mechanisms leading to enhanced soil nitrous oxide fluxes induced by freeze–thaw cycles. *https://doi.org/10.4141/Cjss2012-071*, 93(4), 401–414. <https://doi.org/10.4141/CJSS2012-071>
- Robertson, G. P., & Groffman, P. M. (2015). Nitrogen... - Google Scholar. (n.d.). Retrieved June 2, 2026, from [https://scholar.google.ca/scholar?q=Robertson,+G.+P.,+%26+Groffman,+P.+M.+\(2015\).+Nitrogen+transformations.+In+E.+A.+Paul+\(Ed.\),+Soil+microbiology,+ecology+and+biochemistry+\(4th+ed.,+pp.+421%E2%80%93446\).+Academic+Press.+https://doi.org/10.1016/B978-0-12-415955-6.00014-1&hl=en&as_sdt=0,5](https://scholar.google.ca/scholar?q=Robertson,+G.+P.,+%26+Groffman,+P.+M.+(2015).+Nitrogen+transformations.+In+E.+A.+Paul+(Ed.),+Soil+microbiology,+ecology+and+biochemistry+(4th+ed.,+pp.+421%E2%80%93446).+Academic+Press.+https://doi.org/10.1016/B978-0-12-415955-6.00014-1&hl=en&as_sdt=0,5)
- Schimel, J. P., & Bennett, J. (2004). Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, 85(3), 591–602. <https://doi.org/10.1890/03-8002>
- Sennett, L. B., Brin, L. D., Goyer, C., Zebarth, B. J., & Burton, D. L. (2024). Effects of soil water content at freezing, thaw temperature, and snowmelt infiltration on N₂O emissions and denitrifier gene and transcript abundance during a single freeze-thaw event. *Biology and Fertility of Soils* 2024 60:4, 60(4), 577–591. <https://doi.org/10.1007/S00374-024-01817-W>
- Silva, A. G. B., Sequeira, C. H., Sermarini, R. A., & Otto, R. (2017). Urease inhibitor NBPT on ammonia volatilization and crop productivity: A meta-analysis. *Agronomy Journal*, 109(1), 1–13. <https://doi.org/10.2134/AGRONJ2016.04.0200>;
- Snyder, C. S., Bruulsema, T. W., Jensen, T. L., & Fixen, P. E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems & Environment*, 133(3–4), 247–266. <https://doi.org/10.1016/J.AGEE.2009.04.021>
- Stanford, G., & Smith, S. J. (1972). Nitrogen Mineralization Potentials of Soils. *Soil Science Society of America Journal*, 36(3), 465–472. <https://doi.org/10.2136/SSSAJ1972.03615995003600030029X>

- Statistics Canada. (2014). *Fertilizer Shipments Survey*. <https://www150.statcan.gc.ca/n1/en/catalogue/21-022-X2014001>
- Teepe, R., Brumme, R., & Beese, F. (2001). Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology and Biochemistry*, 33(9), 1269–1275. [https://doi.org/10.1016/S0038-0717\(01\)00084-0](https://doi.org/10.1016/S0038-0717(01)00084-0)
- Teepe, R., Vor, A., Beese, F., & Ludwig, B. (2004). Emissions of N₂O from soils during cycles of freezing and thawing and the effects of soil water, texture and duration of freezing. *European Journal of Soil Science*, 55(2), 357–365. <https://doi.org/10.1111/J.1365-2389.2004.00602.X>;
- Tenuta, M., Gao, X., Flaten, D. N., & Amiro, B. D. (2016). Lower Nitrous Oxide Emissions from Anhydrous Ammonia Application Prior to Soil Freezing in Late Fall Than Spring Pre-Plant Application. *Journal of Environmental Quality*, 45(4), 1133–1143. <https://doi.org/10.2134/JEQ2015.03.0159>;PAGEGROUP:STRING:PUBLICATION
- Tenuta, M., Gao, X., Tiessen, K. H. D., Baron, K., Sparling, B., & Stewardship, N. (2023). Placement and nitrogen source effects on N₂O emissions for canola production in Manitoba. *Agronomy Journal*, 115(5), 2369–2383. <https://doi.org/10.1002/AGJ2.21408>
- Tenuta, M., Mkhabela, M., Tremorin, D., Coppi, L., Phipps, G., Flaten, D., & Ominski, K. (2010). Nitrous oxide and methane emission from a coarse-textured grassland soil receiving hog slurry. *Agriculture, Ecosystems & Environment*, 138(1–2), 35–43. <https://doi.org/10.1016/J.AGEE.2010.03.014>
- Tenuta, M., & Sparling, B. (2011). A laboratory study of soil conditions affecting emissions of nitrous oxide from packed cores subjected to freezing and thawing. *https://doi.org/10.4141/Cjss09051*, 91(2), 223–233. <https://doi.org/10.4141/CJSS09051>

- Thapa, R., Chatterjee, A., Awale, R., McGranahan, D. A., & Daigh, A. (2016). Effect of Enhanced Efficiency Fertilizers on Nitrous Oxide Emissions and Crop Yields: A Meta-analysis. *Soil Science Society of America Journal*, 80(5), 1121–1134. <https://doi.org/10.2136/SSSAJ2016.06.0179>;
- The Fertilizer Institute. (2017). *What are the 4Rs?* Nutrient Stewardship. <https://nutrientstewardship.org/4rs/>
- Thilakarathna, S. K., & Hernandez-Ramirez, G. (2021). How does management legacy, nitrogen addition, and nitrification inhibition affect soil organic matter priming and nitrous oxide production? *Journal of Environmental Quality*, 50(1), 78–93. <https://doi.org/10.1002/JEQ2.20168>;
- Thilakarathna, S. K., Hernandez-Ramirez, G., Puurveen, D., Kryzanowski, L., Lohstraeter, G., Powers, L. A., Quan, N., & Tenuta, M. (2020). Nitrous oxide emissions and nitrogen use efficiency in wheat: Nitrogen fertilization timing and formulation, soil nitrogen, and weather effects. *Soil Science Society of America Journal*, 84(6), 1910–1927. <https://doi.org/10.1002/SAJ2.20145>
- Thomas, B. W., Gao, X., Stoeckli, J. L., Beck, R., Liu, K., Koenig, K. M., Beres, B. L., & Hao, X. (2017). Nitrapyrin Reduced Nitrous Oxide Emissions from Beef Cattle Urine Patches on a Semiarid Tame Pasture. *Soil Science Society of America Journal*, 81(6), 1537–1547. <https://doi.org/10.2136/SSSAJ2017.04.0123>
- Tiessen, K. H. D., Flaten, D. N., Bullock, P. R., Burton, D. L., Grant, C. A., & Karamanos, R. E. (2006). Transformation of fall-banded urea: Application date, landscape position, and fertilizer additive effects. *Agronomy Journal*, 98(6), 1460–1470. <https://doi.org/10.2134/AGRONJ2005.0304>;
- Tiessen, K. H. D., Flaten, D. N., Grant, C. A., Karamanos, R. E., & Entz, M. H. (2005). Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position and fertilizer additives. *https://doi.org/10.4141/S05-017*, 85(5), 649–666. <https://doi.org/10.4141/S05-017>

- Tremorin, D. G., Tenuta, M., Mkhabela, M., Flaten, D. N., & Ominski, K. H. (2012). Nitrous oxide emissions from feces and synthetic urine of cattle grazing forage grass fertilized with hog slurry. *Animal Feed Science and Technology*, 177(3–4), 225–236. <https://doi.org/10.1016/J.ANIFEEDSCI.2012.08.008>
- van Bochove, E., Prévost, D., & Pelletier, F. (2000). Effects of Freeze–Thaw and Soil Structure on Nitrous Oxide Produced in a Clay Soil. *Soil Science Society of America Journal*, 64(5), 1638–1643. <https://doi.org/10.2136/SSSAJ2000.6451638X>
- Wagner-Riddle, C., Congreves, K. A., Abalos, D., Berg, A. A., Brown, S. E., Ambadan, J. T., Gao, X., & Tenuta, M. (2017). Globally important nitrous oxide emissions from croplands induced by freeze–thaw cycles. *Nature Geoscience* 2017 10:4, 10(4), 279–283. <https://doi.org/10.1038/ngeo2907>
- Wagner-Riddle, C., Congreves, K. A., Brown, S. E., Helgason, W. D., & Farrell, R. E. (2024). Overwinter and Spring Thaw Nitrous Oxide Fluxes in a Northern Prairie Cropland Are Limited but a Significant Proportion of Annual Emissions. *Global Biogeochemical Cycles*, 38(4), e2023GB008051. <https://doi.org/10.1029/2023GB008051>;
- Wagner-Riddle, C., Hu, Q. C., van Bochove, E., & Jayasundara, S. (2008). Linking Nitrous Oxide Flux During Spring Thaw to Nitrate Denitrification in the Soil Profile. *Soil Science Society of America Journal*, 72(4), 908–916. <https://doi.org/10.2136/SSSAJ2007.0353>;ISSUE:ISSUE:DOI
- Wang, Z., Owens, J. L., Thomas, B. W., Hao, X., Coles, K., Holzapfel, C., Rahmani, E., Karimi, R., Gill, K. S., & Beres, B. L. (2023). Winter wheat responses to enhanced efficiency granular nitrogen fertilizer in the Canadian Prairies. *Https://Doi.Org/10.1139/Cjps-2022-0209*, 103(4), 368–388. <https://doi.org/10.1139/CJPS-2022-0209>
- Wertz, S., Goyer, C., Zebarth, B. J., Burton, D. L., Tatti, E., Chantigny, M. H., & Fillion, M. (2013). Effects of temperatures near the freezing point on N₂O emissions, denitrification and on the abundance and structure

- of nitrifying and denitrifying soil communities. *FEMS Microbiology Ecology*, 83(1), 242–254.
<https://doi.org/10.1111/J.1574-6941.2012.01468.X>
- Wood, M. D., Gao, X., Tiessen, K. H. D., Tenuta, M., & Flaten, D. N. (2024). Enhanced efficiency urea fertilizers and timing effects on N₂O emissions from spring wheat production in Manitoba. *Agronomy Journal*, 116(1), 51–72. <https://doi.org/10.1002/AGJ2.21448>
- Wrage, N., Velthof, G. L., Van Beusichem, M. L., & Oenema, O. (2001). Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biology and Biochemistry*, 33(12–13), 1723–1732.
[https://doi.org/10.1016/S0038-0717\(01\)00096-7](https://doi.org/10.1016/S0038-0717(01)00096-7)
- Yadav, D., & Wang, J. (2021). An improved UK-DNDC model for evaluations of soil temperature and nitrous oxide emissions from Canadian agriculture. *Plant and Soil* 2021 469:1, 469(1), 15–37.
<https://doi.org/10.1007/S11104-021-05125-2>
- Zhang, J., Zhang, W., Jansson, P. E., & Petersen, S. O. (2022). Modeling nitrous oxide emissions from agricultural soil incubation experiments using CoupModel. *Biogeosciences*, 19(19), 4811–4832.
<https://doi.org/10.5194/BG-19-4811-2022>
- Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and Molecular Biology Reviews*, 61(4), 533–616. <https://doi.org/10.1128/MMBR.61.4.533-616.1997>

2 EFFECTS OF THAWING TEMPERATURE, SOIL MOISTURE, AND FERTILIZER SOURCE ON NITROUS OXIDE EMISSIONS FROM A GLEYED HUMIC VERTISOL FOLLOWING A SIMULATED FREEZE–THAW EVENT

2.1 Abstract

Freeze-thaw cycles affect soil nitrogen dynamics and nitrous oxide (N₂O) emissions in Canadian Prairie clay soils. However, the combined effects of soil moisture, thawing temperature, and stabilized nitrogen fertilizers remain poorly understood. This study evaluated the effects of thawing temperature (4, 8, 12, and 16°C), moisture content (32, 50, 75, and 100% water-filled pore space [WFPS]), and fertilizer source (Urea and SuperU) on N₂O emissions. Repacked soil cores from Glenlea Research Station, Manitoba, were treated with urea or SuperU, frozen at -20 °C, and thawed under assigned temperature and moisture treatment conditions. N₂O flux was measured for 60 hours after thawing, and residual nitrate and ammonium were measured afterward. Soil moisture was the primary factor regulating N₂O emissions. Mean flux increased from 0.14 and 0.13 µg N₂O–N kg⁻¹ h⁻¹ at 32 and 50% WFPS to 0.95 and 1.19 µg N₂O–N kg⁻¹ h⁻¹ at 75 and 100% WFPS, respectively. Cumulative emissions increased from 6.26 and 6.45 µg kg⁻¹ at 32 and 50% WFPS to 48.12 and 82.83 µg kg⁻¹ at 75 and 100% WFPS. Mean N₂O flux also increased from 0.29 at 4°C to 0.54 µg kg⁻¹ h⁻¹ at 16°C. Fertilizer source did not significantly affect cumulative emissions. Nitrate-N peaked at 75% WFPS (18.72 mg kg⁻¹) and declined at 100% WFPS (6.43 mg kg⁻¹). Ammonium-N was higher with SuperU (22.45 mg kg⁻¹) and urea (23.31 mg kg⁻¹) than in the control (6.29 mg kg⁻¹). Effective mitigation should limit reactive nitrogen accumulation before thaw in poorly drained soils.

2.2 Introduction

Agriculture is a major contributor to both global and national greenhouse gas (GHG) emissions. In Canada, agricultural activities account for approximately 72% of anthropogenic nitrous oxide (N₂O) emissions, primarily from the use of synthetic nitrogen fertilizers (Environment Canada, 2014). Nitrous oxide is a highly potent greenhouse gas, with a global warming potential 265 times that of carbon dioxide (CO₂) and an atmospheric lifetime of about 109 years (IPCC, 2014; Prather et al., 2015). It also contributes to the depletion of the stratospheric ozone layer (Chen et al., 1995). By 2019, atmospheric concentrations of N₂O had reached 332 ppb (IPCC, 2023). In response to increasing agricultural emissions, Canada has pledged to reduce GHG emissions from nitrogen (N) fertilizer use by 30% relative to 2020 levels by 2030 (Environment and Climate Change Canada, 2025). Achieving this target requires developing sustainable N management practices that effectively reduce emissions while sustaining or enhancing crop yields (Wood et al., 2024).

Agricultural N₂O emissions present significant sustainability challenges from an economic perspective. Nitrogen losses through volatilization and denitrification reduce the proportion of fertilizer N available for crop uptake, thereby lowering nitrogen use efficiency, requiring increased fertilizer inputs, and thereby raising production costs (Agriculture and Agri-Food Canada, 2022; Butterbach-Bahl et al., 2013). In Canada, N fertilizer use increased by 93% between 2005 and 2021, while associated N₂O emissions rose by 94%, indicating a strong correlation between fertilizer inputs and agricultural emissions (Agriculture and Agri-Food Canada, 2023). Such losses result in economic consequences, as producers incur costs for fertilizer N that may be lost prior to crop uptake, thereby diminishing the return on fertilizer investment. At the same time, climate-related impacts associated with elevated N₂O levels, including droughts, floods, and temperature fluctuations, adversely affect crop productivity and increase yield variability (Mapfumo et al., 2023). These environmental and economic consequences highlight the need for integrated N

management strategies that reduce N losses while sustaining the productivity and resilience of agricultural systems.

This problem is particularly important in the Canadian Prairies, where N fertilizer may be applied in the fall before soil freeze-up (Manitoba Agriculture, 2007; Tiessen et al., 2005). A fall N application may provide an operational advantage by reducing the number of field activities required during the short spring seeding period (Amiro et al., 2017; Mezbahuddin et al., 2020). It may also provide an economic advantage when fertilizer is purchased at lower fall prices, although this benefit varies among years and may be offset by financing, storage, and potential overwinter N losses (Amiro et al., 2017; Mezbahuddin et al., 2020). However, fall-applied N remains in the soil throughout the winter and may become vulnerable to transformation and loss before crop uptake begins in the spring season. To reduce this risk, Prairie recommendations generally encourage fall fertilizer to be banded and applied after soil temperature declines below 5°C, when microbial activity and nitrification are slowed (Lasisi et al., 2020; Manitoba Agriculture, 2007; Tenuta et al., 2016). Nonetheless, N retained during the winter may become susceptible to loss when soils thaw in spring.

Spring thaw is a critical period because N₂O emissions can occur as short-lived but intense pulses (Risk et al., 2013). Freeze–thaw cycles (FTCs), characterized by soil temperature fluctuations near 0°C, are common in Prairie soils, especially in early spring and late fall (Liu et al., 2021). These cycles, influenced by the greater heat capacity of soil relative to air (Zhang et al., 2005; Zheng et al., 1993), impact soil structure, microbial communities, and the availability of nitrogen substrates such as nitrate and labile carbon (Christensen & Tiedje, 1990; Teepe et al., 2001). During freezing, microbial activity decreases but persists in unfrozen water films, preserving microbial viability (Edwards et al., 2006; Teepe et al., 2001). Freeze–thaw processes also modify soil physical properties by disrupting aggregates, exposing organic matter, and altering pore connectivity (Leuther & Schlüter, 2021; Matzner & Borken, 2008; Shi et al., 2024). When soil thaws, microbial activity resumes rapidly, and previously protected or immobilized

substrates become available for transformation (Risk et al., 2013). The release of mineral N and labile carbon can stimulate nitrification and denitrification, resulting in pronounced N₂O emission pulses that contribute significantly to annual emissions (Wagner-Riddle et al., 2008). The magnitude of this response depends strongly on soil moisture. These effects are particularly pronounced in fine-textured soils, where high water retention and limited drainage maintain elevated soil moisture during thaw (Balaine et al., 2013). Under these conditions, soil moisture strongly influences whether N transformations occur under aerated, partially aerated, or oxygen-limited environments (Balaine et al., 2013; Bateman & Baggs, 2005; Linn & Doran, 1984).

Several strategies have been proposed to reduce N losses from fall-applied fertilizer. The 4R nutrient stewardship promotes the use of the right fertilizer source at the right rate, time, and place (Liu et al., 2022). In addition to the 4R management, enhanced-efficiency fertilizers have been developed to slow specific N transformations and losses. Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), delay the hydrolysis of urea fertilizer, whereas nitrification inhibitors such as dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), and nitrapyrin slow the microbial oxidation of ammonium (NH₄⁺) to nitrate (NO₃⁻) (Abalos et al., 2014; Akiyama et al., 2010; Trenkel, 2021; Thapa et al., 2016). SuperU is a stabilized urea fertilizer that contains both NBPT and DCD formulated to reduce N losses following urea application (Ghosh et al., 2019). Although SuperU and other enhanced-efficiency fertilizers have shown potential to reduce N₂O emissions and improve NUE, their performance has been inconsistent under Canadian Prairie conditions (Asgedom et al., 2014; Gao et al., 2015; Tenuta et al., 2023; Wood et al., 2024). On a heavy clay soil in Manitoba, polymercoated ESN reduced the N₂O emission factor 0.7% of applied N compared with 1.7% for conventional urea, whereas SuperU had the same emission factor as conventional urea at 1.7% (Asgedom et al., 2014). In Manitoba, a spring-wheat study found that mid-row banded SuperU and ESN reduced cumulative N₂O emissions by 47% relative to broadcast-incorporated conventional urea during the warmer, wetter early-season conditions of 2011, but this benefit

was not consistently observed under drier conditions (Gao et al., 2015). More recently, a study by Tenuta et al. (2023) in southern Manitoba found that SuperU significantly reduced N₂O emissions in four of the six canola site years. Similarly, Wood et al. (2024) reported that SuperU reduced N₂O emissions by 37–57% in three of six spring wheat site years, while yield, grain protein, and N uptake were unaffected by N source in five of six site-years. These findings indicate that SuperU and other similar products should not be assumed to perform consistently under all Prairie soil conditions.

Despite increasing evidence that FTCs contribute to agricultural N₂O emissions, the combined effects of thawing temperature, soil moisture, and fertilizer source remain insufficiently understood in Prairie clay soils. Most previous studies have investigated freeze–thaw responses under temperatures or moisture that do not reflect field conditions, and relatively few have assessed inhibitor-treated fertilizers under wet-thaw conditions representative of poorly drained, fine-textured soils (Asgedom et al., 2014; Gao et al., 2015; Tenuta et al., 2023; Tenuta & Sparling, 2011; Wood et al., 2024). This knowledge gap limits the ability to determine whether SuperU can consistently reduce post-thaw N₂O from fall-applied N under contrasting moisture and conditions.

This study therefore examined the effects of thawing temperature (4, 8, 12, and 16°C), soil moisture (32, 50, 75, and 100% WFPS), and fertilizer source (urea and SuperU) on N₂O emissions from a Gleyed Humic Vertisol soil following a simulated freeze–thaw event. The study hypothesized that (1) N₂O emissions increase with higher thawing temperatures and elevated soil moisture due to enhanced microbial activity and reduced oxygen availability during thaw, and (2) SuperU reduces N₂O emissions compared to conventional urea by slowing nitrogen transformations and limiting nitrate accumulation available for denitrification.

2.3 Materials and Methods

2.3.1 Study site description

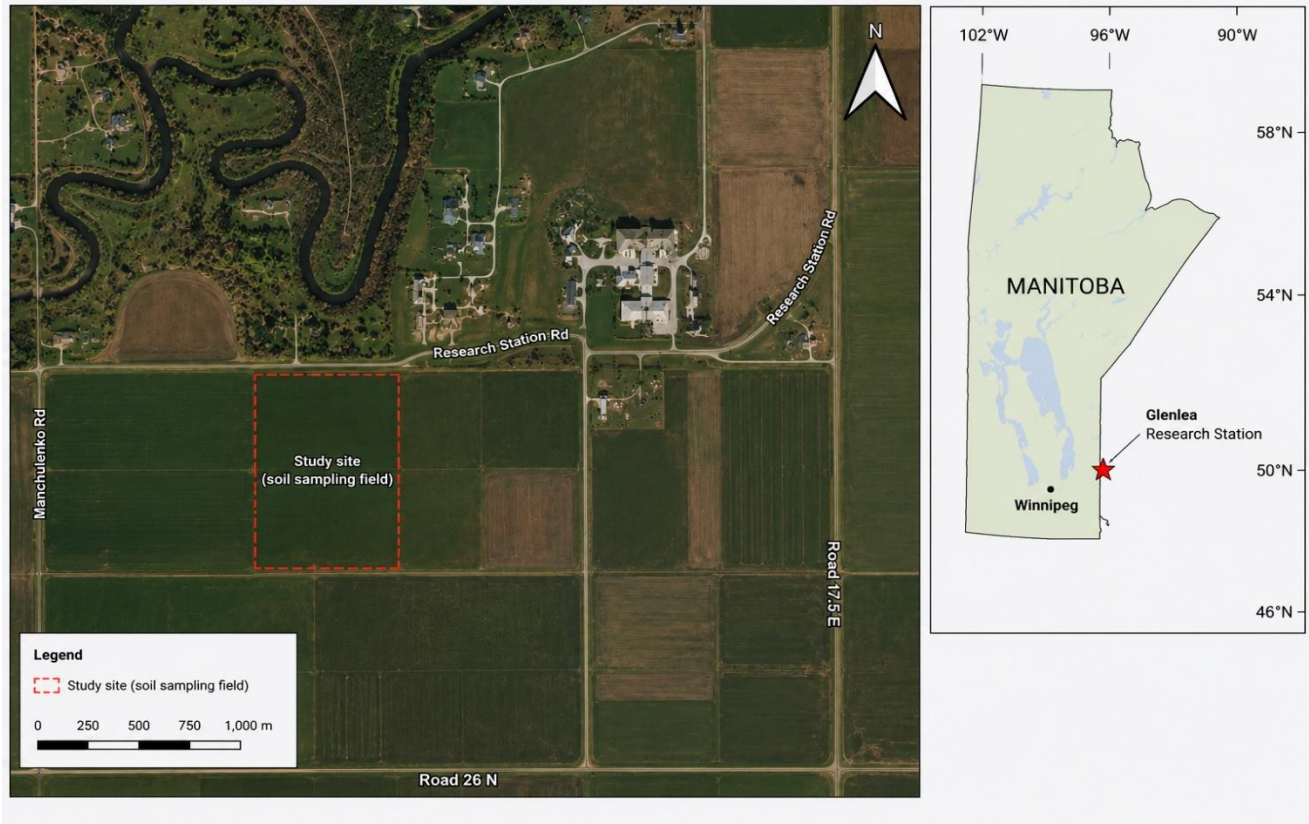


Figure 2.1 Location of the soil sampling site at Glenlea Research Station, Winnipeg, Manitoba, Canada

The soil for the study was collected from the Glenlea Research Station in the Red River Valley, Manitoba ($49^{\circ}39'00.000''$ N and $97^{\circ}07'00.000''$ W), with an elevation of 238.7 m above sea level (Google Earth, 2025). The soil at this location is a Gleyed Humic Vertisol (Soil Classification Working Group, 1998). The soil is imperfectly drained, which promotes water retention and delays drainage during spring snowmelt (Asgedom et al., 2014). According to the 1981–2010 climate normals (Environment and Climate Change Canada, 2010), the Glenlea area exhibits a mean annual air temperature of 2.8°C . Mean temperatures reach approximately 16.0°C during the growing season (May–September) and decrease to -6.7°C during

the non-growing season (October–April), resulting in conditions that promote seasonal soil freezing and thawing (Environment and Climate Change Canada, 2025).

To characterize the regional freeze–thaw regime, daily soil temperature and moisture data from Real-time In-Situ Soil Monitoring for Agriculture (RISMA) station MB10 were used as a proxy for Glenlea, since Glenlea is not an official RISMA station. Therefore, MB10 data provide regional context rather than direct site measurements. MB10 is located in the Sturgeon Creek watershed, 43 km from the study site, where RISMA metadata describe the landscape as predominantly flat with imperfectly to poorly drained lacustrine clay and clay loam soils (Ward, 2019). According to MB10 data, the 0–5 cm soil layer reached winter minimum temperatures ranging from -20.1 to -6.6 °C between 2014/2015 and 2021/2022, while the average initial spring-thaw temperature was 0.45 °C. Fall volumetric water content in the 0–5 cm soil layer from October 15 to November 10 ranged from 0.223 to 0.630 m³ m⁻³ (Agriculture and Agri-Food Canada, 2026). These data indicate a regional freeze–thaw regime characterized by sustained winter freezing, spring thaw near 0 °C, and relatively wet near-surface soil conditions, which are relevant to N₂O production in clayey, imperfectly drained Prairie soils

2.3.2 Soil Sample Collection and Preparation

Soils were collected from a depth of 0–15 cm using shovels and placed into 5-litre plastic buckets. They were then air-dried for one week and sieved through a $2\text{ mm} \times 7\text{ mm}$ diamond-shaped mesh to standardize aggregate size across all treatments (Carter & Gregorich, 2007; Schindlbacher et al., 2004). The sieved soils were homogenized to ensure consistency in physical and chemical properties (Carter & Gregorich, 2007; Schindlbacher et al., 2004). Prior to the experiment, baseline characteristics such as organic matter content, cation exchange capacity (CEC), electrical conductivity (EC), pH, total nitrogen, nitrate (NO₃⁻), ammonium (NH₄⁺), and particle size distribution were determined according to protocols described in section 2.3.3.4.

2.3.3 Baseline soil properties

2.3.3.1 Soil pH and cation exchange capacity

Soil pH and CEC were measured after homogenizing the soil samples. Soil pH was determined using the protocol described by Hendershot et al. (2008) where 10 g of soil was transferred to a 50 mL beaker and 10 mL of deionized water was added. The mixture was agitated for 15 minutes using a mechanical shaker and then allowed to settle undisturbed for 30 minutes. After settling, the pH of the suspension was measured with a pH meter (Thermo Scientific Orion Star A215).

Cation exchange capacity was determined using the NH_4^+ saturation method, following the protocol described by Carter & Gregorich (2007). Briefly, 2 g of ground soil was weighed into a 30 mL centrifuge tube, and 25 mL of 10% ethanol was added to remove soluble salts. The mixture was shaken for 30 minutes and then centrifuged at 10,000 rpm. Subsequently, 20 mL of 1 M NH_4Cl in 60% aqueous ethanol, buffered at pH 8.5 with NH_3 , was added to the soil, and the suspension was agitated for 30 minutes. After shaking, the sample was centrifuged, and the supernatant was decanted into a 100 mL volumetric flask. This extraction process was repeated three times using the same NH_4Cl solution, and once with 15 mL of 0.05 M NH_4Cl (also at pH 8.5 in 60% ethanol) to reduce the NH_4Cl concentration. In total, five extracts were collected and combined. The combined extract was then acidified with 2 mL of 10 M HCl and brought to volume (100 mL) in the flask. The resulting solution was analyzed for exchangeable cations, including Ca^{2+} , Mg^{2+} , and K^+ , to calculate the soil's cation exchange capacity.

2.3.3.2 Organic matter content

Soil organic matter content was determined using the loss-on-ignition (LOI) method, following the procedure described by Nelson & Sommers (1996). Crucibles were first pre-heated in a muffle furnace at 400°C for 2 hours, then cooled and weighed empty. Three grams (3 g) of air-dried soil was placed into each tared crucible. The samples were oven-dried at 105°C for 24 hours, cooled in a desiccator over calcium chloride (CaCl_2), and weighed to determine oven-dry weight (by subtracting the empty crucible

weight). The oven-dried samples were then placed in a muffle furnace and ignited at 400°C for 16 hours. After ignition, the crucibles were cooled, and the weight of the remaining mineral residue was recorded. The weight loss due to combustion was attributed to the organic matter content.

Loss-on-ignition (LOI) was calculated using the following equation

$$\text{Organic matter content (g kg}^{-1}\text{)} = \frac{\text{Weight}_{105} - \text{Weight}_{400}}{(\text{Weight}_{105})} \times 1000 \quad (1)$$

where weight_{105} is the oven-dry weight at 105°C after subtracting the empty weight of the crucible, and weight_{400} is the weight after ignition at 400°C, also after subtracting the empty weight of the crucible.

2.3.3.3 Soil bulk density and water-filled pore space

Soil was packed to a bulk density of 0.815 g cm⁻³, representing field conditions at the study site. This bulk density was used to calculate soil porosity, which was subsequently used to determine the water-filled pore space (WFPS). The equations used for these calculations are as follows:

$$f = 1 - \frac{BD}{PD} \quad (2)$$

where f is total porosity, BD is bulk density (g cm⁻³), and PD is particle density assumed to be 2.65 g cm⁻³

Moisture content (%) was calculated following the procedure described by Fichtner et al. (2019). It was estimated using the following formula:

$$\text{WFPS} = \frac{SWC}{f} \times 100 \quad (3)$$

where WFPS is water-filled pore space (%), SWC is the volumetric soil water content (vol %), and f is porosity.

2.3.3.4 Ammonium and Nitrate

Extraction of ammonium-N ($\text{NH}_4^+\text{-N}$) and nitrate-N ($\text{NO}_3^-\text{-N}$) from the destructively sampled soils was carried out using a 2.0 M KCl solution (Carter & Gregorich, 2007). Air-dried soil samples (5 g) were weighed into 50 mL Eppendorf tubes, after which 25 mL of 2.0 M KCl were added, maintaining a 1:5 soil-to-extractant ratio (g:mL). The tubes were sealed with stoppers and shaken mechanically at 60 strokes per minute for 30 minutes to ensure thorough extraction. Following agitation, the suspensions were filtered through Whatman No. 1 filter paper. The filtrates were analyzed for mineral nitrogen ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) using a continuous-flow analyzer (Skalar, SA 1100, Netherlands) with colorimetric detection.

2.3.3.5 Total nitrogen

Total soil nitrogen was quantified using the Kjeldahl digestion method. Air-dried soil samples (0.2 g) were placed in Kjeldahl digestion tubes, and 4.4 mL of digestion mixture was added. The digestion mixture was prepared according to the procedure outlined by Thomas et al. (1967). The tubes were heated on a digestion block until frothing ceased, then the temperature was gradually increased to 350 °C and maintained for 1 hour. Following digestion, the tubes were cooled to room temperature, and the digest was diluted to 50 mL with deionized water in a volumetric flask. The resulting solution was transferred to vials and analyzed using a Technicon Autoanalyzer.

Table 2.1 Baseline properties for soil samples

Soil property	Value
Bulk density (g cm^{-3})	0.815
Organic matter (g kg^{-1})	115
Particle size distribution	
Sand (%)	27
Silt (%)	28
Clay (%)	45
CEC (cmol (+) kg^{-1})	15
pH (water)	7.2
NO_3^- -N (mg kg^{-1})	13.4
NH_4^+ -N (mg kg^{-1})	6.6
Total N (g kg^{-1})	5.05

Note: CEC = Cation Exchange Capacity

2.3.4 Experimental design

The experiment was a randomized complete block design (RCBD) with a 4×12 split-plot treatment structure, replicated across three blocks (per time), with Figure 2.2 showing the layout of one block. The factors include thawing temperature (4, 8, 12, and 16 °C), moisture content (32%, 50%, 75%, and 100% WFPS), and fertilizer source (SuperU, urea, and an unfertilized control). The main plot factor was temperature, and the subplot factors were moisture level and fertilizer type. Four incubation chambers were used for each of the four thawing temperatures. To avoid bias, each thawing temperature was

randomly assigned to one of the incubation chambers. In total, 48 experimental units were used per time (block), with 12 experimental units assigned to each incubation chamber.

Experimental Layout for One Replicate: Split-Plot Design (4 × 12)

Chamber 1: 12°C				Chamber 2: 8°C			
75% Urea	100% SuperU	50% Control	75% SuperU	32% Control	32% Urea	100% Urea	75% Urea
32% SuperU	100% Urea	50% Urea	75% Control	50% Urea	75% SuperU	50% Control	100% Control
32% Urea	32% Control	100% Control	50% SuperU	32% SuperU	100% SuperU	75% Control	50% SuperU
Chamber 3: 16°C				Chamber 4: 4°C			
50% Control	100% Control	50% SuperU	100% Urea	100% SuperU	100% Urea	75% Urea	50% SuperU
32% Urea	75% Urea	75% Control	75% SuperU	32% SuperU	32% Control	50% Urea	75% Control
32% Control	32% SuperU	50% Urea	100% SuperU	50% Control	100% Control	75% SuperU	32% Urea

Each chamber contains 12 randomized moisture × fertilizer source combinations.

Temperature is assigned at the chamber level (main plot).

Figure 2.2 Treatment layout in Block 1 showing treatment randomization within the incubation chambers

2.3.4.1 Soil moisture treatments

A total of 967 g of air-dry soil was weighed into 2 L freeze-tolerant glass jars with an internal diameter of 9.5 cm to achieve a bulk density of 0.82 g cm^{-3} at a soil column height of 10 cm. Moisture contents were then adjusted to 32%, 50%, 75%, and 100% WFPS to reflect the designated treatment levels. The experimental units were placed in a refrigerator and allowed to equilibrate for 36 hours at 4 °C. The equilibration duration was determined by inserting moisture sensors (S-SMC-M005) near the bottom of the soil-filled jars and continuously monitoring moisture levels until they stabilized, indicating that equilibrium had been reached throughout the soil column. To minimize moisture loss from air circulation

caused by the in-built fan in the freeze–thaw (FT) chamber, the tops of the jars were sealed with parafilm (PM-996, USA). To prevent the accumulation of N₂O in the headspace, five small holes (3 mm in diameter) were punched into the parafilm using a thin plastic rod, following the method described by Tenuta & Sparling (2011). During the thawing period, soil moisture levels were monitored by recording the weight of each jar at each gas sampling interval.

2.3.4.2 Fertilizer treatment

Fertilizers were applied in bands 5 cm deep and 10 cm long in the soil. These bands were created by digging a narrow trench, about 10 cm long and 5 cm deep, in the compacted soil using a spatula. The amount to supply the rate of 12.8 g N m⁻² (128 kg N ha⁻¹) of fertilizer was evenly spread into the trench and then covered with soil. Two types of fertilizer were used: urea and SuperU. The application rate was based on soil test recommendations (Manitoba Agriculture, 2007).

2.3.4.3 Freezing and thawing temperatures

All soil samples were frozen at -20 °C for 48 hours using a freeze-thaw chamber (UTEST, UTD-1442). Given that freezing intensity can affect microbial survival (Henry, 2007), the chamber temperature was gradually reduced over 12 hours to allow the soil microbial community to acclimate to the changing thermal conditions. After reaching the target freezing temperature, each batch was held at this temperature for 48 hours. During this interval, the soil was considered representative of winter conditions because it was frozen. The chosen freezing temperature corresponded to the minimum soil temperature observed at the study site. Following freezing, the soils were thawed at one of four temperatures: 4°C, 8°C, 12 °C, or 16 °C, for a period of 60 hours.

2.3.5 Nitrous oxide emissions

Nitrous oxide emission was measured during the thawing period at each temperature treatment following freezing at -20 °C. Gas samples were collected from the headspace of the plastic jars. Before each sampling event, the headspace was evacuated by repeatedly inserting and removing (10 times) a cylindrical plastic container (250 mL). The jars were then securely sealed with a lid equipped with a septum stopper and allowed to sit for 2 h to allow gas to accumulate for sampling. Gas sampling was conducted over a 60-hour thawing period (2.5 days), with samples taken at 0, 3, 6, 10, 20, 30, 40, 50, and 60 hours. The 60-hour thawing duration is based on the activity pattern of denitrifying bacteria, which typically reach peak performance after approximately 50 hours of incubation at 4 °C (Braker et al., 2010). At each time point, a 20 mL disposable syringe (Becton, Dickson and Company, Franklin Lakes, USA) was used to extract gas from the headspace via the septum. The collected gas was then transferred into 12 mL evacuated exetainers for N₂O analysis using a gas chromatograph (Varian CP-3800, Walnut Creek, USA). Following each sampling event, the lids were replaced with parafilm (PM-996, USA), perforated with five evenly spaced 3 mm holes to minimize moisture loss and prevent gas accumulation. Ambient air samples were collected at room temperature from three different locations within the laboratory for background correction (Parkin & Venterea, 2010; Rochette & Bertrand, 2008). Upon completion of gas sampling, the experimental units were sampled for analysis of soil mineral nitrogen (NO₃⁻ and NH₄⁺) concentrations. The jars were emptied, and the soil was thoroughly mixed and air-dried to determine mineral nitrogen content following the procedure outlined in Section 2.3.3.4.

2.3.6 Statistical Analysis

Treatment effects were evaluated using PROC GLIMMIX in SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). N₂O flux was modeled using a gamma distribution, while cumulative N₂O emissions and residual mineral N concentrations (NH₄⁺-N and NO₃⁻-N) were modeled using a normal distribution. A three-way ANOVA assessed the fixed effects of thawing temperature, soil moisture, fertilizer source, and their interactions on cumulative N₂O emissions and residual mineral nitrogen (NH₄⁺-N and NO₃⁻-N). Blocks were included as random effects, and the block by temperature interaction served as the main-plot error term. For N₂O flux, a repeated-measures three-way ANOVA was performed with thawing temperature, soil moisture, fertilizer source, sampling time, and their interactions as fixed effects. Time was designated as the repeated-measures factor because flux measurements were collected from the same experimental units at multiple time points. Several covariance structures were compared, including unstructured (UN), first-order ante-dependence (ANTE (1)), compound symmetry (CS), first-order autoregressive (AR (1)), and spatial power (SP(POW)). The structure with the lowest Akaike Information Criterion (AIC) was selected, resulting in the use of AR (1) for the final analysis. When significant main effects or interactions were detected, least-squares means were separated using Tukey-adjusted pairwise comparisons. Statistical significance was determined at $P < 0.05$.

2.4 Results and Discussion

2.4.1 N₂O flux

N₂O flux was significantly influenced by soil moisture ($P < 0.0001$) and sampling time ($P < 0.0001$) (Table 2.2). The main effects of thawing temperature ($P = 0.08$) and fertilizer source ($P = 0.05$) were not significant. However, significant moisture $\times$ temperature ($P = 0.01$), moisture $\times$ fertilizer source ($P = 0.004$), temperature $\times$ fertilizer source ($P = 0.01$), and moisture $\times$ temperature $\times$ fertilizer source ($P < 0.0001$) interactions were detected.

At 32% and 50% WFPS, N₂O fluxes generally remained below 1.0 µg N₂O–N kg⁻¹ h⁻¹ across thawing temperatures and fertilizer treatments (Figure 2.3). By comparison, fluxes were higher at 75% and 100% WFPS, ranging from approximately 0.4 to 2.0 and 0.7 to 2.3 µg N₂O–N kg⁻¹ h⁻¹, respectively, depending on thawing temperature and fertilizer treatment.

The effect of thawing temperature became more pronounced under wetter conditions. At 75% WFPS, N₂O fluxes rose from 0.96 to 1.72 µg N₂O–N kg⁻¹ h⁻¹ for the control, from 0.53 to 1.52 µg N₂O–N kg⁻¹ h⁻¹ for SuperU, and from 0.76 to 1.97 µg N₂O–N kg⁻¹ h⁻¹ for urea, as temperature increased from 4 to 16 °C. At 100% WFPS, the corresponding increases were from 0.67 to 1.28 µg N₂O–N kg⁻¹ h⁻¹ for the control, from 1.00 to 1.78 µg N₂O–N kg⁻¹ h⁻¹ for SuperU, and from 0.71 to 2.29 µg N₂O–N kg⁻¹ h⁻¹ for urea. In contrast, mean fluxes at 32% and 50% WFPS remained below 0.50 µg N₂O–N kg⁻¹ h⁻¹. The largest mean flux observed was 2.29 µg N₂O–N kg⁻¹ h⁻¹ for urea at 100% WFPS and 16°C, compared to 1.78 µg N₂O–N kg⁻¹ h⁻¹ for SuperU and 1.28 µg N₂O–N kg⁻¹ h⁻¹ for the control under identical conditions.

N₂O flux was significantly affected by soil moisture ($p < 0.0001$), with fluxes at 75% and 100% WFPS significantly greater than those at 32% and 50% WFPS. Thawing temperature alone was not significant ($p = 0.0784$), but its interaction with soil moisture was significant ($p = 0.0110$), suggesting that temperature effects depended on moisture conditions. These results identify soil moisture as the dominant control of post-thaw N₂O flux

The significant effect of soil moisture on N₂O flux suggests it was the dominant factor regulating N₂O production following thaw. Soil moisture affects microbial activity, substrate mobility, oxygen diffusion, and gas transport, all of which directly influence nitrification and denitrification processes (Bateman & Baggs, 2005; Butterbach-Bahl et al., 2013; Dobbie & Smith, 2003; Linn & Doran, 1984). The consistently low fluxes at 32% and 50% WFPS suggest that limited water availability restricted microbial activity, maintaining sufficient soil aeration to suppress denitrification. In contrast, the significant increases at 75%

and 100% WFPS indicate that wetter conditions reduced oxygen diffusion and promoted anaerobic conditions, thereby favoring N₂O production.

The observed response to soil moisture aligns with findings from previous freeze–thaw studies. Teepe et al. (2001) observed N₂O emission peaks lasting 1–3 days during soil thawing. The initial thaw produced an average peak flux of 184 µg N₂O–N m⁻² h⁻¹, with a maximum of 330 µg N₂O–N m⁻² h⁻¹. During the subsequent thaw, the mean peak flux across four soil columns reached 279 µg N₂O–N m⁻² h⁻¹. These thaw-related peaks were attributed to the rapid release of N₂O trapped during freezing and to potential denitrification processes occurring during thaw. Similarly, Mørkved et al. (2006), Wagner-Riddle et al., (2008), Risk et al. (2013), and Bhowmik et al. (2017) identified soil moisture as a primary determinant of thaw-induced N₂O emissions. Bhowmik et al. (2017) specifically found that N₂O emissions from urea-amended soil increased from 145 µg N₂O kg⁻¹ soil at 40% WFPS and 300 µg N₂O kg⁻¹ soil at 60% WFPS to 471 µg N₂O kg⁻¹ soil at 80% WFPS, consistent with the marked increase observed between 50% and 75% WFPS in the present study. However, the present study extends this relationship to a poorly drained clay soil under controlled thaw temperatures, identifying a sharp moisture response above 50% WFPS and demonstrating that the influence of thawing temperature on N₂O flux depends strongly on soil moisture.

The greater N₂O fluxes observed at 75–100% WFPS under higher thawing temperatures combinations may reflect enhanced microbial N transformation, as increasing temperature can stimulate microbial metabolism and N₂O-producing processes when soil moisture is sufficient (Firestone & Davidson 1989; Butterbach-Bahl et al., 2013). Sennett et al. (2024) reported that cumulative N₂O emissions from soils frozen at 30% WFPS were more than twice as high when thawed at 8 °C compared to 1.5 °C, while emissions remained low at 15% WFPS at both temperatures. These findings are consistent with the present study, which found that increasing temperature resulted in greater increases in N₂O flux at 75% and 100% WFPS than at 32% and 50% WFPS.

Fertilizer responses depended on soil moisture and thawing temperature. At 32% WFPS and 12 °C, the N₂O flux was significantly greater in the control (0.842 µg N₂O–N kg⁻¹ h⁻¹) compared to SuperU (0.065 µg N₂O–N kg⁻¹ h⁻¹) and urea (0.066 µg N₂O–N kg⁻¹ h⁻¹), with no significant difference between SuperU and urea. At 50% WFPS and 12 °C, SuperU resulted in a significantly higher N₂O flux (0.663 µg N₂O–N kg⁻¹ h⁻¹) than both the control and urea. At 100% WFPS and 16 °C, although urea produced the highest numerical flux (2.291 µg N₂O–N kg⁻¹ h⁻¹), differences among fertilizer treatments were not statistically significant. These results demonstrate that although fertilizer source interacted with other factors, no single fertilizer consistently caused the highest or lowest N₂O flux across all moisture and temperature conditions. Efficiency fertilizers such as SuperU are designed to reduce N losses by delaying urea hydrolysis and nitrification through NBPT and DCD inhibitors (Akiyama et al., 2010; Trenkel, 2010; Thapa et al., 2016). However, the effectiveness of these inhibitors is strongly influenced by environmental conditions, including soil moisture, temperature, microbial activity, and the timing of fertilizer application relative to thaw (Abalos et al., 2014; Grant et al., 2020a; Lin, Hernandez-Ramirez, et al., 2021). Similar variability has been reported in Prairie studies, where environmental conditions often exerted stronger control over N₂O emissions than fertilizer source (Gao et al., 2015; Tenuta et al., 2023; Wood et al., 2024).

These findings are particularly relevant for Prairie clay soils, such as the Gleyed Humic Vertisol examined in this study. These soils have high water-holding capacity and restricted drainage, which can sustain elevated WFPS during snowmelt and spring thaw (Soil Classification Working Group, 1998; Tenuta et al., 2019). Consequently, wet spring conditions may significantly increase thaw-induced N₂O emissions by extending periods of oxygen limitation and promoting denitrification. The results indicate that soil moisture exerts a stronger influence on post-thaw N₂O flux than either fertilizer source or moderate differences in thawing temperature.

Table 2.2 Effect of soil moisture, thawing temperature, and fertilizer source on hourly N₂O flux and cumulative emissions

Effects	N ₂ O flux (µg kg ⁻¹ h ⁻¹)	Cumulative N ₂ O (µg kg ⁻¹)
Moisture		
32% WFPS	0.14	6.26
50% WFPS	0.13	6.45
75% WFPS	0.95	48.12
100% WFPS	1.19	82.83
Temperature		
4 °C	0.29	15.19
8°C	0.34	17.55
12 °C	0.38	19.77
16°C	0.54	30.52
Fertilizer source		
Control	0.39	16.98
Urea	0.32	18.65
SuperU	0.43	25.37
Sampling time(hr)		
0	0.07	
3	0.34	
6	0.33	
10	0.39	
20	0.47	
30	0.57	
40	0.64	
50	0.49	
60	0.57	
	<i>p-value</i>	
Time	<0.0001	
Moisture (M)	<0.0001	<.0001
Temperature (T)	0.0784	0.1346
Fertilizer source (FS)	0.0514	0.2941
Moisture x Temperature	0.0110	0.2486
M x FS	0.0042	0.3039
T x FS	0.0063	0.5742
M x T x FS	<.0001	0.2023

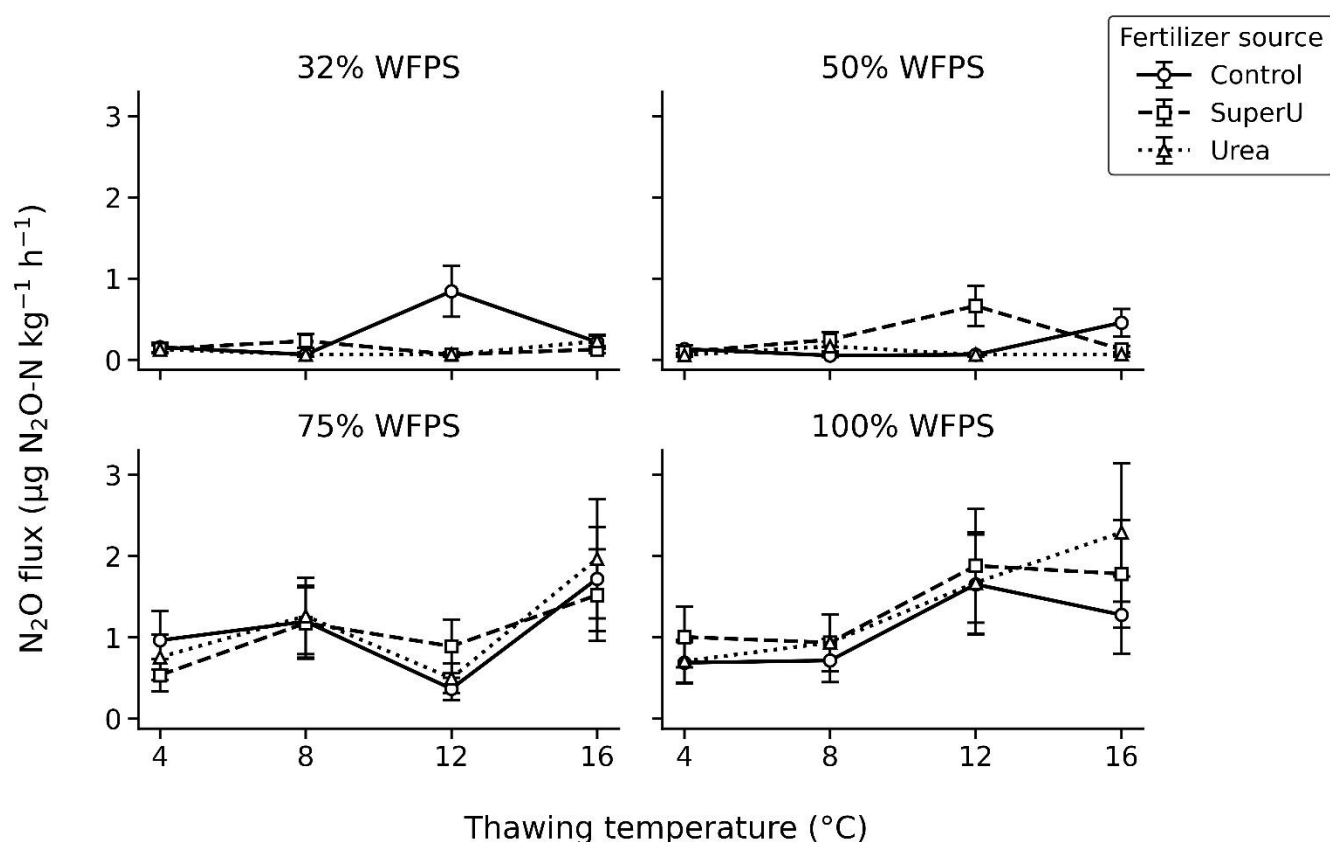


Figure 2.3 Effect of thawing temperature, soil moisture, and fertilizer source on N₂O flux. Error bars represent the standard errors of the mean.

2.4.2 Cumulative N₂O emissions

Soil moisture had a significant effect on cumulative N₂O emissions ($P < 0.0001$), while neither thawing temperature ($P = 0.1346$) nor fertilizer source ($P = 0.2941$) significantly influenced cumulative emissions (Table 2.2). No interaction terms were significant.

Cumulative N₂O emissions increased significantly with higher WFPS (Table 2.2). The lowest emissions occurred at 32% and 50% WFPS, averaging 6.26 and 6.45 µg N₂O-N kg⁻¹, respectively. These treatments did not differ significantly and constituted the low-emission group. In contrast, emissions rose to 48.12 µg N₂O-N kg⁻¹ at 75% WFPS and 82.83 µg N₂O-N kg⁻¹ at 100% WFPS, forming a distinct high-emission group with significantly greater emissions than the 32% and 50% WFPS treatments.

The increase in cumulative emissions between 50% and 75% WFPS was especially pronounced. Emissions at 100% WFPS were approximately 13 times higher than those at 32% WFPS. Although cumulative emissions increased numerically with thawing temperature, differences among temperatures were not statistically significant. Similarly, fertilizer source did not significantly affect cumulative N₂O emissions.

Overall, soil moisture was the primary factor controlling cumulative N₂O emissions, with the greatest losses occurring under wet-thaw conditions. The dominant effect of soil moisture on cumulative N₂O emissions demonstrates that it was the primary regulator of total N₂O loss during thaw. Soil moisture determines oxygen availability and influences the balance between nitrification and denitrification, thereby controlling the extent of N₂O production (Butterbach-Bahl et al., 2013; Dobbie & Smith, 2003; Linn & Doran, 1984). The approximately 1200% increase in cumulative N₂O between the driest (32% WFPS) and wettest (100% WFPS) treatments underscores the critical role of moisture in regulating N₂O losses from thawing soils.

The separation of 32% and 50% WFPS from 75% and 100% WFPS indicates a moisture threshold response. Similar threshold behavior has been widely documented, with N₂O emissions increasing rapidly once WFPS exceeds approximately 60% due to restricted oxygen diffusion and enhanced denitrification (Balaine et al., 2013; Bateman & Baggs, 2005; Dobbie & Smith, 2003). During thaw, this response may be intensified by the release of labile carbon and nitrogen from disrupted aggregates and damaged microbial cells, which provide readily available substrates for microbial metabolism (Christensen & Tiedje, 1990; Teepe et al., 2001; Fu et al., 2019).

The significant effect of moisture observed in this study was expected and aligns with previous freeze–thaw research. Wagner-Riddle et al. (2008) reported that during spring thaw, N₂O fluxes from soil columns labeled with nitrate in the 0–5 cm surface layer were 1.5 to 5 times higher than those labeled at 12–17 cm. The study estimated that the physical release of previously trapped N₂O accounted for only approximately

22% of the initial emission event, suggesting that the majority of the thaw pulse resulted from new N₂O production via denitrification in the wet surface soil. Risk et al. (2013) also identified soil moisture as a major determinant of non-growing-season N₂O losses. Pelster et al. (2019) also demonstrated that moisture conditions can strongly influence annual N₂O budgets by affecting non-growing-season emissions.

Although cumulative N₂O emissions increased from 15.19 µg N₂O–N kg⁻¹ at 4 °C to 30.52 µg N₂O–N kg⁻¹ at 16 °C; however, differences among thawing temperatures were not statistically significant ($p = 0.1346$). Therefore, the observed increase with temperature reflects a numerical trend rather than a statistically significant temperature effect on cumulative N₂O emissions.

Previous studies have shown that warmer thawing conditions stimulate microbial recovery and accelerate nitrogen turnover after freezing (Mørkved et al., 2006; Wertz et al., 2013). However, moisture typically exerts stronger control over cumulative emissions by determining the extent of oxygen limitation and denitrification (Balaine et al., 2013; Bateman & Baggs, 2005; Bhowmik et al., 2017). These results suggest that moisture availability was sufficiently dominant to override differences among thawing temperatures.

Fertilizer source did not significantly affect cumulative emissions. Although enhanced-efficiency fertilizers often reduce N₂O emissions under field conditions by delaying nitrification and nitrate accumulation (Abalos et al., 2014; Akiyama et al., 2010; Thapa et al., 2016), their effectiveness may vary under freeze–thaw conditions. Environmental factors such as soil moisture, temperature, and oxygen availability frequently exert stronger effects on N₂O production than fertilizer formulation alone (Grant et al., 2020; Hernandez-Ramirez, et al., 2021; Wood et al., 2024). Therefore, the absence of a fertilizer-source effect suggests that moisture conditions during thaw largely determined the magnitude of cumulative N₂O loss.

These findings have important implications for Prairie clay soils. Gleyed Humic Vertisols and similar fine-textured soils often retain substantial water after snowmelt due to restricted drainage (Soil Classification Working Group, 1998; Tenuta et al., 2019). As a result, wet spring conditions may create prolonged periods of oxygen limitation that favor N_2O production. These findings suggest that managing reactive nitrogen before thaw and reducing excessive soil wetness, where possible, may offer greater mitigation benefits than altering fertilizer source alone.

2.4.3 Residual nitrate

Residual nitrate concentration was significantly influenced by fertilizer source ($P = 0.004$), soil moisture ($P < 0.0001$), and the interaction between fertilizer source and soil moisture ($P = 0.04$). In contrast, temperature did not have a significant effect (Table 2.3).

The interaction between fertilizer source and moisture level suggests that residual NO_3^- -N concentrations under urea were 12.09, 17.27, 21.60, and 6.73 mg kg^{-1} at 32, 50, 75, and 100% WFPS, respectively. Corresponding concentrations under SuperU were 11.42, 17.10, 21.92, and 6.43 mg kg^{-1} . For both fertilizers, concentrations at 32% and 50% WFPS did not differ significantly. However, concentrations at 75% WFPS were significantly greater than those at 32% WFPS, and concentrations at 100% WFPS were significantly lower than those at 50% and 75% WFPS (Figure 2.4).

The observed increase in nitrate concentration from 32% to 75% WFPS suggests that moderate soil moisture conditions promote nitrification and subsequent nitrate accumulation. Previous studies have reported similar responses, indicating that adequate moisture supports microbial activity while maintaining sufficient oxygen for nitrification (Bateman & Baggs, 2005; Linn & Doran, 1984).

The sharp decline in nitrate concentration at 100% WFPS likely results from increased nitrate consumption via denitrification under oxygen-limited conditions (Firestone, & Davidson, 1989; Balaine et al., 2013). This interpretation is further supported by the higher N₂O emissions observed under saturated conditions. The elevated nitrate concentrations in the urea and SuperU treatments relative to the control indicate greater fertilizer-derived nitrogen availability following thaw.

Table 2.3 Effect of thawing temperature, moisture, and fertilizer source on residual nitrate

Effect	Nitrate (mgkg ⁻¹)	Ammonium (mgkg ⁻¹)
Fertilizer source (FS)		
Control	11.07 b	6.29 b
SuperU	14.22 a	22.45 a
Urea	14.42 a	23.31 a
Moisture (M)		
32% WFPS	11.66 b	18.37 ab
50% WFPS	16.14 a	18.36 ab
75% WFPS	18.72 a	19.17 a
100% WFPS	6.43 c	13.50 b
Temperature (T)		
4 °C	12.17	14.35
8 °C	13.48	18.75
12 °C	14.08	17.74
16 °C	13.23	18.55
	<i>p-value</i>	
FS	0.004	<.0001
M	<.0001	0.026
FS x M	0.036	0.04
T	0.502	0.118
FS x T	0.255	0.036

M x T	0.424	0.402
FS x M x T	0.932	0.981

† Means with different letters are significantly different at $\alpha = 0.05$.

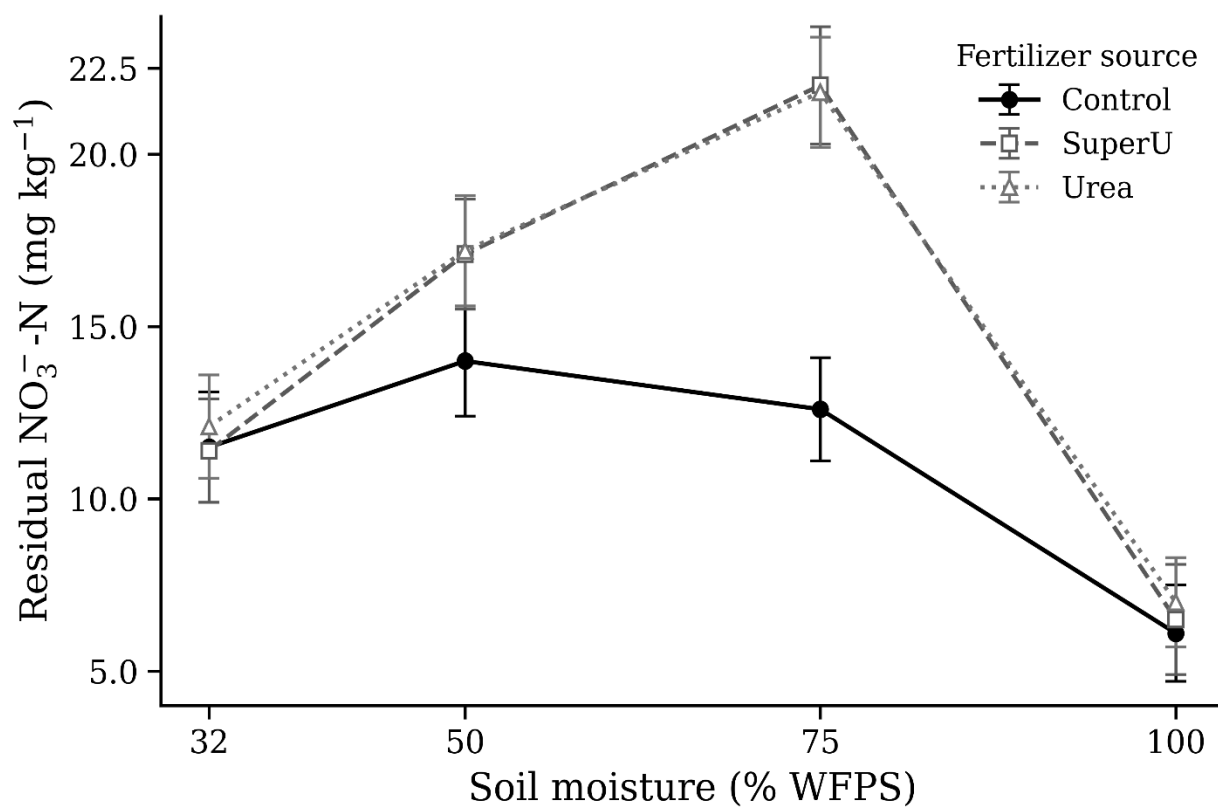


Figure 2.4 Effect of soil moisture and fertilizer source on residual Nitrate after thawing. Error bars represent the standard errors of the mean.

2.4.4 Residual ammonium

Residual ammonium concentration was significantly influenced by fertilizer source ($P < 0.0001$), soil moisture ($P = 0.03$), the interaction between fertilizer source and moisture ($P = 0.04$), and the interaction between fertilizer source and temperature ($P = 0.04$) (Table 2.3). Temperature as an independent factor did not have a significant effect.

Residual $\text{NH}_4^+\text{-N}$ concentrations were substantially higher in the urea (23.3 mg kg^{-1}) and SuperU (22.5 mg kg^{-1}) treatments compared to the control (6.29 mg kg^{-1}). Ammonium concentrations peaked at 75% WFPS (19.17 mg kg^{-1}) and were lowest at 100% WFPS (13.50 mg kg^{-1}). Residual $\text{NH}_4^+\text{-N}$ concentrations under SuperU ranged from 23.86 to 25.70 mg kg^{-1} between 32% and 75% WFPS, followed by a decrease of 10.21 mg kg^{-1} (39.7%) to 15.49 mg kg^{-1} at 100% WFPS. For urea, concentrations ranged from 23.47 to 27.6 mg kg^{-1} between 32% and 75% WFPS, then decreased by 11.0 mg kg^{-1} (40%) to 16.6 mg kg^{-1} at 100% WFPS (Figure 2.5).

The elevated ammonium concentrations observed in the urea and SuperU treatments indicate that fertilizer application increased the pool of available mineral nitrogen following thaw. The reduction in $\text{NH}_4^+\text{-N}$ at 100% WFPS suggests enhanced microbial utilization and transformation of ammonium to nitrate followed by denitrification, contributing to greater N_2O production under saturated conditions (Christensen & Tiedje, 1990; Teepe et al., 2001).

A significant interaction between fertilizer source and temperature ($p = 0.0353$) suggested that residual $\text{NH}_4^+\text{-N}$ levels among fertilizer treatments depended on thawing temperature. At 4°C , residual $\text{NH}_4^+\text{-N}$ was significantly higher with SuperU (23.24 mg kg^{-1}) and urea (16.72 mg kg^{-1}) compared to the control (3.09 mg kg^{-1}) (Figure 2.6). At 8°C , no significant differences were observed among fertilizer treatments. At 12 and 16°C , both SuperU and urea again resulted in significantly greater residual $\text{NH}_4^+\text{-N}$ than the control, with no significant difference between SuperU and urea (Figure 2.6).

However, since temperature was not a significant main effect, fertilizer source exerted a greater influence on $\text{NH}_4^+\text{-N}$ retention than temperature alone. Overall, the mineral nitrogen results suggest that moderate moisture levels favored mineral N accumulation, while saturated conditions promoted nitrogen transformations associated with increased N_2O emissions.

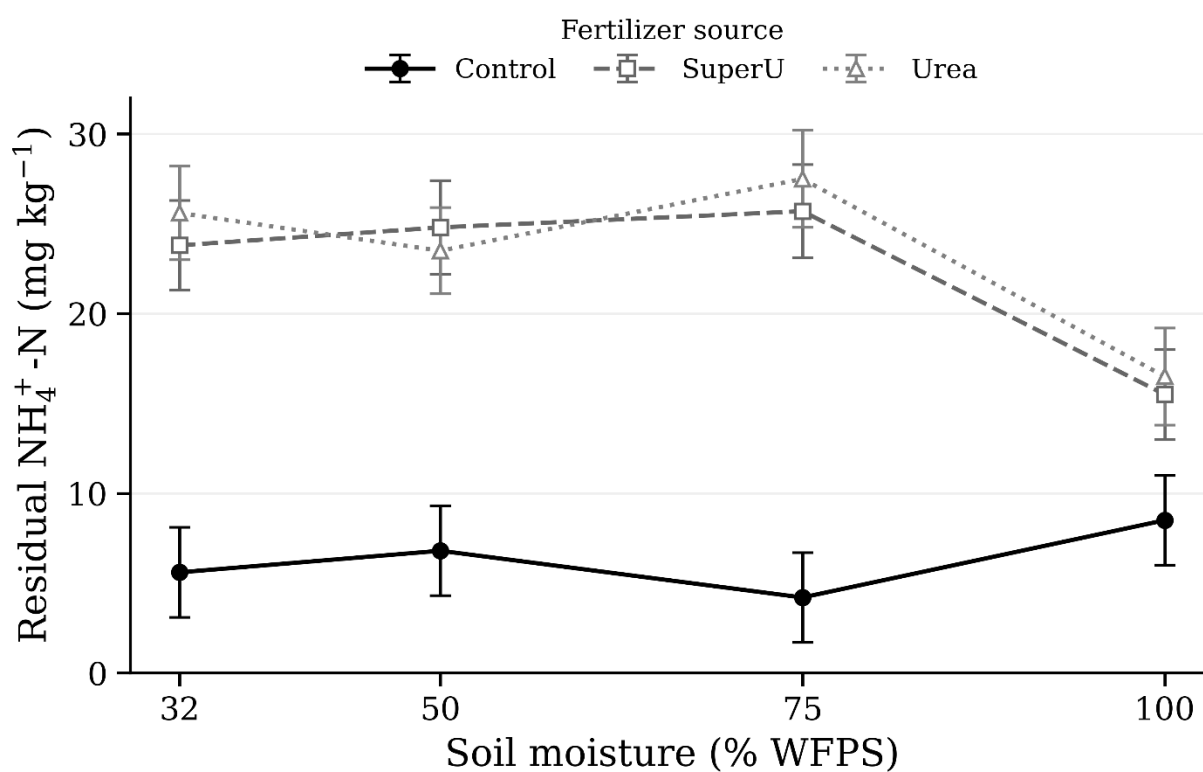


Figure 2.5 Effect of soil moisture and fertilizer source on residual ammonium after thaw. Error bars represent the standard errors of the mean.

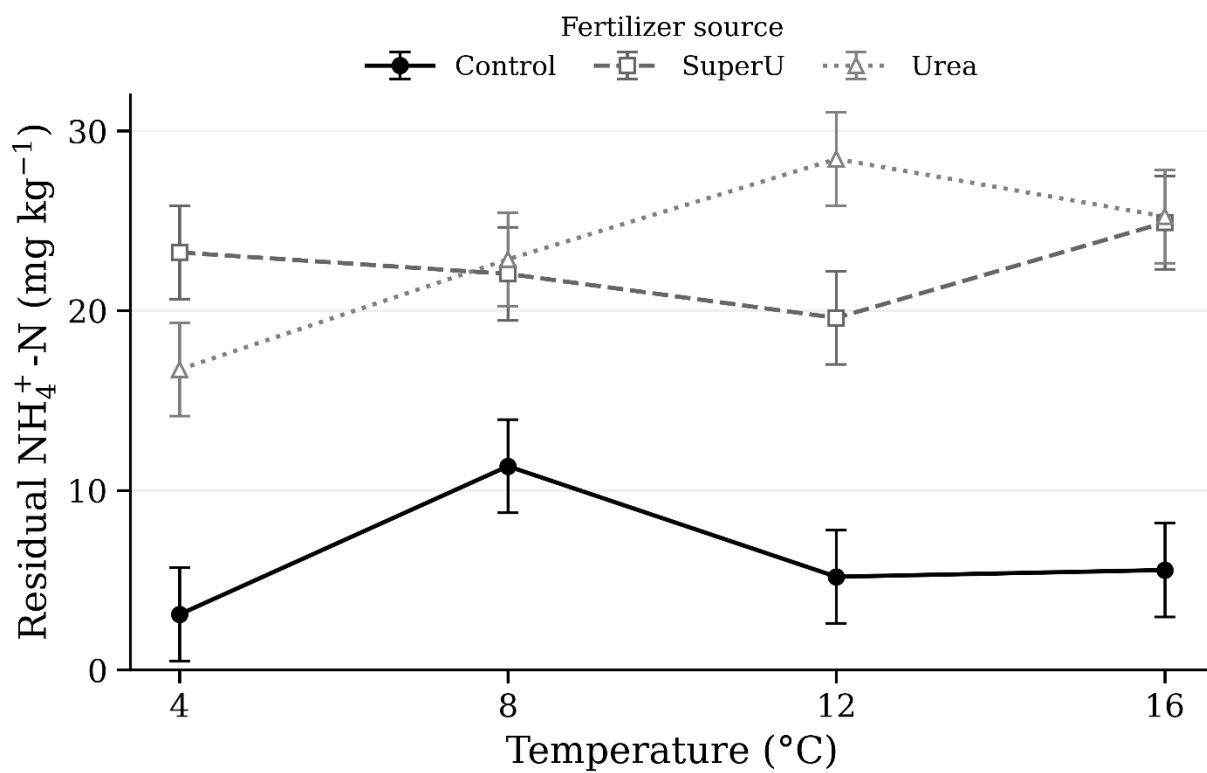


Figure 2.6 Effect of thawing temperature on residual ammonium. Error bars represent the standard errors of the mean.

2.5 Conclusion

The results demonstrate that soil moisture is the primary factor regulating N₂O emissions and post-thaw nitrogen dynamics in a Gleyed Humic Vertisol following a simulated freeze–thaw event. N₂O fluxes were low at 32% and 50% WFPS, at 75% and 100% WFPS. This indicates that moisture availability has a greater impact on emissions than either thawing temperature or fertilizer source. Residual nitrate and ammonium concentrations further demonstrate that soil moisture significantly affects nitrogen transformations during thaw. Residual mineral nitrogen concentrations were highest under moderate moisture conditions, which coincided with the highest N₂O emissions. Residual nitrate increased at 50%–75% WFPS. This pattern suggests that saturated conditions promote rapid nitrogen turnover and denitrification, increasing gaseous nitrogen losses. Although SuperU altered residual mineral nitrogen concentrations, it did not significantly reduce N₂O emissions compared to urea during the 60-hour thawing period. These findings indicate that the effectiveness of enhanced-efficiency fertilizers during thaw is limited when soil conditions strongly favor N₂O production. This study identifies soil moisture as the principal driver of thaw-induced N₂O emissions in Prairie clay soils. The findings suggest that years with wet spring thaw conditions are likely to experience increased N₂O losses, and that management strategies focused on limiting reactive nitrogen availability prior to thaw may offer greater mitigation benefits than altering fertilizer source alone. These results enhance understanding of how soil moisture and thawing temperature interact to regulate N₂O emissions during freeze–thaw events, informing nitrogen management practices in cold-region agricultural systems.

2.6 References

- Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., & Vallejo, A. (2014). Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment*, 189, 136–144. <https://doi.org/10.1016/J.AGEE.2014.03.036>
- Agriculture and Agri-Food Canada. (2022). *Discussion document: Reducing emissions arising from the application of fertilizer in Canada's agriculture sector*. <https://agriculture.canada.ca/en/departement/transparency/public-opinion-research-consultations/share-ideas-fertilizer-emissions-reduction-target>
- Agriculture and Agri-Food Canada. (2026). *Real-Time In-Situ Soil Monitoring for Agriculture (RISMA): MB10 daily soil temperature and soil moisture dataset, 2014–2022*. Real-Time In-Situ Soil Monitoring for Agriculture. <https://agriculture.canada.ca/en/agricultural-production/soil-and-land/real-time-situ-soil-monitoring-agriculture?utm>
- Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: Meta-analysis. *Global Change Biology*, 16(6), 1837–1846. <https://doi.org/10.1111/J.1365-2486.2009.02031.X>;
- Amiro, B., Tenuta, M., Hanis-Gervais, K., Gao, X., Flaten, D., & Rawluk, C. (2017). Agronomists' views on the potential to adopt beneficial greenhouse gas nitrogen management practices through fertilizer management. *Canadian Journal of Soil Science*, 97(4), 801–804. <https://doi.org/10.1139/cjss-2017-0062>
- Asgedom, H., Tenuta, M., Flaten, D. N., Gao, X., & Kebreab, E. (2014). Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. *Agronomy Journal*, 106(2), 732–744. <https://doi.org/10.2134/AGRONJ2013.0096>

- Balaine, N., Clough, T. J., Beare, M. H., Thomas, S. M., Meenken, E. D., & Ross, J. G. (2013). Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. *Soil Science Society of America Journal*, 77(5), 1496–1505. <https://doi.org/10.2136/SSSAJ2013.04.0141>;
- Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 2005 41:6, 41(6), 379–388. <https://doi.org/10.1007/S00374-005-0858-3>
- Bhowmik, A., Fortuna, A. M., Cihacek, L. J., Rahman, S., Borhan, M. S., & Carr, P. M. (2017). Use of laboratory incubation techniques to estimate greenhouse gas footprints from conventional and no-tillage organic agroecosystems. *Soil Biology and Biochemistry*, 112, 204–215. <https://doi.org/10.1016/J.SOILBIO.2017.04.015>
- Braker, G., Schwarz, J., & Conrad, R. (2010). Influence of temperature on the composition and activity of denitrifying soil communities. *FEMS Microbiology Ecology*, 73(1), 134–148. <https://doi.org/10.1111/J.1574-6941.2010.00884.X>
- Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621). <https://doi.org/10.1098/RSTB.2013.0122/22151>
- Carter, M. R., & Gregorich, E. G. (2007). *Soil Sampling and Methods of Analysis Second Edition Edited by*.
- Chen, Y., Tessier, S., MacKenzie, A. F., & Laverdière, M. R. (1995). Nitrous oxide emission from an agricultural soil subjected to different freeze-thaw cycles. *Agriculture, Ecosystems & Environment*, 55(2), 123–128. [https://doi.org/10.1016/0167-8809\(95\)00611-U](https://doi.org/10.1016/0167-8809(95)00611-U)

- CHRISTENSEN, S., & TIEDJE, J. M. (1990). Brief and vigorous N₂O production by soil at spring thaw. *Journal of Soil Science*, 41(1), 1–4. <https://doi.org/10.1111/J.1365-2389.1990.TB00039.X>
- Dobbie, K. E., & Smith, K. A. (2003). Impact of different forms of N fertilizer on N₂O emissions from intensive grassland. *Nutrient Cycling in Agroecosystems* 2003 67:1, 67(1), 37–46. <https://doi.org/10.1023/A:1025119512447>
- Edwards, K. A., McCulloch, J., Kershaw, G. P., & Jefferies, R. L. (2006). Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. *Soil Biology and Biochemistry*, 38(9), 2843–2851. <https://doi.org/10.1016/J.SOILBIO.2006.04.042>
- Environment and Climate Change Canada. (2010). *Canadian Climate Normals 1981–2010: Glenlea, Manitoba (Climate ID 5021054)*. Canadian Climate Normals. https://climate.weather.gc.ca/climate_normals/results_1981_2010_e.html?climate_id=5021054&dispBack=0&lstProvince=MB&searchType=stnProv
- Environment and Climate Change Canada. (2025). *Canadian climate normals 1991–2020: Winnipeg Richardson Airport. Historical Climate Data*. https://climate.weather.gc.ca/climate_normals/results_1991_2020_e.html?climate_id=5023222
- Environment Canada. (2014). *National inventory report 1990–2012: Greenhouse gas sources and sinks in Canada*. https://publications.gc.ca/collections/collection_2018/eccc/En81-4-2014-3-eng.pdf
- Fichtner, T., Goersmeyer, N., & Stefan, C. (2019). Influence of Soil Pore System Properties on the Degradation Rates of Organic Substances during Soil Aquifer Treatment (SAT). *Applied Sciences* 2019, Vol. 9, Page 496, 9(3), 496. <https://doi.org/10.3390/APP9030496>
- Firestone, M. K., & Davidson, E. A. (1989). Microbiological basis of NO and N₂O production and consumption in soil. *Exchange of trace gases between terrestrial ecosystems and the atmosphere*, 47,7-

21. Retrieved June 2, 2026, from
https://scholar.google.ca/scholar?hl=en&as_sdt=0%2C5&q=Firestone%2C+M.+K.%2C+%26+Davidson%2C+E.+A.+%281989%29.+Microbiological+basis+of+NO+and+N+2+O+production+and+consumption+in+soil.+Exchange+of+trace+gases+between+terrestrial+ecosystems+and+the+atmosphere%2C+47%2C+7-21.&btnG=

Fu, Q., Yan, J., Li, H., Li, T., Hou, R., Liu, D., & Ji, Y. (2019). Effects of biochar amendment on nitrogen mineralization in black soil with different moisture contents under freeze-thaw cycles. *Geoderma*, 353, 459–467. <https://doi.org/10.1016/J.GEODERMA.2019.07.027>

Gao, X., Asgedom, H., Tenuta, M., & Flaten, D. N. (2015). Enhanced efficiency urea sources and placement effects on nitrous oxide emissions. *Agronomy Journal*, 107(1), 265–277. <https://doi.org/10.2134/AGRONJ14.0213;PAGEGROUP:STRING:PUBLICATION>

Ghosh, U., Chatterjee, A., & Hatterman-Valenti, H. (2019). Enhanced Efficiency Fertilizers in Minimizing Nitrogen Losses in Irrigated Russet Potato. *Agrosystems, Geosciences and Environment*, 2(1), 1–9. <https://doi.org/10.2134/AGE2019.06.0047;ISSUE:ISSUE:DOI>

Google Earth. (2025). *Google Earth*. <https://earth.google.com>

Grant, R. F., Lin, S., & Hernandez-Ramirez, G. (2020). Modelling nitrification inhibitor effects on N₂O emissions after fall-and spring-Applied slurry by reducing nitrifier NH₄⁺ oxidation rate. *Biogeosciences*, 17(7), 2021–2039. <https://doi.org/10.5194/BG-17-2021-2020>

Hendershot, W. H., Lalonde, H., & Duquette, M. (2008). Soil reaction and exchangeable acidity. In M. R. Carter & E. G. Gregorich (Eds.), *Soil sampling and methods of analysis* (2nd ed., pp. 173–178). CRC Press.

- Henry, H. A. L. (2007). Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry*, 39(5), 977–986.
<https://doi.org/10.1016/J.SOILBIO.2006.11.017>
- IPCC. (2014). *Climate Change 2013 – The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (T. F. ; Q. D. ; P. G.-K. ; T. M. ; A. S. K. ; B. J. ; Stocker & A. ; X. Y. ; B. V. ; M. P. M. Nauels, Eds.). Cambridge University Press.
<https://doi.org/10.1017/CBO9781107415324>
- IPCC. (2023). Summary for Policymakers. In Core Writing Team, H. ; Lee, & J. Romero (Eds.), *Climate Change 2023: Synthesis Report: Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 1–34). IPCC.
<https://doi.org/10.59327/IPCC/AR6-9789291691647.001>
- Lasisi, A. A., Akinremi, O. O., Zhang, Q., & Kumaragamage, D. (2020). Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors I. Ammonia volatilization and mitigation by NBPT. *Soil Science Society of America Journal*, 84(3), 949–962.
<https://doi.org/10.1002/SAJ2.20062>;
- Leuther, F., & Schlüter, S. (2021). Impact of freeze-thaw cycles on soil structure and soil hydraulic properties. *SOIL*, 7(1), 179–191. <https://doi.org/10.5194/SOIL-7-179-2021>
- Lin, S., Hernandez-Ramirez, G., Lin, S., & Hernandez-Ramirez, G. (2021). Nitrogen turnover and N₂O production in incubated soils after receiving field applications of liquid manure and nitrification inhibitors. *https://doi.org/10.1139/Cjss-2020-0102*, 101(2), 290–304. <https://doi.org/10.1139/CJSS-2020-0102>
- Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal*, 48(6), 1267–1272.
<https://doi.org/10.2136/SSSAJ1984.03615995004800060013X>

- Liu, B., Ma, R., & Fan, H. (2021). Evaluation of the impact of freeze-thaw cycles on pore structure characteristics of black soil using X-ray computed tomography. *Soil and Tillage Research*, 206. <https://doi.org/10.1016/j.still.2020.104810>
- Liu, M., Xu, W., Zhang, H., Chen, H., Han, G., & Yu, X. (2022). Livestock Production, Greenhouse Gas Emissions, Air Pollution, and Grassland Conservation: Quasi-Natural Experimental Evidence. *SSRN Electronic Journal*. <https://doi.org/10.2139/SSRN.4306904>
- Manitoba Agriculture. (2007). *Manitoba soil fertility guide*. https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/pubs/soil_fertility_guide.pdf
- Mapfumo, E., Chanasyk, D. S., Puurveen, D., Elton, S., & Acharya, S. (2023). Historic climate change trends and impacts on crop yields in key agricultural areas of the prairie provinces in Canada: a literature review. *https://doi.org/10.1139/Cjps-2022-0215*, 103(3), 243–258. <https://doi.org/10.1139/CJPS-2022-0215>
- Matzner, E., & Borken, W. (2008). Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, 59(2), 274–284. <https://doi.org/10.1111/J.1365-2389.2007.00992.X>
- M.E. Trenkel, T. (2021). *Slow- and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture*.
- Mezbahuddin, S., Spiess, D., Hildebrand, D., Kryzanowski, L., Itenfisu, D., Goddard, T., Iqbal, J., & Grant, R. (2020). Assessing Effects of Agronomic Nitrogen Management on Crop Nitrogen Use and Nitrogen Losses in the Western Canadian Prairies. *Frontiers in Sustainable Food Systems*, 4, 512292. <https://doi.org/10.3389/FSUFS.2020.512292>

- Mørkved, P. T., Dörsch, P., Henriksen, T. M., & Bakken, L. R. (2006). N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biology and Biochemistry*, 38(12), 3411–3420. <https://doi.org/10.1016/J.SOILBIO.2006.05.015>
- Nelson, D. W., & Sommers, L. E. (2018). Total carbon, organic carbon, and organic matter. *Methods of Soil Analysis, Part 3: Chemical Methods*, 961–1010. <https://doi.org/10.2136/SSSABOOKSER5.3.C34;PAGEGROUP:STRING:PUBLICATION>
- Parkin, T. B., & Venterea, R. T. (2010). USDA-ARS GRACEnet Project Protocols, Chapter 3: Chamber-based trace gas flux measurements. In R. F. Follett (Ed.), *Sampling Protocols* (pp. 1–39). USDA-ARS. <https://www.ars.usda.gov/ARSUserFiles/np212/chapter%203.%20gracenet%20Trace%20Gas%20Sampling%20protocols.pdf>
- Pelster, D. E., Chantigny, M. H., Rochette, P., Bertrand, N., Angers, D. A., Zebarth, B. J., & Goyer, C. (2019). Rates and intensity of freeze–thaw cycles affect nitrous oxide and carbon dioxide emissions from agricultural soils. *https://doi.org/10.1139/Cjss-2019-0058*, 99(4), 472–484. <https://doi.org/10.1139/CJSS-2019-0058>
- Prather, M. J., Hsu, J., DeLuca, N. M., Jackman, C. H., Oman, L. D., Douglass, A. R., Fleming, E. L., Strahan, S. E., Steenrod, S. D., Søvde, O. A., Isaksen, I. S. A., Froidevaux, L., & Funke, B. (2015). Measuring and modeling the lifetime of nitrous oxide including its variability. *Journal of Geophysical Research: Atmospheres*, 120(11), 5693–5705. <https://doi.org/10.1002/2015JD023267>
- Risk, N., Snider, D., & Wagner-Riddle, C. (2013). Mechanisms leading to enhanced soil nitrous oxide fluxes induced by freeze–thaw cycles. *https://doi.org/10.4141/Cjss2012-071*, 93(4), 401–414. <https://doi.org/10.4141/CJSS2012-071>
- Rochette, P., & Bertrand, N. (2008). Soil-surface gas emissions. In M. R. Carter & E. G. Gregorich (Eds.), *Soil sampling and methods of analysis* (2nd ed., pp. 851–861). CRC Press.

- Schindlbacher, A., Zechmeister-Boltenstern, S., & Butterbach-Bahl, K. (2004). Effects of soil moisture and temperature on NO, NO₂, and N₂O emissions from European forest soils. *Journal of Geophysical Research: Atmospheres*, 109(D17). <https://doi.org/10.1029/2004JD004590>
- Sennett, L. B., Brin, L. D., Goyer, C., Zebarth, B. J., & Burton, D. L. (2024). Effects of soil water content at freezing, thaw temperature, and snowmelt infiltration on N₂O emissions and denitrifier gene and transcript abundance during a single freeze-thaw event. *Biology and Fertility of Soils* 2024 60:4, 60(4), 577–591. <https://doi.org/10.1007/S00374-024-01817-W>
- Shi, Y., Zhang, L., Mu, Y., Ma, W., Kong, X., & Yang, C. (2024). Dynamic characteristics of soil pore structure and water-heat variations during freeze-thaw process. *Engineering Geology*, 343, 107785. <https://doi.org/10.1016/J.ENGGEOL.2024.107785>
- Soil Classification Working Group. (1998). *The Canadian system of soil classification*. Agriculture and Agri-Food Canada. <https://sis.agr.gc.ca/cansis/publications/manuals/1998-cssc-ed3/index.html>
- Teepe, R., Brumme, R., & Beese, F. (2001). Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology and Biochemistry*, 33(9), 1269–1275. [https://doi.org/10.1016/S0038-0717\(01\)00084-0](https://doi.org/10.1016/S0038-0717(01)00084-0)
- Tenuta, M., Amiro, B. D., Gao, X., Wagner-Riddle, C., & Gervais, M. (2019). Agricultural management practices and environmental drivers of nitrous oxide emissions over a decade for an annual and an annual-perennial crop rotation. *Agricultural and Forest Meteorology*, 276–277, 107636. <https://doi.org/10.1016/J.AGRFORMET.2019.107636>
- Tenuta, M., Gao, X., Flaten, D. N., & Amiro, B. D. (2016). Lower Nitrous Oxide Emissions from Anhydrous Ammonia Application Prior to Soil Freezing in Late Fall Than Spring Pre-Plant Application. *Journal of Environmental Quality*, 45(4), 1133–1143. <https://doi.org/10.2134/JEQ2015.03.0159>;

- Tenuta, M., Gao, X., Tiessen, K. H. D., Baron, K., Sparling, B., & Stewardship, N. (2023). Placement and nitrogen source effects on N₂O emissions for canola production in Manitoba. *Agronomy Journal*, 115(5), 2369–2383. <https://doi.org/10.1002/AGJ2.21408>
- Tenuta, M., & Sparling, B. (2011). A laboratory study of soil conditions affecting emissions of nitrous oxide from packed cores subjected to freezing and thawing. *https://doi.org/10.4141/Cjss09051*, 91(2), 223–233. <https://doi.org/10.4141/CJSS09051>
- Thapa, R., Chatterjee, A., Awale, R., McGranahan, D. A., & Daigh, A. (2016). Effect of Enhanced Efficiency Fertilizers on Nitrous Oxide Emissions and Crop Yields: A Meta-analysis. *Soil Science Society of America Journal*, 80(5), 1121–1134. <https://doi.org/10.2136/SSSAJ2016.06.0179>
- Thomas, R. L., Sheard, R. W., & Moyer, J. R. (1967). Comparison of Conventional and Automated Procedures for Nitrogen, Phosphorus, and Potassium Analysis of Plant Material Using a Single Digestion. *Agronomy Journal*, 59(3), 240–243. <https://doi.org/10.2134/AGRONJ1967.00021962005900030010X>
- Tiessen, K. H. D., Flaten, D. N., Grant, C. A., Karamanos, R. E., & Entz, M. H. (2011). Efficiency of fall-banded urea for spring wheat production in Manitoba: Influence of application date, landscape position and fertilizer additives. *https://doi.org/10.4141/S05-017*, 85(5), 649–666. <https://doi.org/10.4141/S05-017>
- Wagner-Riddle, C., Hu, Q. C., van Bochove, E., & Jayasundara, S. (2008). Linking Nitrous Oxide Flux During Spring Thaw to Nitrate Denitrification in the Soil Profile. *Soil Science Society of America Journal*, 72(4), 908–916. <https://doi.org/10.2136/SSSAJ2007.0353>;
- Ward, A. (2019). *Real-Time In-Situ Soil Monitoring for Agriculture (RISMA) network metadata*. <https://publications.gc.ca/site/eng/9.910268/publication.html>

- Wertz, S., Goyer, C., Zebarth, B. J., Burton, D. L., Tatti, E., Chantigny, M. H., & Fillion, M. (2013). Effects of temperatures near the freezing point on N₂O emissions, denitrification and on the abundance and structure of nitrifying and denitrifying soil communities. *FEMS Microbiology Ecology*, 83(1), 242–254. <https://doi.org/10.1111/J.1574-6941.2012.01468.X>
- Wood, M. D., Gao, X., Tiessen, K. H. D., Tenuta, M., & Flaten, D. N. (2024). Enhanced efficiency urea fertilizers and timing effects on N₂O emissions from spring wheat production in Manitoba. *Agronomy Journal*, 116(1), 51–72. <https://doi.org/10.1002/AGJ2.21448>
- Zhang, Y., Chen, W., Smith, S. L., Riseborough, D. W., & Cihlar, J. (2005). Soil temperature in Canada during the twentieth century: Complex responses to atmospheric climate change. *Journal of Geophysical Research D: Atmospheres*, 110(3), 1–15. <https://doi.org/10.1029/2004JD004910>;
- Zheng, D., Hunt Jr, E. R., & Running, S. W. (1993). A daily soil temperature model based on air temperature and precipitation for continental applications. *Climate Research*, 183-191. Retrieved June 2, 2026, from <https://www.int-res.com/articles/cr/2/c002p183.pdf>

3 KINETIC AND THERMODYNAMIC RESPONSES OF THAW-INDUCED NITROUS OXIDE EMISSIONS TO THAWING TEMPERATURE, SOIL MOISTURE, AND FERTILIZER SOURCE IN A GLEYED HUMIC VERTISOL

3.1 Abstract

Freeze–thaw cycles strongly influence nitrous oxide (N₂O) emissions from agricultural soils. However, the effects of thawing temperature, soil moisture, and fertilizer source on the kinetics and thermodynamics of N₂O production in Prairie clay soils remain poorly understood. This study investigated the impacts of thawing temperature (4, 8, 12, and 16°C), soil moisture (32%, 50%, 75%, and 100% water-filled pore space [WFPS]), and fertilizer source (control, urea, and SuperU) on the kinetics and thermodynamic properties of N₂O emissions following a simulated freeze–thaw event using a freeze-thaw chamber. Cumulative N₂O emissions from the 60-hour incubation period were modeled using a first-order kinetic approach to estimate the rate constant (*k*), half-life (*t*_{1/2}), activation energy (*E*_a), and thermodynamic parameters including Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS). Soil moisture affected all kinetic and thermodynamic parameters, while fertilizer source had no significant effect; temperature effects depended on *k* and (*t*_{1/2}) depended on moisture. As WFPS increased from 32% to 100%, *k* declined from 0.03 to 0.02 h⁻¹ and *t*_{1/2} increased from 24.68 to 33.82 h (*p* < 0.0001). Moisture also affected *E*_a, ΔG , ΔH , and ΔS (*p* ≤ 0.006), with *E*_a declining to approximately zero, ΔG increasing, ΔH becoming negative, and ΔS becoming more negative. Fertilizer source had no significant effect on kinetic or thermodynamic parameters. Soil moisture was the dominant control on thaw-induced N₂O dynamics, consistent with denitrification in wetter soils. This highlights the importance of limiting high soil moisture to reduce N₂O emissions during thaw in Prairie clay soils.

3.2 Introduction

Nitrous oxide (N_2O) emissions from agricultural soils exhibit significant temporal variability due to the rapid response of nitrification and denitrification processes to fluctuations in soil temperature, moisture, oxygen availability, and mineral nitrogen supply. This variability is especially pronounced during the non-growing season. A synthesis of year-round measurements from Canadian croplands showed that non-growing-season emissions accounted for an average of 35.5% of annual N_2O emissions (Pelster et al., 2022). In the Canadian Prairies, spring thaw represents a critical period for N_2O loss due to rapid shifts in temperature, moisture, oxygen availability, and microbial activity (Tenuta et al., 2019; Wood et al., 2024). While cumulative N_2O emissions estimate total nitrogen loss, they do not capture the rate of emission development or the mechanisms underlying transient emission pulses. Thus, characterizing the temporal dynamics of N_2O production is essential for advancing nitrogen management and improving emission predictions under increasingly variable climatic conditions.

The temporal behavior of N_2O emissions can be described using kinetic models that quantify the rates and durations of microbial reactions. First-order kinetic models are commonly applied to characterize soil organic matter decomposition, nitrogen mineralization, carbon turnover, and greenhouse gas production because they provide biologically meaningful parameters describing reaction progress (Stanford & Smith, 1972; Lasisi et al., 2020; Paul, 2015). For N_2O emissions, the rate constant (k) represents the speed of emission accumulation, whereas the half-life ($t_{1/2}$) describes the time required for cumulative emissions to reach half of their maximum value (Sharma et al., 2025). These parameters provide insight into the persistence and temporal dynamics of emission events that cannot be obtained from cumulative emissions alone (Sharma et al., 2025). In effect, kinetic analyses can improve understanding of how environmental and management factors influence not only the magnitude of N_2O emissions but also the rate at which they develop following thaw (Sennett et al., 2024; Sharma et al., 2025).

While kinetic modeling describes the temporal characteristics of N₂O production, thermodynamic analysis provides information on the energetic requirements of the underlying microbial processes (Atkins & de Paula, 2018; Von Stockar et al., 2006). The Arrhenius relationship describes the temperature dependence of reaction rates through activation energy (E_a), whereas transition-state theory provides estimates of Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS), which collectively describe reaction spontaneity, heat exchange, and molecular organization during enzyme-mediated reactions (Justi, 2002; Atkins & de Paula, 2018). These principles have been widely applied to characterize enzyme-catalyzed reactions and soil biochemical processes (Davidson & Janssens, 2006; Paul, 2015). Applying these principles to N₂O production offers the opportunity to better understand the energetic constraints regulating microbial nitrogen transformations during freeze–thaw events.

The kinetics and thermodynamics of N₂O production are strongly influenced by soil environmental conditions. Soil moisture regulates oxygen diffusion, substrate transport, and microbial activity, thereby controlling the balance between nitrification and denitrification (Firestone & Davidson, 1989; Linn & Doran, 1984; Butterbach-Bahl et al., 2013). As water-filled pore space (WFPS) increases, oxygen diffusion decreases, favoring denitrification and altering both the rate and energetic requirements of N₂O-producing reactions (Bateman & Baggs, 2005; Balaine et al., 2013). Temperature also regulates microbial metabolism by influencing enzyme activity and reaction rates (Davidson & Janssens, 2006; Schipper et al., 2010). Because soil moisture and temperature operate simultaneously, their combined effects are likely to determine not only the magnitude of N₂O emissions but also the rate at which emissions develop and the energetic pathways through which they are produced.

Freeze–thaw events further alter these environmental controls by simultaneously changing soil physical, chemical, and biological properties. Ice formation disrupts soil aggregates, damages microbial cells, and releases labile carbon and nitrogen substrates that become rapidly available after thaw (Christebsen & Tiedje, 1990; Leuther & Schlüter, 2021; Matzner & Borken, 2008; Teepe et al., 2001). As microbial

activity resumes, nitrification and denitrification rates increase, resulting in large but short-lived N_2O emission pulses (Chen et al., 2021; Congreves et al., 2018; Wagner-Riddle et al., 2008; Wu et al., 2020). In Gleyed Humic Vertisols of the Canadian Prairies, high clay content, poor internal drainage, and prolonged soil saturation after snowmelt create conditions that favour oxygen limitation and enhance microbial nitrogen transformations (Pelster et al., 2019; Tenuta et al., 2019; Wood et al., 2024). Therefore, both the rates and energetics of N_2O production are expected to vary substantially across different combinations of thawing temperature and soil moisture.

Enhanced-efficiency fertilizers have been developed to reduce nitrogen losses by slowing microbial nitrogen transformations. Urease inhibitors, such as N-(n-butyl) thiophosphoric triamide (NBPT), delay urea hydrolysis, while nitrification inhibitors, including dicyandiamide (DCD), 3,4-dimethylpyrazole phosphate (DMPP), and nitrapyrin, suppress the oxidation of ammonium to nitrate (Trenkel, 2021; Abalos et al., 2014; Akiyama et al., 2010; Thapa et al., 2016). SuperU integrates both urease and nitrification inhibitors within a stabilized urea fertilizer and has shown variable effectiveness across different soil and climatic conditions (Grant et al., 2020; Tenuta et al., 2023; Wood et al., 2024). Although these products are often evaluated using cumulative N_2O emissions, their effects on the kinetics and thermodynamics of N_2O production during freeze–thaw events remain largely uncharacterized.

Despite advances in understanding freeze–thaw-induced N_2O emissions, most research has emphasized the magnitude of emissions rather than the mechanistic processes that govern their dynamics. Previous studies have examined the effects of freeze–thaw cycles, soil moisture, thawing temperature, and enhanced-efficiency fertilizers on N_2O emissions, primarily through assessments of N_2O fluxes and cumulative emissions. However, based on the available literature, the combined effects of soil moisture, thawing temperature, and fertilizer source on the kinetic and thermodynamic characteristics of N_2O production in Prairie clay soils remain poorly understood. Addressing this knowledge gap is essential for

improving mechanistic understanding of freeze–thaw emissions and for developing nitrogen management strategies that remain effective under increasingly variable spring climatic conditions.

Therefore, the objective of this study was to evaluate the effects of thawing temperature (4, 8, 12, and 16°C), soil moisture (32%, 50%, 75%, and 100% WFPS), and fertilizer source (control, urea, and SuperU) on the kinetic and thermodynamic characteristics of thaw-induced N₂O emissions from a Gleyed Humic Vertisol. Specifically, first-order kinetic modeling was used to estimate the rate constant (k) and half-life ($t_{1/2}$), while Arrhenius and transition-state analyses were used to determine activation energy (E_a), Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS). It was hypothesized that (1) high soil moisture will decrease the rate constant and activation energy and increase the half-life N₂O production, while the magnitude of these changes will differ among thawing temperatures and (2) Compared with conventional urea, SuperU will decrease the rate constant, increase the half-life, and increase the activation energy and Gibbs free energy, indicating slower N₂O production and a greater energy requirement.

3.3 Materials and Methods

3.3.1 Experimental procedures

The procedures for soil collection, experimental design, fertilizer treatments, freeze–thaw incubation, gas sampling, and cumulative N₂O emission measurements in this chapter followed those outlined in Chapter 2 (Sections 2.3.1–2.3.5). In summary, Gleyed Humic Vertisol samples from the Glenlea Research Station in Manitoba were exposed to a simulated freeze–thaw event using a freeze-thaw chamber (UTEST, UTD-1442) at thawing temperatures (4, 8, 12, and 16 °C), soil moisture (32, 50, 75, and 100% water-filled pore space [WFPS]), and fertilizer treatments (control, urea, and SuperU). The cumulative N₂O emissions recorded over the 60-hour incubation period were used for subsequent kinetic and thermodynamic analyses.

3.3.2 Kinetic modeling

A first-order kinetic model was selected because N₂O production was expected to decline as substrates for microbial nitrogen transformations became depleted during thaw. We also evaluated exponential and pseudo-first-order models; however, they did not consistently converge across treatments. The first-order model yielded stable estimates of k and $t_{1/2}$, which were subsequently used in Arrhenius and Eyring analyses. Cumulative N₂O production kinetics were quantified using a first-order kinetic model adapted from the analytical framework of Lasisi and Akinremi (2020), with modifications to characterize cumulative N₂O emissions instead of urea hydrolysis. Before model fitting, the cumulative N₂O dataset was examined for missing values in fertilizer source, soil moisture, thawing temperature, block, sampling time, and cumulative N₂O emissions, as complete response and treatment information was needed to identify each experimental unit and fit its kinetic response over time. No missing values were detected. We assessed the cumulative N₂O trajectories for monotonicity and observed no decreases over time; therefore, no adjustment of cumulative values was necessary. Two experimental units with zero cumulative N₂O throughout the incubation were excluded, as a positive final cumulative value was required to

normalize the response for kinetic model fitting. Incubation temperatures were converted from degrees Celsius to Kelvin for subsequent kinetic and thermodynamic analyses.

For each experimental unit, the final cumulative N₂O emission (U₀) was defined as the maximum cumulative emission measured during the 60-hour incubation. Cumulative emissions at each sampling time (U_t) were normalized by dividing by U₀ to express emissions as a proportion of the final cumulative emission. This normalization enabled direct comparison of emission dynamics among treatments (Lasisi and Akinremi, 2020).

Normalized cumulative emissions were fitted separately for each fertilizer, thawing temperature, soil moisture, and block combination using nonlinear regression (PROC NLIN, SAS version 9.4). Parameter estimation employed the Marquardt iterative algorithm, which ensures stable convergence for nonlinear least-squares estimation of kinetic parameters (Archontoulis & Miguez, 2015).

The first-order kinetic model was formulated as follows:

$$\frac{U_t}{U_0} = 1 - e^{-kt} \quad (4)$$

where

U_t is the cumulative N₂O emissions at time t

U₀ is the final cumulative N₂O emissions

k is the first-order rate constant (h⁻¹)

t is the incubation time (h)

The half-life of cumulative N₂O production (t_{1/2}) was calculated as

$$t_{1/2} = \frac{0.693}{k} \quad (5)$$

where $t_{1/2}$ represents the time required for cumulative N₂O emissions to reach 50% of the final cumulative emission.

3.3.3 Activation energy analysis

Temperature sensitivity of cumulative N₂O production was assessed by calculating the activation energy (E_a) from first-order rate constants using the Arrhenius equation (IUPAC, 2025), following the soil kinetic as outlined by (Holtan-Hartwig et al., 2002).

$$k = A \exp \left(-\frac{E_a}{RT} \right) \quad (6)$$

where the pre-exponential factor, A, denotes the activation energy (J mol⁻¹), R is the universal gas constant (8.314 J mol⁻¹ K⁻¹), and T refers to the absolute temperature (K).

Activation energy was estimated for each fertilizer, soil moisture, and block combination using nonlinear regression (PROC NLIN) with the Marquardt estimation algorithm. The estimated values were converted to kJ mol⁻¹ prior to statistical analysis. Activation energy quantified the sensitivity of cumulative N₂O production to thawing temperature.

3.3.4 Thermodynamic characterization of cumulative N₂O

Thermodynamic parameters characterizing the aspects of microbial N₂O production were estimated using transition-state theory (Eyring, 1935), following the approach of Lasisi and Akinremi (2020).

The Gibbs free energy of activation (ΔG) was calculated as follows:

$$\Delta G = -RT \ln \left(\frac{k_s h}{k_B T} \right) \quad (7)$$

Here, k_s denotes the first-order rate constant, converted from h^{-1} to s^{-1} ; h represents Planck's constant; k_B is Boltzmann's constant; R refers to the universal gas constant; and T indicates absolute temperature (K).

The mean Gibbs free energy was calculated across the four thawing temperatures for each combination of fertilizer, soil moisture, and block.

Enthalpy (ΔH) and entropy (ΔS) were estimated using the Gibbs–Helmholtz relationship

$$\Delta G = \Delta H - T\Delta S \quad (8)$$

Linear regression of Gibbs free energy versus absolute temperature was conducted separately for each fertilizer, soil moisture, and block combination using PROC REG. The ΔH resulted from the intercept of the regression, and ΔS resulted from the slope of the equation. The ΔH , ΔS , and ΔG constitute a set of variables that characterizes both the energy involved in and temperature effects on reactions, as well as the extent of cellular order in biochemical processes, which are also used to show energetic features of microbial N_2O production in thaw (Lasisi and Akinremi, 2020).

3.3.5 Statistical analysis

The estimated kinetic and thermodynamic parameters were analyzed using PROC GLIMMIX in SAS version 9.4. The first-order rate constant (k) and half-life ($t_{1/2}$) were estimated at each thawing temperature. These parameters were analyzed using a three-way ANOVA, with thawing temperature, soil moisture, fertilizer source, and their interactions specified as fixed effects, and block and the block-by-temperature interaction included as random effects to account for the split-plot design. Given that k

and $t_{1/2}$ were positive and right skewed; Gamma distributions were used for their analysis for k and Lognormal distribution for $t_{1/2}$. Activation energy (E_a) was estimated from the rate constants across the four thawing temperatures for each combination of block, soil moisture, and fertilizer source. Therefore, temperature was not included as a factor in the statistical model. Soil moisture, fertilizer source, and their interaction were treated as fixed effects, while block was treated as a random effect. Activation energy was analyzed using a normal distribution. Thermodynamic parameters, including Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS), were estimated across thawing temperatures. Each parameter was analyzed separately, with soil moisture, fertilizer source, and their interaction as fixed effects, and block as a random effect. A normal distribution was used for each thermodynamic parameter. Degrees of freedom were estimated using the Kenward–Roger method. When significant treatment effects were detected, least-squares means were compared using Tukey-adjusted pairwise comparisons. Treatment effects were considered significant at $P < 0.05$.

3.4 Results and Discussion

3.4.1 N₂O Emission Kinetics and Activation Energy

First-order kinetics were fitted to cumulative N₂O flux data measured over a 60-hour post-thaw incubation period. At 16 °C, k was highest for all treatment combinations at 32% WFPS (0.044 h⁻¹) but declined sharply with increasing moisture to 0.020 h⁻¹ at 100% WFPS (Figure. 3.1). At lower temperatures (4, 8, and 12 °C), k remained comparatively stable across the moisture gradient, showing a shallower and less consistent decline. The effect of thawing temperature on k varied considerably with soil moisture content. At 32% WFPS, k reached its maximum value at 16 °C (0.0438 h⁻¹), which was significantly higher than at 4 °C. In contrast, at 75% WFPS, k was highest at 4 °C (0.0337 h⁻¹) and was significantly greater than at 8 and 16 °C, while the difference from 12 °C was not significant. At 100% WFPS, k was numerically highest at 4 °C (0.0276 h⁻¹), although differences among thawing temperatures were not statistically

significant (Figure 3.1). This reversal in temperature response across the moisture gradient explains the significant temperature-by-moisture interaction. Correspondingly, $t_{1/2}$ at 16 °C rose from 19.0 hr at 32% WFPS to 36.0 hr at 100% WFPS, the steepest increase of any temperature treatment (Figure 3.2). At 4 °C, $t_{1/2}$ was generally longer at the two lower moisture levels (32–50% WFPS) compared to the two higher moisture levels (75–100% WFPS). The maximum value occurred at 32% WFPS (31.5 h), while the minimum was observed at 75% WFPS (21.5 h), with a subsequent partial increase at 100% WFPS (26.0 h). This pattern suggests that moisture effects on $t_{1/2}$ at 4 °C were not strictly linear, likely due to the combined effects of freeze–thaw-induced soil structural changes, substrate availability, and reduced microbial activity at near-freezing temperatures (Congreves et al., 2018; Wagner-Riddle et al., 2008).

The significant interaction suggests that warmer post-thaw conditions increased the moisture sensitivity of N₂O emission kinetics, consistent with temperature stimulating microbial activity at low moisture, where oxygen and substrate were accessible, but offering no comparable kinetic advantage once pore space when pore spaces are saturated with water and diffusion is limited.

Thawing temperature as a main effect was not significant for k ($p = 0.15$) or $t_{1/2}$ ($p = 0.21$) (Table 3.1), indicating that temperature did not exert a consistent directional influence on N₂O emission kinetics across all moisture levels. This is consistent with the significant Temperature $\times$ Moisture interaction discussed above: temperature's influence on emission kinetics was conditional on soil moisture rather than acting as an independent driver, as has been observed in other freeze-thaw studies on fine-textured soils (Congreves et al., 2018; Wagner-Riddle et al., 2008).

Fertilizer source had no significant effect on k ($p = 0.75$) or $t_{1/2}$ ($p = 0.62$) (Table 3.1), with k values of 0.03 hr⁻¹ for all three fertilizer treatments and $t_{1/2}$ values of 27.63, 28.35, and 28.89 hr for the control, SuperU, and urea treatments, respectively. The absence of a significant SuperU effect indicates that the dual inhibitors (NBPT and DCD) in SuperU did not meaningfully alter the temporal dynamics of N₂O

emissions under freeze-thaw conditions. The limited response to SuperU under high soil moisture conditions likely reflects constraints on both inhibitor components. Reduced oxygen availability in wet soils may slow the oxidation of NBPT to its active metabolite, N-(n-butyl) phosphoric triamide oxide (NBPTO), thereby limiting urease inhibition. In contrast, DCD directly inhibits ammonia oxidation during nitrification (Yildirim et al., 2023). As soil moisture increases and oxygen availability decreases, N₂O production shifts toward denitrification, which may reduce DCD's effectiveness in mitigating N₂O emissions (Bateman & Baggs, 2005).

The activation energy (E_a), derived from the Arrhenius relationship between k and temperature, represents the minimum energy barrier that must be overcome for the N₂O-generating reactions to proceed and is therefore presented here as a kinetic, rather than equilibrium thermodynamic, parameter (Table 3.1). Only moisture had a significant effect on E_a ($p = 0.006$) (Table 3.1). Activation energy declined sharply from 38.14 kJ·mol⁻¹ at 32% WFPS to values that were statistically insignificant relative to zero at 50, 75, and 100% WFPS, suggesting that the energy barrier to N₂O formation was substantially lower under wetter soil conditions. This is consistent with the role of moisture in reducing diffusion barriers and promoting the establishment of anaerobic microsites where denitrification enzymes operate with minimal activation constraints (Balaine et al., 2013; Davidson et al., 2000). The simultaneous decline in both E_a and k with increasing moisture represents a kinetic paradox: a lower energy barrier would ordinarily be expected to accelerate the reaction, yet k decreased. This is resolved by recognizing that the overall rate constant in the Arrhenius equation depends on both E_a and the pre-exponential frequency factor, which in a biological context reflects the availability of active microbial sites and accessible substrate. At high WFPS, water-saturated pores restricted oxygen and substrate diffusion to denitrifying microsites, sufficiently reducing the effective frequency factor to outweigh the kinetic advantage of a lower energy barrier (Balaine et al., 2013; Congreves et al., 2018).

Table 3.1 Effect of moisture, temperature, and fertilizer source on activation energy (E_a), rate constant (k), and half-life ($t_{1/2}$) of N_2O emissions in clay soil following a freeze–thaw event

Effect	E_a (kJ·mol ⁻¹)	k (hr ⁻¹)	$t_{1/2}$ (hr)
Moisture			
32% WFPS	38.14	0.03	24.65
50% WFPS	11.76	0.03	24.83
75% WFPS	1.17	0.02	30.12
100% WFPS	0	0.02	34.73
Temperature			
4 °C		0.03	25.88
8 °C		0.02	31.21
12 °C		0.02	29.42
16 °C		0.03	26.95
Fertilizer Source			
Control	10.60	0.03	27.63
SuperU	8.90	0.03	28.35
Urea	18.80	0.03	28.89
	p-value		
Moisture	0.006	<0.0001	<0.0001
Temperature		0.15	0.19
Source	0.54	0.75	0.70
Temperature × Moisture		<0.0001	<0.0001
Source × Moisture	0.17	0.92	0.96
Source × Temperature		0.97	0.99
Source × Temperature × Moisture		0.14	0.40

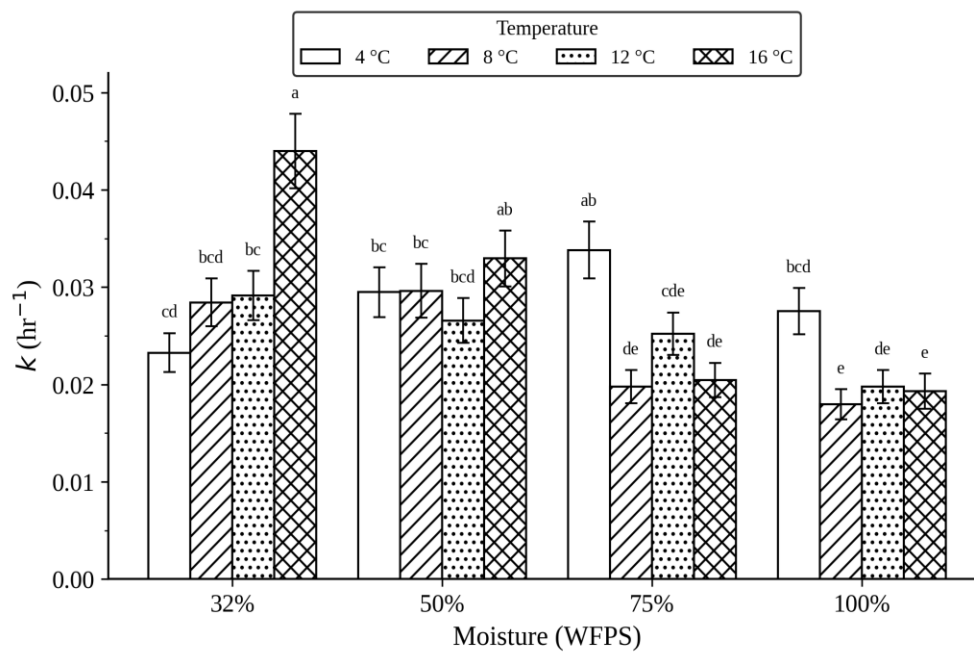


Figure 3.1. Interaction between thawing temperature and moisture on the N₂O emission rate constant (k) in clay soil following a freeze-thaw event. Error bars are standard errors of the mean. Bars with different letters are significantly different at a probability value of <0.05 (Tukey–Kramer).

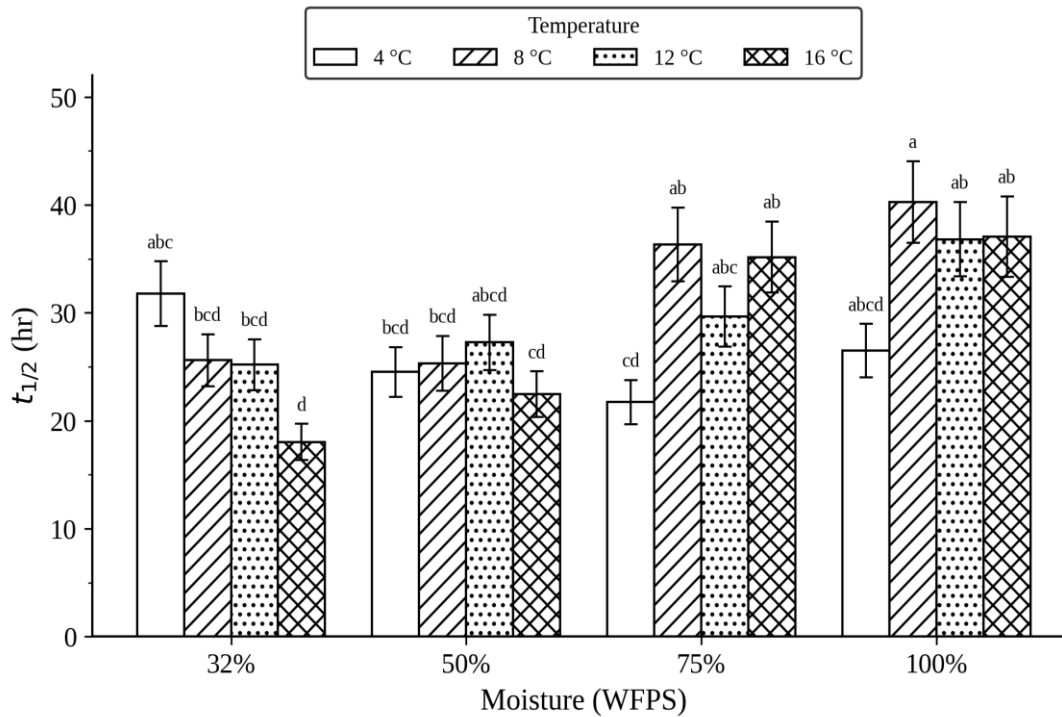


Figure. 3.2. Interaction between thawing temperature and moisture on the N₂O emission half-life ($t_{1/2}$) in clay soil following a freeze–thaw event. Error bars are standard errors of the mean. Bars with different letters are significantly different at a probability value of <0.05 (Tukey–Kramer).

To check how well the model actually captured the data, the fitted first-order kinetic curves were plotted against the observed cumulative N₂O flux for each Temperature $\times$ Moisture combination (Figure 3.3). Since fertilizer source had no significant effect on either k or $t_{1/2}$, a single curve representing the average rate constant is shown for each combination. Across all sixteen combinations, the fitted curves tracked the observed emission buildup closely, supporting the appropriateness of the first-order kinetic model for describing post-thaw N₂O emission dynamics.

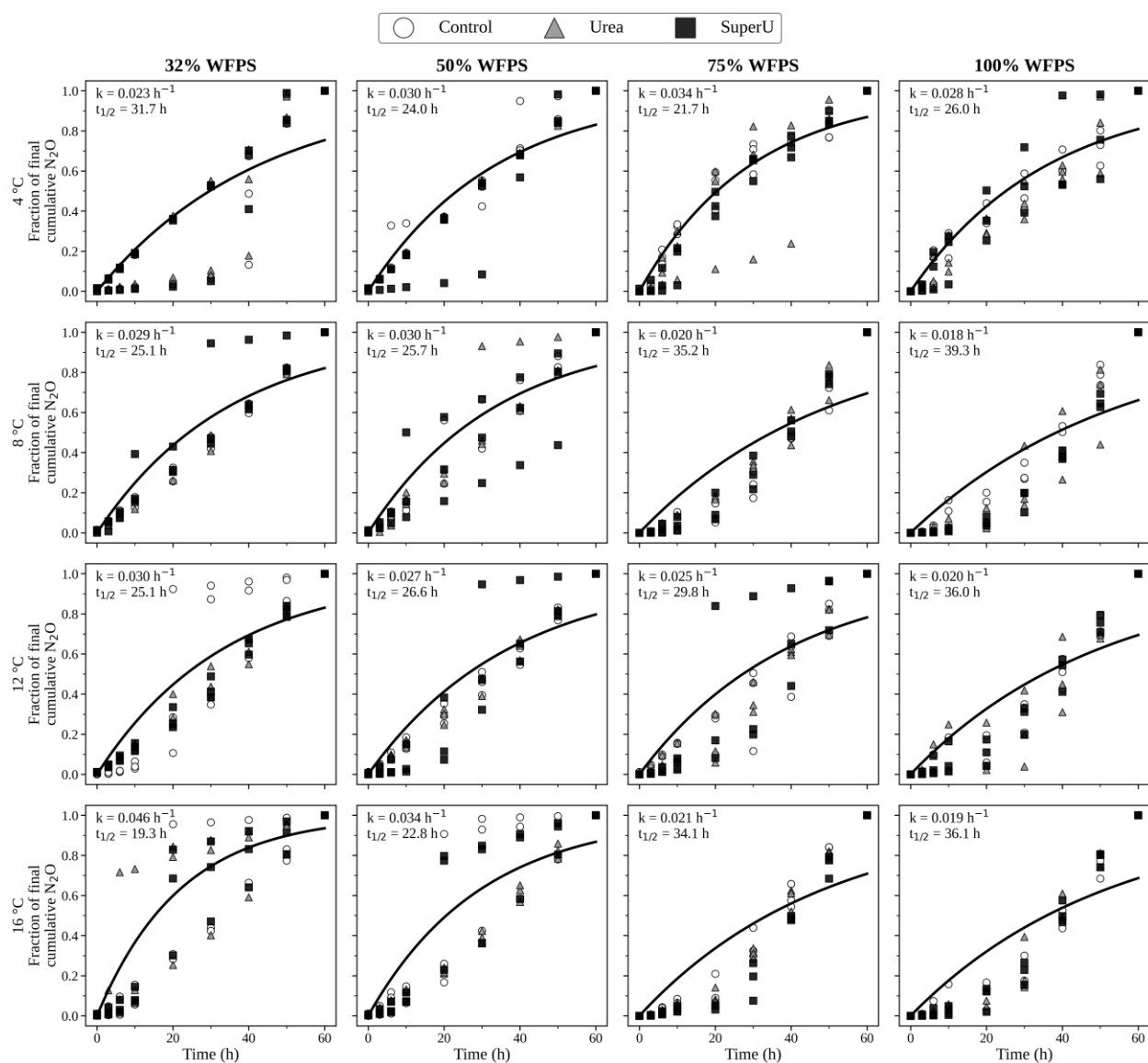


Figure 3.3. First-order kinetic curves fitted to cumulative N_2O flux buildup across the sixteen thawing temperature $\times$ soil moisture combinations (0-60 h post-thaw). Flux values are expressed as a fraction of the final cumulative total, and each curve reflects the average rate constant for that combination.

3.4.2 Thermodynamics of N₂O Emissions: Gibbs Free Energy, Enthalpy, and Entropy

The change in Gibbs free energy (ΔG) was positive across all treatments, ranging from 96.80 to 97.64 kJ·mol⁻¹ (Table 3.2), confirming that N₂O-producing reactions were thermodynamically nonspontaneous and depended on continuous microbial catalysis, consistent with the enzymatic nature of denitrification and nitrification (Conrad, 1996; Lasisi et al., 2021). Moisture had a significant effect on ΔG ($p = 0.0002$; Table 3.2), with values at 75% WFPS (97.30 kJ·mol⁻¹) and 100% WFPS (97.64 kJ·mol⁻¹) significantly higher than at 32% WFPS (96.82 kJ·mol⁻¹) and 50% WFPS (96.80 kJ·mol⁻¹), suggesting a modestly greater thermodynamic energy requirement for N₂O formation under wetter conditions. Fertilizer source ($p = 0.68$) and its interaction with moisture ($p = 0.95$) had no significant effect on ΔG (Table 3.2).

The enthalpy change (ΔH) was significantly affected by moisture ($p < 0.0001$; Table 3.2), shifting from a positive value at 32% WFPS (26.31 kJ·mol⁻¹) to near zero at 50% WFPS (0.79 kJ·mol⁻¹) and negative at 75 and 100% WFPS (-22.85 and -19.42 kJ·mol⁻¹, respectively) (table 3.2). This transition from endothermic to exothermic enthalpy with increasing WFPS suggests a fundamental shift in the dominant N₂O-producing pathway: at low moisture, where oxygen is relatively available, nitrification-associated oxidative pathways requiring greater enthalpic input may predominate, whereas at high moisture, where anaerobiosis increasingly favours denitrification, the stepwise reduction of nitrogen oxides to N₂O proceeds with a net release of energy (Butterbach-Bahl et al., 2013; Conrad, 1996). Neither fertilizer source ($p = 0.89$) nor its interaction with moisture ($p = 0.22$) significantly affected ΔH (Table 3.2).

The entropy change (ΔS) was negative across all moisture levels, declining significantly with increasing WFPS ($p < 0.0001$; Table 3.2), from -0.25 kJ·mol⁻¹·K⁻¹ at 32% WFPS to -0.42

$\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$ at 75% WFPS, with 100% WFPS ($-0.41 \text{ kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1}$) not significantly different from 75% WFPS (table 3.2). The consistently negative ΔS indicates that the transition state of the N_2O -producing reaction was more ordered than the reactants, consistent with the formation of an enzyme-substrate complex during microbially mediated catalysis (Lasisi et al., 2021). The increasingly negative values at higher moisture suggest a progressively more constrained transition state under water-saturated, diffusion-limited conditions, where substrate and enzyme access are restricted, and the activated complex is more spatially organized. Fertilizer source ($p = 0.90$) and its interaction with moisture ($p = 0.23$) were not significant for ΔS (Table 3.2), consistent with the pattern across all kinetic and thermodynamic parameters that SuperU did not alter the underlying reaction energetics under freeze–thaw conditions in this clay soil.

Table 3.2 Effect of moisture and fertilizer source on Gibbs free energy (ΔG), enthalpy (ΔH), and entropy (ΔS) of N_2O emissions in clay soil following a freeze–thaw event

Effect	$\Delta G (\text{kJ}\cdot\text{mol}^{-1})$	$\Delta H (\text{kJ}\cdot\text{mol}^{-1})$	$\Delta S (\text{kJ}\cdot\text{mol}^{-1}\cdot\text{K}^{-1})$
Moisture			
32% WFPS	96.82	26.31	-0.25
50% WFPS	96.80	0.79	-0.34
75% WFPS	97.30	-22.85	-0.42
100% WFPS	97.64	-19.42	-0.41
Fertilizer Source			
Control	97.06	-5.50	-0.36
SuperU	97.16	-3.81	-0.36
Urea	97.20	-2.06	-0.35

		p-value	
Moisture	0.0002	<0.0001	<0.0001
Source	0.68	0.89	0.90
Source × Moisture	0.95	0.22	0.23

3.5 Conclusion

Soil moisture is identified as the primary regulator of the kinetic and thermodynamic processes that govern thaw-induced N₂O production in Prairie clay soil. Increased soil moisture consistently altered both the rate and persistence of cumulative N₂O emissions, lowered the activation energy barrier to microbial N₂O production, and changed the thermodynamic properties of the reaction. While thawing temperature affected emission kinetics through its interaction with soil moisture, it did not independently influence the activation energy or thermodynamic properties of N₂O production. Furthermore, the lack of significant effects from different fertilizer sources on kinetic and thermodynamic parameters suggests that thaw-induced N₂O dynamics are primarily controlled by soil moisture and temperature, rather than by fertilizer source. The integration of kinetic and thermodynamic analyses in this study advances understanding of freeze–thaw-induced N₂O emissions beyond traditional assessments that focus solely on emission magnitude. The results suggest that physical factors influencing oxygen availability and microbial activity during thaw have a greater impact on N₂O production than fertilizer stabilization technologies. These findings underscore the need for management practices that limit excessive soil moisture and enhance soil aeration during the thaw period to mitigate spring N₂O emissions from Prairie agricultural soils. More broadly, this research establishes a mechanistic framework for improving predictions of N₂O

emissions under variable climatic conditions and informs the refinement of nitrogen management strategies for cold-climate cropping systems.

3.6 References

- Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., & Vallejo, A. (2014). Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment*, 189, 136–144. <https://doi.org/10.1016/J.AGEE.2014.03.036>
- Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: Meta-analysis. *Global Change Biology*, 16(6), 1837–1846. <https://doi.org/10.1111/J.1365-2486.2009.02031>.
- Archontoulis, S. V., & Miguez, F. E. (2015). Nonlinear regression models and applications in agricultural research. *Agronomy Journal*, 107(2), 786–798. <https://doi.org/10.2134/AGRONJ2012.0506>;
- Atkins, P., & de Paula, J. (2018). *Atkins' physical chemistry* (11th ed.). Oxford University Press.
- Balaine, N., Clough, T. J., Beare, M. H., Thomas, S. M., Meenken, E. D., & Ross, J. G. (2013). Changes in Relative Gas Diffusivity Explain Soil Nitrous Oxide Flux Dynamics. *Soil Science Society of America Journal*, 77(5), 1496–1505. <https://doi.org/10.2136/SSSAJ2013.04.0141>;
- Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 2005 41:6, 41(6), 379–388. <https://doi.org/10.1007/S00374-005-0858-3>

- Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621). <https://doi.org/10.1098/RSTB.2013.0122/22151>
- Chen, Z., Ge, S., Zhang, Z., Du, Y., Yao, B., Xie, H., Liu, P., Zhang, Y., Wang, W., & Zhou, H. (2021). Soil Moisture but Not Warming Dominates Nitrous Oxide Emissions During Freeze–Thaw Cycles in a Qinghai–Tibetan Plateau Alpine Meadow With Discontinuous Permafrost. *Frontiers in Ecology and Evolution*, 9, 676027. <https://doi.org/10.3389/FEVO.2021.676027/TEXT>
- CHRISTENSEN, S., & TIEDJE, J. M. (1990). Brief and vigorous N₂O production by soil at spring thaw. *Journal of Soil Science*, 41(1), 1–4. <https://doi.org/10.1111/J.1365-2389.1990.TB00039.X>
- Congreves, K. A., Wagner-Riddle, C., Si, B. C., & Clough, T. J. (2018). Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. *Soil Biology and Biochemistry*, 117, 5–15. <https://doi.org/10.1016/J.SOILBIO.2017.10.040>
- Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H₂, CO, CH₄, OCS, N₂O, and NO). *Microbiological Reviews*, 60(4), 609–640. <https://doi.org/10.1128/MR.60.4.609-640.1996>;
- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. In *Nature* (Vol. 440, Number 7081, pp. 165–173). <https://doi.org/10.1038/nature04514>
- Davidson, E. A. ;, Keller, M. ;, Erickson, H. E. ;, Verchot, L. V. ;, & Veldkamp, E. (2000). Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, 50(8), 667–680.

- Eyring, H. (1935). The Activated Complex in Chemical Reactions. *The Journal of Chemical Physics*, 3(2), 107–115. <https://doi.org/10.1063/1.1749604>
- Firestone, M. K., & Davidson, E. A. (1989). Microbiological basis of NO and N₂O production and consumption in soil. *Exchange of trace gases between terrestrial ecosystems and the atmosphere*, 47, 7-21. Retrieved June 2, 2026, from https://scholar.google.ca/scholar?hl=en&as_sdt=0%2C5&q=Firestone%2C+M.+K.%2C+%26+Davidson%2C+E.+A.+%281989%29.+Microbiological+basis+of+NO+and+N2+O+production+and+consumption+in+soil.+Exchange+of+trace+gases+between+terrestrial+ecosystems+and+the+atmosphere%2C+47%2C+7-21.&btnG=
- Grant, R. F., Lin, S., & Hernandez-Ramirez, G. (2020). Modelling nitrification inhibitor effects on N₂O emissions after fall-and spring-Applied slurry by reducing nitrifier NH₄⁺ oxidation rate. *Biogeosciences*, 17(7), 2021–2039. <https://doi.org/10.5194/BG-17-2021-2020>
- Holtan-Hartwig, L., Dörsch, P., & Bakken, L. R. (2002). Low temperature control of soil denitrifying communities: kinetics of N₂O production and reduction. *Soil Biology and Biochemistry*, 34(11), 1797–1806. [https://doi.org/10.1016/S0038-0717\(02\)00169-4](https://doi.org/10.1016/S0038-0717(02)00169-4)
- Justi, R. (2002). Teaching and Learning Chemical Kinetics. *Chemical Education: Towards Research-Based Practice*, 293–315. https://doi.org/10.1007/0-306-47977-X_13
- Lasisi, A. A., Akinremi, O. O., & Kumaragamage, D. (2021). Efficiency of fall versus spring applied urea-based fertilizers treated with urease and nitrification inhibitors II. Crop yield and nitrogen use efficiency. *Soil Science Society of America Journal*, 85(2), 299–313. <https://doi.org/10.1002/SAJ2.20126>;

- Lasisi, A. A. & Akinremi, O. O. (2020). Kinetics and thermodynamics of urea hydrolysis in the presence of urease and nitrification inhibitors. *Https://Doi.Org/10.1139/Cjss-2020-0044*, 101(2), 192–202. <https://doi.org/10.1139/CJSS-2020-0044>
- Leuther, F., & Schlüter, S. (2021). Impact of freeze-thaw cycles on soil structure and soil hydraulic properties. *SOIL*, 7(1), 179–191. <https://doi.org/10.5194/SOIL-7-179-2021>
- Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal*, 48(6), 1267–1272. <https://doi.org/10.2136/SSSAJ1984.03615995004800060013X>;
- Matzner, E., & Borken, W. (2008). Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, 59(2), 274–284. <https://doi.org/10.1111/J.1365-2389.2007.00992.X>
- M.E. Trenkel, T. (2021). *Slow- and Controlled-Release and Stabilized Fertilizers: An Option for Enhancing Nutrient Use Efficiency in Agriculture*.
- Paul, E. A. (2015). Soil Microbiology, Ecology, and Biochemistry. In *Soil Microbiology, Ecology and Biochemistry* (pp. 1–14). Elsevier. <https://doi.org/10.1016/b978-0-12-415955-6.00001-3>
- Pelster, D. E., Chantigny, M. H., Rochette, P., Bertrand, N., Angers, D. A., Zebarth, B. J., & Goyer, C. (2019). Rates and intensity of freeze–thaw cycles affect nitrous oxide and carbon dioxide emissions from agricultural soils. *Https://Doi.Org/10.1139/Cjss-2019-0058*, 99(4), 472–484. <https://doi.org/10.1139/CJSS-2019-0058>
- Pelster, D. E., Thiagarajan, A., Liang, C., Chantigny, M. H., Wagner-Riddle, C., Congreves, K., Lemke, R., Glenn, A., Tenuta, M., Hernandez-Ramirez, G., Bittman, S., Hunt, D., Owens, J., &

- MacDonald, D. (2022). Ratio of non-growing season to growing season N₂O emissions in Canadian croplands: an update to national inventory methodology. *Https://Doi.Org/10.1139/Cjss-2022-0101*, 103(2), 344–352. <https://doi.org/10.1139/CJSS-2022-0101>
- Schipper, L. A., Robertson, W. D., Gold, A. J., Jaynes, D. B., & Cameron, S. C. (2010). Denitrifying bioreactors—An approach for reducing nitrate loads to receiving waters. *Ecological Engineering*, 36(11), 1532–1543. <https://doi.org/10.1016/J.ECOLENG.2010.04.008>
- Sennett, L. B., Brin, L. D., Goyer, C., Zebarth, B. J., & Burton, D. L. (2024). Effects of soil water content at freezing, thaw temperature, and snowmelt infiltration on N₂O emissions and denitrifier gene and transcript abundance during a single freeze-thaw event. *Biology and Fertility of Soils* 2024 60:4, 60(4), 577–591. <https://doi.org/10.1007/S00374-024-01817-W>
- Sharma, B., Ghimire, R., Sapkota, S., Shrestha, P., Brewer, C. E., & Adhikari, S. (2025). Nitrous oxide mitigation potential of biochar derived from agricultural and forest biomass: Effects of feedstock composition and pyrolysis temperature. *Journal of Environmental Quality*, 54(6), 1746–1758. <https://doi.org/10.1002/JEQ2.70054>;
- Stanford, G., & Smith, S. J. (1972). Nitrogen Mineralization Potentials of Soils. *Soil Science Society of America Journal*, 36(3), 465–472. <https://doi.org/10.2136/SSSAJ1972.03615995003600030029X>
- Teepe, R., Brumme, R., & Beese, F. (2001). Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology and Biochemistry*, 33(9), 1269–1275. [https://doi.org/10.1016/S0038-0717\(01\)00084-0](https://doi.org/10.1016/S0038-0717(01)00084-0)
- Tenuta, M., Amiro, B. D., Gao, X., Wagner-Riddle, C., & Gervais, M. (2019). Agricultural management practices and environmental drivers of nitrous oxide emissions over a decade for an annual and an

annual-perennial crop rotation. *Agricultural and Forest Meteorology*, 276–277, 107636.
<https://doi.org/10.1016/J.AGRFORMET.2019.107636>

Tenuta, M., Gao, X., Tiessen, K. H. D., Baron, K., Sparling, B., & Stewardship, N. (2023). Placement and nitrogen source effects on N₂O emissions for canola production in Manitoba. *Agronomy Journal*, 115(5), 2369–2383. <https://doi.org/10.1002/AGJ2.21408>

Thapa, R., Chatterjee, A., Awale, R., McGranahan, D. A., & Daigh, A. (2016). Effect of Enhanced Efficiency Fertilizers on Nitrous Oxide Emissions and Crop Yields: A Meta-analysis. *Soil Science Society of America Journal*, 80(5), 1121–1134. <https://doi.org/10.2136/SSSAJ2016.06.0179>;

The IUPAC Compendium of Chemical Terminology. (2025). *The IUPAC Compendium of Chemical Terminology*. <https://doi.org/10.1351/GOLDBOOK>

Von Stockar, U., Maskow, T., Liu, J., Marison, I. W., & Patiño, R. (2006). Thermodynamics of microbial growth and metabolism: An analysis of the current situation. *Journal of Biotechnology*, 121(4), 517–533. <https://doi.org/10.1016/J.JBIOTEC.2005.08.012>

Wagner-Riddle, C., Hu, Q. C., van Bochove, E., & Jayasundara, S. (2008). Linking Nitrous Oxide Flux During Spring Thaw to Nitrate Denitrification in the Soil Profile. *Soil Science Society of America Journal*, 72(4), 908–916. <https://doi.org/10.2136/SSSAJ2007.0353>;ISSUE:ISSUE:DOI

Wood, M. D., Gao, X., Tiessen, K. H. D., Tenuta, M., & Flaten, D. N. (2024). Enhanced efficiency urea fertilizers and timing effects on N₂O emissions from spring wheat production in Manitoba. *Agronomy Journal*, 116(1), 51–72. <https://doi.org/10.1002/AGJ2.21448>

- Wu, X., Li, T., Wang, D., Wang, F., Fu, B., Liu, G., & Lv, Y. (2020). Soil properties mediate the freeze-thaw-related soil N₂O and CO₂ emissions from temperate grasslands. *CATENA*, 195, 104797. <https://doi.org/10.1016/J.CATENA.2020.104797>
- Yildirim, S. C., Walker, R. M., Roessner, U., & Wille, U. (2023). Assessing the Efficacy, Acute Toxicity, and Binding Modes of the Agricultural Nitrification Inhibitors 3,4-Dimethyl-1H-pyrazole (DMP) and Dicyandiamide (DCD) with *Nitrosomonas europaea*. *ACS Agricultural Science & Technology*, 3(2), 222–231. <https://doi.org/10.1021/ACSAGSCITECH.2C00303>

4 OVERALL SYNTHESIS

4.1 Research Findings

Nitrous oxide (N_2O) emissions during freeze–thaw events result from complex interactions between soil physical, chemical, and biological processes. Soil moisture, temperature, and nitrogen availability are the main environmental factors involved (Butterbach-Bahl et al., 2013; Wagner-Riddle et al., 2017). While many studies have examined these factors separately, few have examined how they interact to influence both the amount of N_2O released and the microbial processes underlying it. This study addresses that gap by combining standard N_2O measurements with kinetic and thermodynamic analyses to better understand thaw-induced N_2O production in a Gleyed Humic Vertisol.

Across both chapters, soil moisture was the main factor controlling thaw-induced N_2O emissions. It affected not only the amount of N_2O released but also the rates and energy requirements of the microbial processes involved. Chapter 2 showed that higher soil moisture led to much greater hourly N_2O flux and total emissions. Chapter 3 found that more moisture also changed the rate constant, half-life, activation energy, Gibbs free energy, enthalpy, and entropy for N_2O production. Together, these results show that soil moisture influences both the amount and the manner in which N_2O is produced.

The key role of soil moisture supports the hole-in-the-pipe model which suggests that soil water content controls how much oxygen can move through the soil and, in turn, affects whether nitrification or denitrification causes nitrogen losses (Davidson, 1993; Davidson et al., 2000; Firestone & Davidson, 1989). When soil moisture increases, oxygen movement is limited, which favors denitrification and boosts N_2O production (Bateman & Baggs, 2005; Butterbach-Bahl et al., 2013; Linn & Doran, 1984). This study supports that idea, as the highest N_2O emissions was

recorded at 100% WFPS. The related increases in activation energy, Gibbs free energy, enthalpy, and entropy show that high soil moisture changes both the main microbial pathway and the energy needed for N₂O production.

These findings are particularly relevant, especially for the Canadian Prairies, where spring snowmelt often leads to waterlogged clay soils (Tenuta et al., 2019; Wood et al., 2024). In these conditions, freeze–thaw cycles disrupt soil aggregates, exposing organic carbon and nitrogen and increasing microbial activity when the soil thaws (Matzner & Borken, 2008; Congreves et al., 2018). This quick release of dissolved organic carbon and mineral nitrogen provides readily available substrate for microbes, causing short but strong bursts of N₂O emissions (Henry, 2007; Teepe et al., 2001). These results support earlier studies showing that soil moisture and oxygen, not just temperature, are the main factors controlling spring thaw N₂O emissions (Hung & Whalen, 2020; Wagner-Riddle et al., 2017). For management, this suggests that reducing soil moisture during the spring thaw could be more effective than focusing solely on fertilizer use.

While thawing temperature did affect total N₂O emissions by interacting with soil moisture, it had little independent effect on the rate or energy needs of microbial processes. Both parts of the study showed that temperature's impact depended mostly on soil moisture, and activation energy, Gibbs free energy, enthalpy, and entropy stayed about the same across the tested temperature range (4–16 °C). This means that once the soil thaws and has enough moisture and nutrients, microbes quickly become active again, and soil moisture, not temperature, is the main factor controlling their activity. Other studies have found similar results, showing that temperature mainly affects the rate at which microbes work, while oxygen and available nutrients determine which nitrogen processes dominate (Butterbach-Bahl et al., 2013; Davidson & Janssens, 2006; Dobbie & Smith,

2003). So, in this study, temperature mostly changed how soil moisture influenced microbial activity, rather than directly driving N₂O production.

A key finding of this study was that the enhanced-efficiency fertilizer SuperU did not significantly reduce thaw-induced N₂O emissions. In both parts of the research, the type of fertilizer used did not affect hourly N₂O flux, total emissions, or any of the measured kinetic and thermodynamic factors. This means that high soil moisture and low oxygen during thaw were strong enough to inhibit the effects of urease and nitrification inhibitors. When soils are saturated, denitrification is the main biochemical pathway through which N₂O is produced, making these inhibitors less effective (Abalos et al., 2014; Akiyama et al., 2010; Wood et al., 2024). Also, repeated freeze–thaw cycles may change how long inhibitors last and how they spread in the soil, further reducing their impact during thaw. These results show that enhanced-efficiency fertilizers are not always effective and should be seen as tools that work best under high moisture conditions.

A key contribution of this study is the use of kinetic and thermodynamic analyses alongside standard N₂O emission measurements. Earlier freeze–thaw research focused solely on how much N₂O was released and on which environmental factors were involved. This study showed that examining rate constants, half-life, activation energy, Gibbs free energy, enthalpy, and entropy provides deeper insight into the microbial processes underlying thaw-induced N₂O production. No studies have used this combined approach for agricultural soils in freeze–thaw conditions, especially in the Canadian Prairies. As a result, this work goes beyond just measuring emissions by describing the energy aspects of microbial N₂O production and offering data that could help improve greenhouse gas models for cold climates.

Overall, the findings from Chapters 2 and 3 clearly establish soil moisture as the primary regulator of thaw-induced N₂O emissions and their underlying kinetic and thermodynamic behaviour in a

Gleyed Humic Vertisol. By combining standard emission measurements with detailed kinetic and thermodynamic analysis, this research offers a new understanding of what drives spring thaw N₂O emissions in Prairie soils. These findings improve scientific understanding of freeze–thaw N₂O production and provide a stronger foundation for nitrogen management, model development, and efforts to reduce agricultural greenhouse gas emissions in Canada as the climate changes.

4.2 Implication of the study

This study sheds light on the drivers of thaw-induced N₂O emissions in Prairie agricultural soils and on their implications for sustainable nitrogen management. In both studies, soil moisture was the main factor controlling N₂O production, while thawing temperature mainly affected emissions through its interaction with soil moisture. The results show that soil conditions during thaw matter more than the type of fertilizer used. Keeping soil moisture at or below 50% WFPS before applying nitrogen in the fall may help lower the risk of high N₂O emissions after spring thaw. While it is not always possible to control soil moisture directly in the field, producers should consider soil drainage, landscape position, and expected spring moisture when planning fall fertilizer applications. These results support earlier studies showing that oxygen availability, which depends on soil moisture, is the main factor controlling denitrification and related N₂O emissions (Butterbach-Bahl et al., 2013; Wood et al., 2024).

These findings are also relevant for how enhanced-efficiency fertilizers are used in cold-climate farming. Even though SuperU contains both urease and nitrification inhibitors, it did not significantly lower N₂O emissions or change how microbes produced N₂O under freeze–thaw conditions in the lab. This means that when soils remain saturated for a long time, these fertilizers may not work as well because denitrification becomes the primary pathway for nitrogen loss. So, fertilizers with inhibitors should be part of a broader nitrogen management plan, not used on their

own to reduce emissions. Their effectiveness should always be checked in relation to soil moisture, especially in poorly drained Prairie clay soils (Abalos et al., 2014; Akiyama et al., 2010; Wood et al., 2024).

Scientifically, this study adds to our understanding of freeze–thaw-induced N₂O emissions by combining standard emission measurements with kinetic and thermodynamic analysis. Unlike earlier research, which mainly focused on total emissions, this study also estimated first-order rate constants, half-lives, activation energies, Gibbs free energies, enthalpies, and entropies. These extra details help explain the microbial processes behind N₂O production. This combined approach gives a clearer picture of how environmental factors affect both the amount and energy aspects of N₂O emissions, and it could help improve behavior that predict greenhouse gas emissions for refining future climate scenarios (Butterbach-Bahl et al., 2013; Davidson & Janssens, 2006).

This research also supports broader environmental and policy goals. Since agriculture is the main source of anthropogenic N₂O emissions in Canada, better nitrogen management is key to meeting the country's greenhouse gas reduction targets (Environment and Climate Change Canada, 2024). By showing that soil moisture is the main factor behind thaw-induced N₂O emissions, this study offers scientific support for improving the 4R Nutrient Stewardship framework and Canada's Fertilizer Emissions Reduction Target, which aims to reduce emissions from fertilizer use while improving productivity (Fertilizer Canada, 2023; Environment and Climate Change Canada, 2020). The findings also support the United Nations Sustainable Development Goal (SDG) 2 (Zero Hunger) by helping improve nitrogen use efficiency, SDG 12 (Responsible Consumption and Production) through better fertilizer management, and SDG 13 (Climate Action) by helping develop ways to reduce greenhouse gas emissions from agriculture.

These findings are useful for fertilizer manufacturers, agronomists, and policymakers. The limited success of SuperU during freeze–thaw cycles suggest a need for more efficient fertilizers that perform well even when soils remain wet and undergo repeated freezing and thawing. For agronomists and extension specialists, the results highlight the importance of considering soil moisture, not just fertilizer type and timing, when recommending fall nitrogen applications. Overall, this research provides a stronger scientific foundation for developing nitrogen management strategies that address climate challenges, improve nitrogen use efficiency, and reduce N₂O emissions from Prairie farms.

4.3 Study limitations

The study was conducted under controlled laboratory conditions, which do not fully capture the spatial and temporal variability of freeze–thaw events observed in the field. Soil moisture and thawing temperature were maintained at fixed levels, whereas these parameters fluctuate dynamically in field settings due to snowmelt, drainage, precipitation, and changes in soil temperature. Additionally, the experiment utilized soil from a single Gleyed Humic Vertisol and examined only one freeze–thaw event, thereby limiting the generalizability of the findings to other soil types, locations, and multiple freeze–thaw cycles.

The 60-hour thaw period captured the immediate N₂O pulse following thaw but may not have been sufficient to assess the full duration of fertilizer-inhibitor effects. Therefore, the lack of a significant SuperU effect should be interpreted within the context of this limited observation period.

The study did not directly measure microbial processes, oxygen availability, denitrification or nitrification rates, or changes in soil pore structure and gas diffusivity. As a result, the mechanisms

proposed to explain the significant moisture response and the temperature-by-moisture interaction remain inferential. Additionally, fall and spring nitrogen applications were not directly compared; thus, recommendations to delay fertilizer application until spring should be treated as management implications requiring field validation rather than direct conclusions from this experiment.

4.4 Recommendations

Based on the findings from the studies, it is recommended that:

1. Fall nitrogen application should be avoided on Gleyed Humic Vertisol fields that are already wet, poorly drained, or likely to remain saturated during spring thaw. In this study, N₂O emissions increased sharply at 75% and 100% WFPS, while SuperU did not reduce emissions relative to urea. Therefore, fall N should be targeted to fields with lower moisture risk, good internal drainage, and no persistent ponding, while wetter depressional areas should be deferred to spring application.
2. SuperU should not be used alone to reduce thaw-induced N₂O emissions from wet Gleyed Humic Vertisols. Since SuperU did not reduce N₂O emissions much compared to urea, it is best to use it alongside careful decisions about soil moisture, drainage, and when and where to apply fertilizer. In fields that often get saturated, waiting until spring to apply nitrogen or choosing better-drained areas for application may help more than just switching fertilizer types.
3. Future research should examine alternative nitrogen management practices, such as various enhanced-efficiency fertilizers, nitrification and urease inhibitors, fertilizer placement techniques, and application schedules, to determine strategies that maintain effectiveness during extended soil saturation in the spring thaw period.

4. Future research should measure how freeze and thaw cycles change soil pore structure, hydraulic conductivity, gas movement, and water distribution in Gleyed Humic Vertisols. Researchers should connect measurements of air-filled porosity, pore connectivity, oxygen levels, infiltration, and preferential flow with N₂O emissions. This will help determine whether high emissions in wet conditions are mostly due to increased microbial N₂O production, limited gas movement, or delayed release of N₂O trapped in the soil profile.

4.5 References

- Abalos, D., Jeffery, S., Sanz-Cobena, A., Guardia, G., & Vallejo, A. (2014). Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. *Agriculture, Ecosystems & Environment*, 189, 136–144. <https://doi.org/10.1016/J.AGEE.2014.03.036>
- Akiyama, H., Yan, X., & Yagi, K. (2010). Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N₂O and NO emissions from agricultural soils: Meta-analysis. *Global Change Biology*, 16(6), 1837–1846. <https://doi.org/10.1111/J.1365-2486.2009.02031.X>;
- Bateman, E. J., & Baggs, E. M. (2005). Contributions of nitrification and denitrification to N₂O emissions from soils at different water-filled pore space. *Biology and Fertility of Soils* 2005 41:6, 41(6), 379–388. <https://doi.org/10.1007/S00374-005-0858-3>
- Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R., & Zechmeister-Boltenstern, S. (2013). Nitrous oxide emissions from soils: How well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 368(1621). <https://doi.org/10.1098/RSTB.2013.0122/22151>
- Congreves, K. A., Wagner-Riddle, C., Si, B. C., & Clough, T. J. (2018). Nitrous oxide emissions and biogeochemical responses to soil freezing-thawing and drying-wetting. *Soil Biology and Biochemistry*, 117, 5–15. <https://doi.org/10.1016/J.SOILBIO.2017.10.040>
- Davidson, E. A. (1993). Soil Water Content and the Ratio of Nitrous Oxide to Nitric Oxide Emitted from Soil. *Biogeochemistry of Global Change*, 369–386. https://doi.org/10.1007/978-1-4615-2812-8_20

- Davidson, E. A., & Janssens, I. A. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. In *Nature* (Vol. 440, Number 7081, pp. 165–173).
<https://doi.org/10.1038/nature04514>
- Davidson, E. A. ;, Keller, M. ;, Erickson, H. E. ;, Verchot, L. V. ;, & Veldkamp, E. (2000). Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, 50(8), 667–680.
- Dobbie, K. E., & Smith, K. A. (2003). Impact of different forms of N fertilizer on N₂O emissions from intensive grassland. *Nutrient Cycling in Agroecosystems* 2003 67:1, 67(1), 37–46.
<https://doi.org/10.1023/A:1025119512447>
- Environment and Climate Change Canada. (2024). *National inventory report 1990–2022: Greenhouse gas sources and sinks in Canada*.
https://publications.gc.ca/collections/collection_2024/eccc/En81-4-2022-1-eng.pdf
- Firestone, M. K., & Davidson, E. A. (1989). Microbiological basis of NO and N₂O production and consumption in soil. *Exchange of trace gases between terrestrial ecosystems and the atmosphere*, 47,7-21. Retrieved June 2, 2026, from
https://scholar.google.ca/scholar?hl=en&as_sdt=0%2C5&q=Firestone%2C+M.+K.%2C+%26+Davidson%2C+E.+A.+%281989%29.+Microbiological+basis+of+NO+and+N+2+O+production+and+consumption+in+soil.+Exchange+of+trace+gases+between+terrestrial+ecosystems+and+the+atmosphere%2C+47%2C+7-21.&btnG=
- Henry, H. A. L. (2007). Soil freeze–thaw cycle experiments: Trends, methodological weaknesses and suggested improvements. *Soil Biology and Biochemistry*, 39(5), 977–986.
<https://doi.org/10.1016/J.SOILBIO.2006.11.017>

- Hung, C. Y., & Whalen, J. K. (2020). Biophysical controls on nitrous oxide emissions following rain-induced thawing of frozen soil microcosms by simulated rainfall. *Soil Biology and Biochemistry*, 149, 107960. <https://doi.org/10.1016/J.SOILBIO.2020.107960>
- Linn, D. M., & Doran, J. W. (1984). Effect of Water-Filled Pore Space on Carbon Dioxide and Nitrous Oxide Production in Tilled and Nontilled Soils. *Soil Science Society of America Journal*, 48(6), 1267–1272. <https://doi.org/10.2136/SSSAJ1984.03615995004800060013X>;
- Matzner, E., & Borken, W. (2008). Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, 59(2), 274–284. <https://doi.org/10.1111/J.1365-2389.2007.00992.X>
- Teepe, R., Brumme, R., & Beese, F. (2001). Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology and Biochemistry*, 33(9), 1269–1275. [https://doi.org/10.1016/S0038-0717\(01\)00084-0](https://doi.org/10.1016/S0038-0717(01)00084-0)
- Tenuta, M., Amiro, B. D., Gao, X., Wagner-Riddle, C., & Gervais, M. (2019). Agricultural management practices and environmental drivers of nitrous oxide emissions over a decade for an annual and an annual-perennial crop rotation. *Agricultural and Forest Meteorology*, 276–277, 107636. <https://doi.org/10.1016/J.AGRFORMET.2019.107636>
- Wagner-Riddle, C., Congreves, K. A., Abalos, D., Berg, A. A., Brown, S. E., Ambadan, J. T., Gao, X., & Tenuta, M. (2017). Globally important nitrous oxide emissions from croplands induced by freeze–thaw cycles. *Nature Geoscience* 2017 10:4, 10(4), 279–283. <https://doi.org/10.1038/ngeo2907>

Wood, M. D., Gao, X., Tiessen, K. H. D., Tenuta, M., & Flaten, D. N. (2024). Enhanced efficiency urea fertilizers and timing effects on N₂O emissions from spring wheat production in Manitoba. *Agronomy Journal*, 116(1), 51–72. <https://doi.org/10.1002/AGJ2.21448>